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Identification of nitroimidazole-oxime derivatives targeting the polo-box domain of polo-like kinase 1

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ABSTRACT

Recent progress in the development of small molecular skeleton-derived polo-like kinase (PLK1) catalytic domain (K_D) inhibitors has led to the synthesis of multiple ligands with high binding affinity. However, few systematic analyses have been conducted to identify key PLK1-PBD domain and characterize their interactions with potent PLK1 inhibitors. Therefore, we designed a series of PLK1-PBD inhibitors with an *in silico* scaffold modification strategy. A docking simulation combined with a primary screen *in vitro* were performed to filter for the lead compound, which was then substituted, synthesized and evaluated by a variety of bioassays. The biological profile of **4v** suggests that this compound may be developed as a potential anticancer agent.

1. Introduction

Polo-like kinase 1 (PLK1), a key player in mitosis,[1,2] modulates the transition through the G2/M checkpoint, is crucial in cell proliferation and has been considered as a target for tumor therapy.[3,4] Until now, two distinct drug targets have been identified in PLK1: a N-terminal catalytic domain (K_D) and a C-terminal domain having 2 highly homologous sequences, termed polo-box domain (PBD). For a long period, discovery of PLK1 inhibitors has been focused on targeting the N-terminal catalytic domain.[5,6] However, protein kinases have high similarities in the ATP-binding pockets,[7] and these efforts suffered from a lack of specificity. An alternative approach with potential for identifying potent and highly selective kinase inhibitors is to target the interfaces of protein-protein complexes of interest.[8] Therefore, the special structure of the PBD has become an highly selective target.[9,10]

Several series of peptides were designed and synthesized targeting PLK1-PBD with high affinity and specificity.[11–16] However, inadequate proteolytic resistance and cell permeability of the peptides hinder the development of these peptide-based inhibitors into novel therapeutic compounds. In order to overcome the shortcomings of peptide-based inhibitors, some small molecules have been designed and synthesized targeting PLK1-PBD.[17–22] Unfortunately, the limited number of PLK1-PBD inhibitors and their modest selectivity greatly limit the design based on the structure. Therefore, clarifying the binding mode between PBD and small molecules, as well as improving their specificity recognition, is the key issues to be resolved.

To continue our efforts aimed at discovering and developing PLK1-PBD inhibitors[23], in this study, we used a structure-based approach to design a novel and potent small molecule named **4v**, which exhibits a high binding affinity for PLK1-PBD.

2. Results and discussion

2.1. Structure-based design of small molecules

To identify the minimal set of key residues required for a high binding affinity, we examined the previously reported cocrystal structures of PLK1-PBD[24] (PDB ID: 4HCO) in complex with a ligand. Then we have found that three binding pockets are critical to bind PLK1-PBD with high affinity, combined with our previous research[23]: (1) Electrostatic-binding region (EBR) in which the Lys540-His538 pincer clinches phosphopeptides by the phosphate. (2) Hydrogen-bonding region (HBR) is surrounded by Trp414, Phe535 and Arg516 residues through participating in a hydrogen bonding interactions. (3) Hydrophobic motif (HM) formed by Val415, Tyr417, Tyr485 and Leu490 through hydrophobic interactions with the above residues (Fig. 1). These observations suggest

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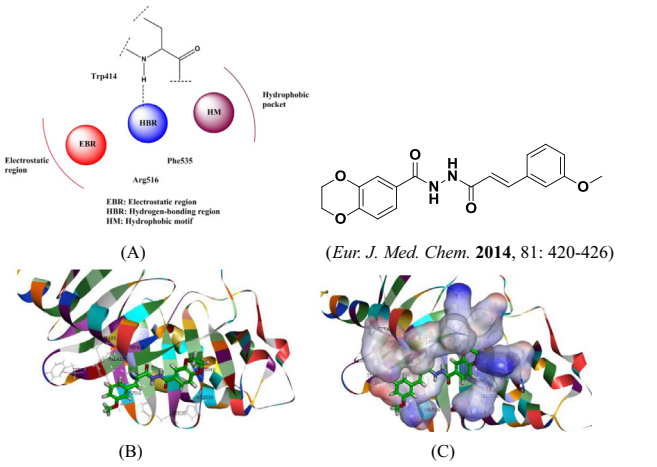


