Discovery of Novel Mammalian target of rapamycin (mTOR) Inhibitors by Support Vector Machine

Panpan Wang¹, *, Xiaobo Xu¹, Yinghong Li², Bo Li³, Qinglan Pei¹, Pei Yu¹, Chenxi Jing¹, Meng Lu¹
¹College of Chemistry and Pharmaceutical Engineering, Huanghuai University, Zhumadian, China
²Chongqing Key Laboratory on Big Data for Bio Intelligence, Chongqing University of Posts and Telecommunications, Chongqing, China
³College of Life Sciences, Chongqing Normal University, Chongqing, China

*Corresponding author: wangpanpan@huanghuai.edu.cn

Abstract. Mammalian target of rapamycin (mTOR) is a protein serine/threonine kinase playing the central downstream role in multiple mitogenic signalling pathways. As a central regulator of cell growth, proliferation, differentiation and survival, mTOR has been reported to modulate proliferation and angiogenesis in neoplastic processes. Currently, sirolimus and its analogues are the only five mTOR inhibitors approved for clinical use, which shows a great capacity in anticancer therapy. However, endocrine resistance in cancer therapy has been observed in sirolimus analogues, and the unavailability of new mTOR inhibitor besides similar structure of sirolimus analogues makes the resistance even worse. It is urgent to discover new mTOR inhibitors as candidates for development of effective anticancer drugs. In this study, support vector machine (SVM) as a virtual screening strategy was proposed. SVM models of mTOR inhibitors were constructed by training data published before 2012, and the ones published after 2012 as test set were used to verify according to cross validation. The selected model performed false hit rates of 0.12% and 0.46% by screening PubChem and MDDR chemical libraries respectively. As results, 9 novel novel scaffolds for mTOR were identified, and 6 of them have been reported their anticancer-related therapeutic capacity. In summary, SVM performed its ability to identify novel mTOR inhibitors, which can supply some candidates for mTOR anticancer drugs, and supply effective method for anticancer drug discovery in future.

1. Introduction
Mammalian target of rapamycin (mTOR) is a protein serine/threonine kinase affecting multiple mitogenic signalling pathways [1], and plays the central downstream part in the life-essential PI3K/Akt/mTOR signalling [2]. mTOR responds to multiple signals from energy, growth factors and oxygen environment to exert its effects on cell growth, proliferation and survival, including mRNA biogenesis [3], biological macromolecule synthesis [4] and autophagy [5]. Moreover, emerging evidence sheds light on the control of mTOR by many other intracellular pathways such as WNT, Hippo…
and Notch signalling [4], and misregulation of these pathways is frequently found in cancers, such as hepatocellular carcinoma [6], squamous cell lung carcinoma [6], metastatic breast cancer [7] and so on. As a central regulator of cell growth, proliferation, differentiation and survival [8], mTOR has been reported to play a central role in modulating proliferation and angiogenesis in neoplastic processes [9] and arresting tumour genesis [10]. Sirolimus and its analogues, reported as specific mTOR inhibitor, are currently one of the most effective classes of immunosuppressants following kidney transplantation and anticancer agents [11], and, according to the latest version of the Therapeutic Target Database (TTD) [12, 13], they are the only five mTOR inhibitors approved for clinical use (Table 1). Sirolimus was originally developed as an antifungal agent, but abandoned when its immunosuppressive and antiproliferative properties in mammalian cells are discovered [14]. After the discovery of sirolimus, four of its analogues (everolimus, temsirolimus, umirolimus and zotarolimus) have been approved for clinical use, which shows a great capacity of sirolimus analogues in cancer therapy.

Table 1. Current developmental status of mTOR inhibitors, examples of approved and clinical trial drugs.

