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Target identification, lead optimization and antitumor evaluation of some new 1,2,4-triazines as c-Met kinase inhibitors

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ABSTRACT

In silico target fishing approach using PharmMapper server identified c-Met kinase as the selective target for our previously synthesized compound **NCI 748494/1**. This approach was validated by *in vitro* kinase assay which showed that **NCI 748494/1** possessed promising inhibitory activity against c-Met kinase (IC_{50} = 31.70 μM). Assessment of ADMET profiling, drug-likeness, drug score as well as docking simulation for the binding pose of that compound in the active site of c-Met kinase domain revealed that **NCI 748494/1** could be considered as a promising drug lead. Based on target identification and validation, it was observed that there is structure similarity between **NCI 748494/1** and the reported type II c-Met kinase inhibitor BMS-777607. Optimization of our lead **NCI 748494/1** furnished newly synthesized 1,2,4-triazine derivatives based on well-established structure-activity relationships, whereas three compounds namely; **4d**, **7a** and **8c** displayed excellent *in vitro* cytotoxicity against three c-Met addicted cancer cell lines; A549 (lung adenocarcinoma), HT-29 (colon cancer) and MKN-45 (gastric carcinoma); with IC_{50} values in the range 0.01–1.86 μM. *In vitro* c-Met kinase assay showed **8c** to possess the highest c-Met kinase inhibition profile (IC_{50} = 4.31 μM). Docking of the active compounds in c-Met kinase active site revealed strong binding interactions comparable to the lead **NCI 748494/1** and BMS-777607, suggesting that c-Met inhibition is very likely to be the mechanism of the antitumor effect of these derivatives.

1. Introduction

Cancer is among the leading causes of death worldwide. It is the second most common cause of death after cardiovascular diseases [1]. The most common causes of cancer death are cancers of lung, liver, stomach, colorectal, breast and oesophageal cancer. Despite the extensive research and rapid progress in cancer treatment, there is still an ever growing need for selective, efficient and safe therapeutic strategies. Consequently, identifying new targets and new chemotherapeutic agents for cancer therapy is of great importance [2]. Target-based cancer therapies have significantly progressed over the past decade. Numerous FDA-approved agents are designed to block specific signalling pathways important for tumor growth, progression, invasion and/or angiogenesis [3].

Target identification and validation is the first key step in the drug discovery and development chain. '*In silico reverse screening*' [4], '*target fishing*' [5] or '*inverse virtual screening*' [6] has become a very popular tool to identify the most likely targets of a query molecule. This approach, in contrast to virtual screening [7,8], allows the prediction of the bioactivity of the query molecule or its mechanism of action [9,10]. PharmMapper server is a freely accessed web server designed to identify potential target candidates for the given small molecules by finding the best mapping poses of the query molecules against all the targets in PharmTarget database [11].

In a previous work, compound **NCI 748494/1** (Fig. 1) was synthesized in our laboratory and evaluated for its antitumor activities at the National Cancer Institute (NCI), Bethesda, Maryland, USA. The compound exhibited potential antitumor activities against most of the tested subpanel cancer cell lines. It displayed high growth inhibitory potential (GI_{50} MG-MID 3.98 μM), together with reasonable cytostatic (TGI MG-MID 35.48 μM) and mild cytotoxic (LC_{50} MG-MID 97.72 μM) activities [12]. In this context, our aim in this work was to first identify the potential target involved in the observed antitumor activity displayed by our previously synthesized compound **NCI 748494/1** and then further design and synthesize optimized derivatives with developed antitumor activity.

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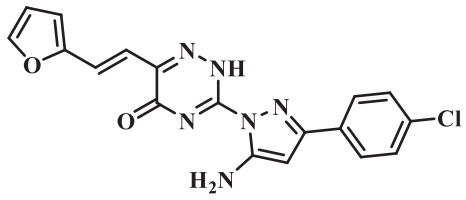

Fig. 1. Structure of the previously synthesized compound **NCI 748494/1**.

2. Results and discussion

2.1. Target identification and validation

PharmMapper identified three targets; c-Met kinase, VEGFR-2 and CDK-2 that displayed the highest fit scores and were considered as possible targets for the antitumor agent **NCI 748494/1** (Table 1). Then, validation was carried out by conducting protein kinase assay at Kinexus Corporation, Vancouver, B.C., Canada. The assay was performed at six concentrations of **NCI 748494/1** (0.1 μM, 1 μM, 10 μM, 50 μM, 100 μM and 500 μM) in singlicate using radiometric assay method to determine its IC_{50} against the three targets. Kinase activity was assessed using a highly standardized assay methodology [13]. The % activity change of c-Met kinase highly decreased by increasing compound **NCI 748494/1** concentration. While, the profiling data for **NCI 748494/1** against CDK-2 and VEGFR-2 showed low and no significant inhibition with increasing its concentration, respectively (Table 2). The IC_{50} value of **NCI 748494/1** against c-Met kinase was calculated to be 31.70 μM, whereas, against CDK-2/cyclin A2 and CDK-2/cyclin E1 were found to be 153.1 and 130.9 μM, respectively. Thus, **NCI 748494/1** could be considered as a promising selective c-Met kinase inhibitor.