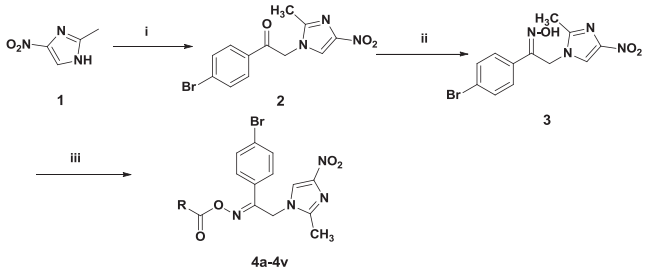
Fig. 1. Three binding pockets are critical to bind PLK1-PBD with high affinity: (A) 2D figure. (B) 3D figure. (C) 3D figure.

that appropriate design of small molecules would provided drug-like small molecule targeting PLK1-PBD with high binding affinity.

According to our previous research and the three binding pockets, we have selected benzene ring and pyridine ring as the basic scaffold. At the same time, a variety of substituents have been introduced, aim to investigate the position effect, space effect, electron effect, hydrophilicity and hydrophobicity against to the biological activity of the target compounds. Meanwhile, nitroimidazole-oxime skeleton has a good affinity with Lys540-His538 pincer clinches in the EBR region according to the Docking simulations. Therefore, in this research, we presented a nitroimidazole-oxime as our scaffold which provides rigidity around the central unit.

2.2. Chemistry

Twenty-two nitroimidazole-oxime analogs were synthesized and all of them were synthesized for the first time. The synthesis of compounds has been followed the general pathway outlined in Scheme 1. All of the synthetic compounds **4a**–**4v** are being reported for the first time and gave satisfactory analytical and spectroscopic data.



4a: R=4-FPh **4f:** R=4-BrPh **4k**: R=2-NO_2Ph **4p**: R=2-MeOPh **4u**: R=iPy
4b: R=4-CH_3Ph **4g:** R=3-FPh **4l:** R=3-MePh **4q**: R=2-ClPy **4v**:R=6MePy
4c: R=4-ClPh **4h**: R=3-ClPh **4m**: R=2-ClPh **4r:** R=6-ClPy
4d: R=4-OMePh **4i:** R=3-BrPh **4n**: R=2-MePh **4s:** R=6-MeOPy
4e: R=4-NO_2Ph **4j:** R=2-BrPh **4o**: R=Ph **4t:** R=Py

Scheme 1. General synthesis of compounds **4a**–**4v**. Reagents and conditions: (i) 2,4′-dibromo-acetophenone, K_2CO_3, TBAB, ethanol, reflux, 0.5–1 h. (ii) Hydroxylamine hydrochloride, K_2CO_3, ethanol, reflux, 2–4 h. (iii) Substituted benzoic acid, niacin or isonicotinic acid, EDC·HCl, HoBt, rt, 8–10 h.

Table 1
In vitro anticancer activities against four human tumor cell lines.