| Drug name   | Developer    | Condition                | Clinical status |
|-------------|--------------|--------------------------|-----------------|
| Everolimus  | Novartis     | Immunosuppressant Renal cell cancer | Launched        |
| Sirolimus   | Pfizer       | Immunosuppressant Hepatocellular carcinoma | Launched        |
| Temsirolimus| Wyeth        | Advanced kidney cancer   | Launched        |
| Umirolimus  | Biosensors   | Restenosis               | Launched        |
| Zotarolimus | Medtronic    | Restenosis               | Launched        |
| AZD-2014    | AstraZeneca  | Breast cancer            | Phase II        |
| GDC-0980    | Genentech    | Endometrial carcinoma    | Phase II        |
| PBI-05204   | Phoenix biotec| Pancreatic cancer      | Phase II        |
| PF-05212384 | Pfizer       | Endometrial neoplasms    | Phase II        |
| PX-866      | Oncothyreon  | Prostate cancer          | Phase II        |
| SAR245409   | Sanofi       | Neoplasm malignant      | Phase II        |
| CC-115      | Celgene      | Chronic lymphocytic leukemia | Phase I        |
| CC-223      | Celgene      | Non-small cell lung cancer | Phase I        |
| DS-3078     | Daiichi Sankyo| Lymphoma              | Phase I        |
| DS-7423     | Daiichi Sankyo| Colorectal cancer      | Phase I        |
| LY3023414   | Eli Lilly    | Non-small cell lung cancer | Phase I        |
| ME-344      | MEI Pharma   | Ovarian cancer           | Phase I        |
| VS-5584     | Verastem     | Lymphoma                 | Phase I        |

However, endocrine resistance in cancer therapy has been observed in sirolimus analogues [15]. The unavailability of new mTOR inhibitor besides similar structure of sirolimus analogues (Fig. 1) makes the resistance even worse. Currently, there are several novel mTOR inhibitors in clinical trial as identified from ClinicalTrials.gov (https://www.clinicaltrials.gov/), majority of which exert anticancer therapeutic effects via their anti-proliferative and anti-angiogenic properties (Table 1). However, all of
these candidates are only in early stage clinical development (none in phase III, 6 in phase II and 7 in phase I) so far. Moreover, the published mTOR inhibitors are distributed in sparsely chemical space. Particularly, there were more than 1,000 mTOR inhibitor as reported [16], and only 45 of them are collected in the Therapeutic Target Database (TTD) [12, 13]. Thus, it is urgent to discovery novel mTOR inhibitors as candidates for development of effective anticancer drugs.

Figure 1. mTOR inhibitors: structure and approved drugs (the shared common structure of all drugs were coloured by blue).

To find novel active compounds, great efforts have been spend to explore larger chemical space [17,18]. As a useful method for identifying bioactive compound, virtual screening (VS) was applied in searching candidates by screening large compounds libraries [19]. According to some previous studies, the predicted property of VS is frequently restricted by the limited reported active inhibitors sparsely covered in some active chemical regions [20]. However, support vector machine (SVM) performed potent ability in searching novel compounds among numbered active inhibitors at low false hit rates [20]. Until now, SVM method has been effectively identified novel candidates for ERK [21], RAF [22], HIV-1 protease [23], ABL [18], mGluR1 [24], dopamine receptor [25] in the development of infection, nervous disorder and many cancers drugs.

This study constructed SVM model by reported mTOR inhibitors to identify novel scaffolds for target. Firstly, mTOR inhibitors published before 2012 were separated as training set to construct models, and the ones reported at later 2012 were considered as test set. Secondly, the VS performance of selected model was measured by independent testing and large compounds libraries scanning. In the end, the identified novel scaffolds were further discussed as far as their reported therapeutic effects.

In order to validate the model constructed, 5-fold cross validation is applied. Active data and inactive data including putative non-inhibitors were randomly separated into 5 approximately equal sets, 4 out
of 5 were considered as training set and another was considered as testing set, the process like this was redone for 5 compositions of training and test sets. For constructed model, its best parameters were identified by average accuracy, its yield and false hit rate were evaluated by screening PubChem [26] and MDDR large chemical libraries including many inactive compounds different from the published mTOR inhibitors. In order to avoid the artificially enhancing the prediction enrichments, a set of mTOR non-inhibitors with similar structure to reported compounds was used to strictly test to illustrate that the prediction enrichments don’t only separated characteristics of easy to distinguish [27].

2. Methods

2.1. The collection of Active data and Construction of Training and Test Sets

This work collected 1141 mTOR inhibitors form CHEMBL [28], 806 of them were active owing to their IC\textsubscript{50}/Ki no more than 500nM. 757 of 806 compounds considered as training set were reported before 2012, and the rest 49 compounds as testing data were published later 2012. All active inhibitors distributed diverse scaffolds, which guaranteed the relatively feasibility of constructed model. As illustrated in Fig. 2, 757 inhibitors were classified into 24 scaffolds with quantities of active inhibitors. Moreover, Fig. 3 illustrates 20 examples of 294 compound in scaffold 1 with their inhibitory activities for mTOR.

![Figure 2. 24 molecular scaffolds for 757 mTOR inhibitors and the corresponding number as shown in bracket for each scaffold.](image-url)
Figure 3. 20 examples of 294 compound in scaffold 1 with their activities for mTOR (the shared common structure of all inhibitors were coloured by blue).