Targeting c-Met (cellular mesenchymal epithelial transition factor), one of the receptor tyrosine kinases [14–16], with small molecule inhibitors has captured extensive attention in the treatment of many different types of solid tumors [17,18]. Of interest are type II ATP competitive inhibitors; Foretinib (phase III), Cabozantinib, Kirin Brewery (phase II), BMS-777607 (phase II), MGCD265 (phase II) and Crizotinib (Fig. 2) [19–22]. Most type II c-Met kinase inhibitors share a common structure-activity relationship that suggests moiety A: that is responsible for hydrogen bond formation with the backbone of c-Met kinase; moiety B: represented by the aryl fragment, probably extends into a hydrophobic pocket and additional hydrogen bonds, which are also crucial for inhibitory activity, which are formed by the linkers connecting moiety A to moiety B [23–25]. Modifications usually take place in the linker atoms as well as moiety A [26–33]. Whereas, there is little change to moiety B that is usually retained as a phenyl or substituted phenyl rings [34]. Literature survey revealed very few reports on 1,2,4-triazines regarding their c-Met kinase inhibitory activity. Different 1,2,4-triazines (Fig. 3) [35–38] were reported to possess high c-Met

Table 1
Pharmacophore candidates of compound **NCI 748494/1** by PharmMapper.

Rank	PDB ID	Fit score	Target name
2	3F82	4.737	Hepatocyte growth factor receptor (c-Met kinase)
13	2RL5	4.218	Vascular endothelial growth factor receptor 2 (VEGFR-2)
27	1GZ8	3.964	Cyclin-dependent kinase-2 (CDK-2)

Table 2
% Activity change of protein kinases in the presence of compound **NCI 748494/1** using radiometric assay method.

Target ID	% Activity Change 0.1 μM	% Activity Change 1 μM	% Activity Change 10 μM	% Activity Change 50 μM	% Activity Change 100 μM	% Activity Change 500 μM	Stauro-sporine 1 μM
c-Met	3	−5	−22	−56	−89	−94	−89
VEGFR-2	−5	−4	−5	−2	−2	−2	−91
CDK-2/Cyclin A2	0	0	−2	−16	−37	−83	−98
CDK-2/Cyclin E1	2	2	−9	−25	−47	−77	−96

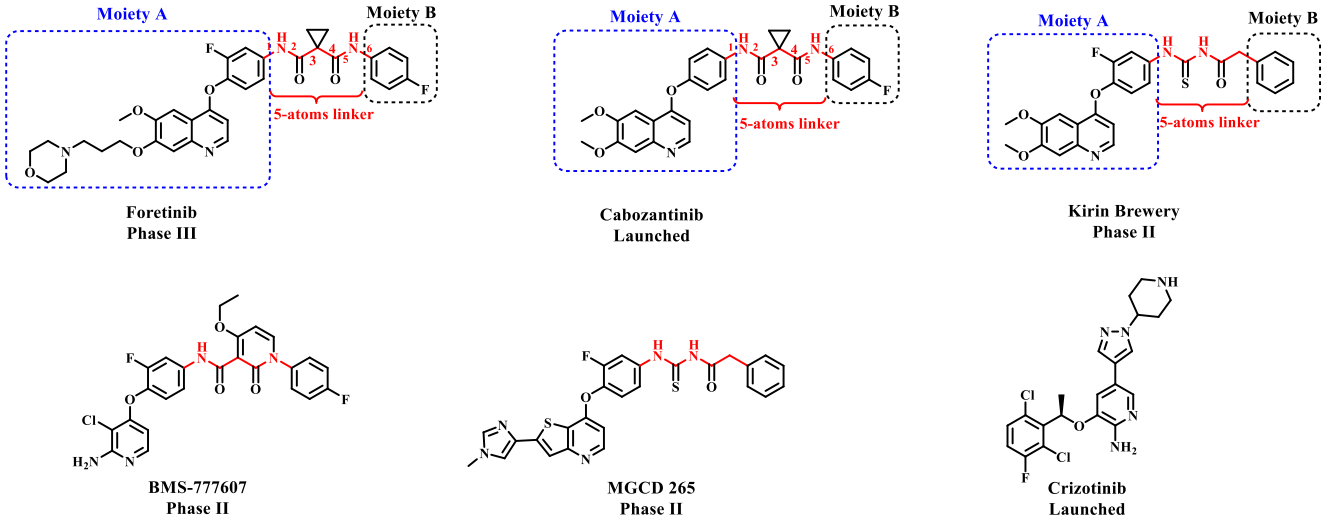

Fig. 2. Structures of several type II c-Met kinase inhibitors launched or in clinical trials.