Compounds	IC_{50} ± SD (μg/mL)			
	MGC-803	HepG-2	MCF-7	Hela
3	>20	17.39 ± 0.12	>20	>20
4a	1.24 ± 0.05	1.22 ± 0.08	2.12 ± 0.03	7.24 ± 0.06
4b	0.81 ± 0.03	0.96 ± 0.02	1.54 ± 0.05	0.96 ± 0.02
4c	0.45 ± 0.02	1.34 ± 0.05	2.79 ± 0.07	8.79 ± 0.03
4d	0.34 ± 0.04	1.25 ± 0.07	3.15 ± 0.02	6.54 ± 0.04
4e	0.37 ± 0.02	1.43 ± 0.09	0.86 ± 0.03	12.41 ± 0.19
4f	1.48 ± 0.06	2.56 ± 0.04	2.56 ± 0.05	5.67 ± 0.06
4g	0.21 ± 0.03	0.97 ± 0.02	1.87 ± 0.03	4.55 ± 0.04
4h	0.18 ± 0.02	1.15 ± 0.03	0.84 ± 0.06	4.09 ± 0.04
4i	0.04 ± 0.01	0.86 ± 0.05	1.37 ± 0.02	2.57 ± 0.08
4j	2.63 ± 0.87	4.38 ± 0.01	3.79 ± 0.09	>20
4k	2.54 ± 0.04	6.59 ± 0.5	7.13 ± 0.04	11.35 ± 0.07
4l	3.25 ± 0.02	10.65 ± 0.14	2.82 ± 0.03	12.35 ± 0.09
4m	0.16 ± 0.07	1.33 ± 0.05	1.27 ± 0.08	8.53 ± 0.06
4n	0.84 ± 0.06	2.37 ± 0.02	2.15 ± 0.06	9.76 ± 0.05
4o	3.66 ± 0.05	13.96 ± 0.28	>20	>20
4p	3.53 ± 0.04	>20	3.13 ± 0.07	>20
4q	0.73 ± 0.05	0.76 ± 0.03	0.76 ± 0.04	1.28 ± 0.01
4r	0.82 ± 0.03	0.71 ± 0.02	0.79 ± 0.02	0.96 ± 0.02
4s	0.03 ± 0.01	0.92 ± 0.03	0.89 ± 0.02	2.35 ± 0.04
4t	0.01 ± 0.01	0.73 ± 0.01	0.78 ± 0.03	1.51 ± 0.05
4u	0.02 ± 0.01	0.81 ± 0.02	0.87 ± 0.01	1.63 ± 0.02
4v	0.005 ± 0.003	0.75 ± 0.01	0.72 ± 0.02	0.86 ± 0.03
5-FU	1.8 ± 0.02	2.24 ± 0.07	2.19 ± 0.03	3.82 ± 0.08

2.3. MTT assay for cell proliferation

To test the antiproliferative activities of the synthesized compounds, the target compounds were evaluated *in vitro* antiproliferation assays against four human cancer cell lines (MCF7, HeLa, MGC803 and HepG2). The results were summarized in Table 1. With few exception, the active analogs showed a remarkable potential antiproliferative activity against MGC803 (Fig. 2), suggesting that nitroimidazole-oxime derivatives could significantly enhance antiproliferative potency. For the given compounds, it was observed that compound **4v** showed the most potent antiproliferative activity (IC_{50} = 0.002 μg/mL).

Structure–activity relationship (SAR) analysis indicated that different acids substitutes led to different antitumor activity, and the potency order was niacin > benzoic acid. compounds **4q** and **4r** with substituted Cl group on niacin ring showed moderate antitumor activity with IC_{50} of 0.71–1.28 μg/mL against four human cancer cell lines. Based on the data obtained, compounds **4t** and **4u** with niacin and Isonicotinic ring were found to be better activity toward BGC823 cancer cell line with IC_{50} values, 0.01 and 0.02 μg/mL respectively.

2.4. Cytotoxicity test

Generally speaking, the inhibitory activity of the compounds is due to cell apoptosis or toxic effect, so we performed cytotoxicity test before detecting kinase activity. All of the compounds were detected for their cytotoxicity on Human Kidney epithelia cells (293T). The pharmacological results of these compounds were summarized in Table 2. What we can see from the data is that most of the compounds were low toxic.

2.5. PLK1-PBD inhibitory assay

To validate whether the above anti-proliferative effect was produced by interaction of PLK1 protein and the synthesized compounds, the selected compounds were evaluated for their abilities to inhibit the activity of PLK1 protein kinases relevant to cancer. As expected, all compounds displayed the best inhibitory