Due to the numbered non-inhibitors, the sufficient putative data were generated to mark the whole inactive compound families by the same way as reported by Liu et al [18], which can effectively reduce false-hits [29]. This study generated 65,806 putative data to develop SVM model, and its virtual hit and false hit rate were evaluated by scanning PubChem, MDDR compounds and 450 MDDR compounds with structurally similar to the published mTOR inhibitors. The similarities of compounds were judged by molecular similarity analyses and visual inspection [20].
2.2. Molecular Descriptors
Molecular descriptors are quantitative representations of structural and physicochemical features of molecules, which have been extensively used in deriving quantitative structure activity relationships (QSAR) and VS tools [18, 23-25, 30, 31]. This work calculated 98 molecular descriptors including 18 descriptors representing simple molecular properties, 3 descriptors describing chemical properties, 35 descriptors showing molecular connectivity and shape, 42 descriptors expressing electro-topological state [18, 23-25, 30, 31].

2.3. The Construction of SVM Model and Molecular Similarity Analysis
In this work, SVM as a supervised learning and classification method was applied to discovering mTOR active compounds from inactive. SVM model was constructed by training set, which can efficiently consider novel molecules as active or inactive, thus SVM is a binary classifier [18].

This study performed Tanimoto similarity searching to analyze the molecular similarities. The similarities of compounds to at least one reported mTOR inhibitor of training set could be calculated by the Tanimoto similarity coefficient as illustrated below [32].

\[
sim(i, j) = \frac{\sum_{d=1}^{l} x_{di}x_{dj}}{\sum_{d=1}^{l} (x_{di})^2 + \sum_{d=1}^{l} (x_{dj})^2 - \sum_{d=1}^{l} x_{di}x_{dj}}
\]

Where \( l \) is the number of molecular fingerprints. Two compounds were considered as similar with more than 0.9.

2.4. The Property of SVM Model in Scanning Large Compound Libraries
The performance factors were calculated by virtual screening large chemical libraries [33]. For example, yield performed the ratio of virtual hits in reported positives, hit rate is the ratio of known positives in virtual hits, false hit rate is the proportion of published negatives in virtual hits, and enrichment factor is the wide improvement of hit rate than random selected method from large compounds libraries.

3. Results and Discussion

3.1. The Construction of SVM Model by 5-Fold Cross Validation
The constructed SVM model was tested by 5-fold cross validation in identifying mTOR inhibitors. In each fold, the predicting accuracies of inhibitors were 92.05–98.68% with 95.11% of average accuracies, and the values of non-inhibitors were 99.90–99.97% with 99.92%. Based on a comprehensive information retrieval until now, there was no report about identifying mTOR inhibitors, so it is difficult to compare with SVM model constructed in this work to assess its performance. But in fact, the identifying yield of mTOR inhibitor is equivalent to or higher than that of reports about some targets [18, 29, 32-35]. Additionally, model performed thin false hit rate by 99.924% predicted yield for non-inhibitor, which could improve effectively success chance for identifying mTOR inhibitors in further \textit{vitro} and \textit{vivo} research. Based on all analysis as shown above, this work constructed an effective SVM model with good prediction capability in identifying known mTOR inhibitor.

3.2. Independent Testing and VS Large Compound Libraries
The SVM model was constructed by published mTOR inhibitors before 2012, and its performance were measured by the ones reported later 2012 as independent testing. The yield is 71.43% with 35 compound predicted correctly, which is equivalent to the published by other VS methods [36]. It may be unreasonable to contrast the yield in this study to other reported researches owing to the differences molecular types, descriptors and parameters. Nevertheless, in terms of 23 compounds in testing set with similar to known mTOR inhibitors, 95.65% (22 inhibitors) were correctly predicted. Moreover, among
26 novel inhibitors in testing set, 50% (13 inhibitors) were found. These results demonstrated the certain capacity of SVM mode in identifying new mTOR compounds.

Aside from the nice prediction performance evaluate as shown above, low false hit rate is another obvious characteristic for developed SVM model. Excluding mTOR inhibitors, 15,817 virtual hits were considered as active inhibitors in scanning PubChem database including 13.56M molecules, corresponding to just 0.12% of all PubChem library. Moreover, 0.46% of false hit rate was estimated in scanning MDDR molecules covered the protein families without mTOR related. All these results demonstrated that SVM model constructed in this work possessed reasonable ability in decreasing false positive inhibitors.

