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Research paper

Locking PDK1 in DFG-out conformation through 2-oxo-indole containing molecules: Another tools to fight glioblastoma

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ABSTRACT

The phosphoinositide-dependent kinase-1 (PDK1) is one of the main components of the PI3K/Akt pathway. Also named the "master kinase" of the AGC family, PDK1 plays a critical role in tumorigenesis, by enhancing cell proliferation and inhibiting apoptosis, as well as in cell invasion and metastasis formation. Although there have been done huge efforts in discovering specific compounds targeting PDK1, nowadays no PDK1 inhibitor has yet entered the clinic. With the aim to pick out novel and potent PDK1 inhibitors, herein we report the design and synthesis of a new class of molecules obtained by merging the 2-oxo-indole nucleus with the 2-oxo-pyridonyl fragment, two moieties with high affinity for the PDK1 hinge region and its DFG-out binding site, respectively. To this purpose, a small series of compounds were synthesised and a tandem application of docking and Molecular Dynamic (MD) was employed to get insight into their mode of binding. The OXID-pyridonyl hybrid **8**, possessing the lower IC_{50} (IC_{50} = 112 nM), was also tested against recombinant kinases involved in the PI3K/PDK1/Akt pathway and was subjected to vitro studies to evaluate the cytotoxicity and the inhibition of tumour cell migration. All together the results let us to consider **8**, as a lead compound of a new generation of PDK1 inhibitors and encourage us to further studies in this direction.

1. Introduction

The PI3K/PDK1/Akt signaling axis is centrally involved in controlling cell growth, proliferation, survival, tissue invasion and angiogenesis. The main effectors of this pathway are often mutated or amplified in solid tumors and at least 50% of all human cancer types are associated with deregulation of this cellular signal transduction cascade [1].

Among the main components of this pathway the phosphoinositide-dependent kinase (PDK1) is a serine/threonine kinase which phosphorylates multiple substrates including phosphoinositide-3-kinase protein kinase B/Akt, ribosomal S6 kinase proteins (S6K, RSK), serum and glucocorticoid-inducible kinase (SGK) and protein kinase C isoforms (PKC) [2,3]. Consistently, PDK1 plays a key role in regulating cell growth migration [4], proliferation [3], and survival [5] through both Akt-dependent and Akt-independent mechanisms. The Akt-dependent pathway is characterized by the implication of downstream proteins, like mammalian target of rapamycin (mTOR), Ras proteins and glycogen synthase kinase-3 (GSK-3), all activated by the serine/threonine-specific protein kinase Akt. Conversely, the Akt-independent signal acts via PLCγ1, a phospholipase thought to play a critical role in both cell invasion and metastasis formation [6,7].

PDK1 was originally identified as an upstream kinase for protein

Abbreviation used: OXIDs, 2-oxindole derivatives; TBTU, N,N,N′,N′-Tetramethyl-O-(benzotriazol-1-yl)uronium tetrafluoroborate; DIPEA, N,N-Diisopropylethylamine; DMF, N,N-Dimethylformamide; TFA, Trifluoroacetic acid; NaH, Sodium hydride; DCM, dichloromethane.

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kinase B (PKB/Akt), but nowadays it is recognized as a "master kinase" for regulating many cellular signaling pathways through the interaction with membrane phospholipids, such as phosphatidylinositol (3,4)-bisphosphate (PIP2) and phosphatidylinositol (3,4,5)-trisphosphate (PIP3), and subsequent phosphorylation of downstream effectors.

Pharmacologically, PDK1 plays a pleiotropic role in growth and development [8,9]. Recent findings revealed that elevated activation of PDK1 induces tumorigenesis by enhancing cell proliferation and inhibiting apoptosis. In addition, increasing evidence show that PDK1 plays a pivotal role in cell migration and metastasis formation [10]. Its role in these processes was proved in different cell types and organisms including endothelial cells [10], smooth muscle cells [11], T lymphocytes [12], neutrophils [13] and several tumour cell lines such as breast [14], glioblastoma (GBM) [15] and pancreatic cancers [16].

The important role of PDK1 has stimulated intense interest in identifying new selective molecules [17]. However, although there have been done huge efforts in discovering specific compounds targeting PI3K and Akt, PDK1 has been rather overlooked. Just recently, the increasing awareness in this kinase prompted many research groups to publish and patent several series of molecules able to inhibit this important node of the PI3K/PDK1/Akt pathway [18–20].