Additionally, the false hit of the SVM model was measured according to predicting 450 MDDR inhibitors with similar to the reported mTOR inhibitors. SVM screened 209 virtual hits from 450 MDDR similarity inhibitors with virtual hit rate 46.44%, which displayed that SVM model performed high capability in identifying mTOR inhibitors from similar inactive compounds. Moreover, 111 mTOR non-inhibitors indicated in ChEMBL database were collected, all of which are structurally dissimilar to known inhibitor (Tanimoto similarity coefficient is less than 0.9). Our SVM model can predict all of them as non-inhibitor, which illustrates a good ability of SVM model in identifying mTOR inhibitors from inactive compounds with dissimilar to active molecules.

### 3.3. Identifying Novel mTOR Inhibitors Based on SVM Model

SVM perform potent ability of identifying novel lead compounds from out of compounds families covered by the reported inhibitors [18, 30]. In this study, 776 compounds from MDDR were identified as active inhibitors by constructed SVM model. 533 of them performed structurally similar with reported mTOR inhibitors. These 533 inhibitors were classified in scaffold 4, 5, 10, 11, 13, 15, 17, 19, 22, 23 and 24 as Fig. 1 with 20, 1, 1, 74, 8, 380, 19, 4, 13, 5 and 8 compounds respectively.

In this work, novel scaffolds of mTOR inhibitors were identified as shown in Fig. 4. 6 scaffolds showed anticancer effects, although there were no directly inhibit mTOR for all of them. As collected in Table 2, Scaffold N1 was camptothecin derivatives, which was reported to be applied for treating metastatic colorectal cancer (CRC), and approved for CRC combined with cytotoxic agents in Japan and Taiwan [37]. Scaffold N2 was 4-amino pyrimidine derivatives, which was reported their valuable pharmacological properties, especially on inhibiting signal transduction of tyrosinkinases, and can be used in the treatment of disorders, especially tumours [38]. Scaffold N3 (2-Amino-6-anilino-purine derivatives) was proposed to inhibit the signal transduction of tyrosinkinases for cancer [39]. Scaffold N4 (pyridopyrimidinones derivatives) was reported as antitumor therapeutic agents [40]. Scaffold N5 (multi-cyclic fused quinoline derivatives) provided a serial of compounds exhibited weak to moderate topoisomerase inhibitory activity, and can be used in the treatment of tumours [41]. Scaffold N8 (oxazolopyrimidines) is reported to be potentially useful against tumour and deregulated angiogenesis [42].

In summary, some found novel mTOR candidates have already reported their effects on cancer-related diseases, but more accurate lead compounds need to be further evaluated. Some large compounds libraries like Zinc, PubChem and MDDR supply a comprehensive candidate’s dataset to discovery novel lead scaffolds, therefore, it is necessary to explore efficient virtual screening tool to promote the development of novel drugs with high robustness and low false hit rate.
Figure 4. 9 selected molecular scaffolds considered as novel mTOR inhibitor in this study.

Table 2. 6 selected molecular scaffolds considered as novel mTOR inhibitor with reported anticancer effects.

| Index | Scaffold-type               | Novel scaffold identified | Reported therapeutic effects of the scaffold                                                                 |
|-------|----------------------------|---------------------------|-------------------------------------------------------------------------------------------------------------|
| N1    | Camptothecin derivatives   |                           | Applied for treating metastatic colorectal cancer (CRC), and approved for CRC combined with cytotoxic agents in Japan and Taiwan [37]. |
| N2    | 4-Amino pyrimidine         |                           | Having pharmacological properties, especially on inhibiting signal transduction of tyrosinkinases, and can be used in the treatment of disorders, especially tumours [38]. |
4. Conclusion
This work proposed SVM as a VS strategy to discovery mTOR candidates and performed satisfactory prediction performance. SVM model of mTOR inhibitors were constructed by training data published before 2012, which could predict successfully 71.43% by independent testing. This result shown the satisfactory ability in identifying novel mTOR inhibitors. Moreover, the model showed low false-hit rates with 0.12% by screening 13.56M PubChem, and 0.46% by scanning 168K MDDR. Additionally, the model performed good performance in discovering mTOR inhibitors from inactive compounds by correctly judged all 111 experimentally mTOR non-inhibitors as inactive. Finally, this work proposed some novel candidates with anticancer effete, indicates that it is necessary to scan comprehensively in larger chemical databases to discovery more leads for mTOR.

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