As regards the structure of PDK1, it is well-known that is constituted by 556 amino acids forming three main different domains: the catalytic or kinase domain (N-terminal), the Pleckstrin homology or PH domain (C-terminal) and the hydrophobic domain [21]. The kinase domain is highly flexible in the PDK1 *inactive form*, while it rearranges, organizing the ATP-binding site in its *active form*. These conformational changes involve also a particular portion in the kinase domain (DFG motif), which is flipped "out" in the *inactive form* (DFG-out conformation) with respect to its active conformation (DFG-in conformation). Once activated, PDK1 phosphorylates its substrates through the PDK1 interacting fragment (PIF) pocket, a small phosphate binding groove in the PDK1 kinase domain [22].

Molecules affecting PDK1 activity might be classified in (a) DFG-in inhibitors, (b) DFG-out inhibitors, (c) PH domain inhibitors and (d) PIF pocket inhibitors (Fig. 1).

Although many inhibitors PDK1 have been synthesized until now, only few of them achieved a confident rate of selectivity due to the high degree of homology within the kinase family [19].

Among the most studied inhibitors, BX517, an indolinone-based compound, shows to inhibit PDK1 with a high potency (IC_{50} = 6 nM) and to interact with the hinge region of PDK1 in the DFG-in conformation. Unfortunately, further studies were halted due to its poor solubility and unfavorable ADME properties [23,24]. The best results in terms of potency and selectivity were obtained with MP7, a compound synthetized by Sunesis and Biogen Idec using a fragment-based approach known as "tethering with extenders". MP7 was designed to bind the inactive DFG-out conformation: its high potency (IC_{50} = 2 nM) and impressive *in vitro* selectivity against 256 kinases tested, could be attributed, at least in part, to this fine interaction [18,19]. Cellular studies were performed to evaluate the antiproliferative activity. Quite surprisingly MP7 failed to inhibit the growth of monolayer cultures in several cancer cell lines (pancreas, breast, brain). On the contrary, it showed to reduce the anchorage-independent cell growth, an essential step to ensure to cancer cells both survival and metastasis formation [25].

Because the deregulation of PI3K/PDK1/Akt signalling pathway is very common in tumors, in recent years our efforts have focused on the development of new inhibitors as useful pharmacological tools to study the biology of PDK1 in GBM [26]. Starting from the 2-oxindole nucleus, the hinge binding moiety of BX-517, we recently synthesized FC85 (Fig. 2) and some 2-oxindole-derivatives (OXIDs) [26–28]. They showed a significant antiproliferative activity against GBM cell lines (U87MG, U118MG and ANGM-CSS), characterized by both a strong resistance to chemotherapy and a marked deregulation of signal transduction pathways [29–31]. The most active OXIDs were profiled against 56 kinases involved in the Akt-signaling cascade: preliminary results showed that some of them possessed a significant affinity against PDK1 (submicromolar range) but lacked of selectivity [26].

With the aim to search for new and potent PDK1 inhibitors, we made a small library of compounds by merging the 2-oxindole nucleus of BX517 with the DFG-out binding fragment of MP7. The

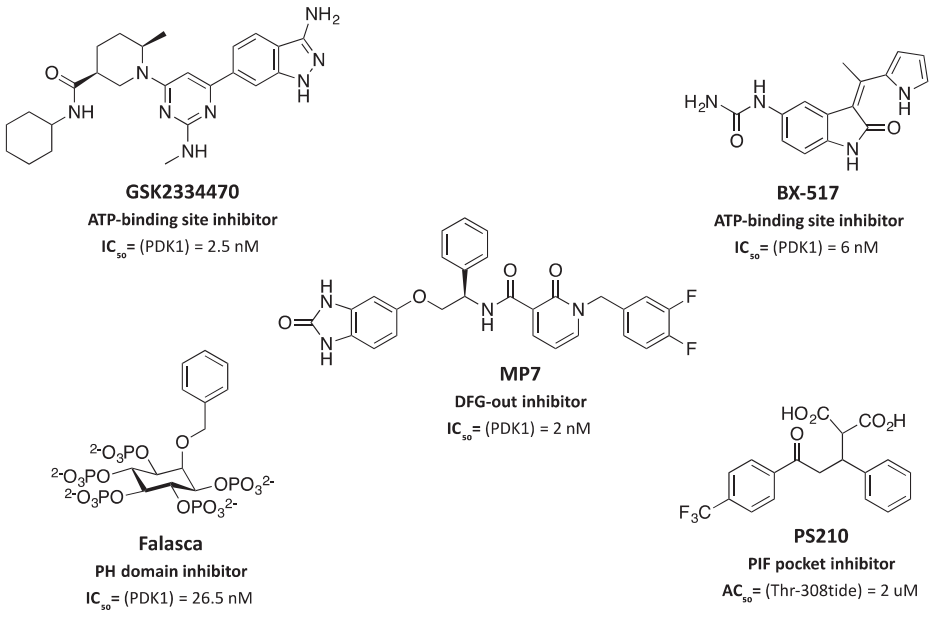

Fig. 1. Representative structures of PDK1 inhibitors.