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Research paper

Part III: Novel checkpoint kinase 2 (Chk2) inhibitors; design, synthesis and biological evaluation of pyrimidine-benzimidazole conjugates

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ABSTRACT

Recently a dramatic development of the cancer drug discovery has been shown in the field of targeted cancer therapy. Checkpoint kinase 2 (Chk2) inhibitors offer a promising approach to enhance the effectiveness of cancer chemotherapy. Accordingly, in this study many pyrimidine-benzimidazole conjugates were designed and twelve feasible derivatives were selected to be synthesized to investigate their activity against Chk2 and subjected to study their antitumor activity alone and in combination with the genotoxic anticancer drugs cisplatin and doxorubicin on breast carcinoma, (ER+) cell line (MCF-7). The results indicated that the studied compounds inhibited Chk2 activity with high potency ($IC_{50} = 5.56$ nM - 46.20 nM). The studied candidates exhibited remarkable antitumor activity against MCF-7 ($IG_{50} = 6.6$ μM - 24.9 μM). Compounds **10a-c, 14** and **15** significantly potentiated the activity of the studied genotoxic drugs, whereas, compounds **9b** and **20**–**23** antagonized their activity. Moreover, the combination of compound **10b** with cisplatin revealed the best apoptotic effect as well as combination of compound **10b** with doxorubicin led to complete arrest of the cell cycle at S phase where more than 40% of cells are in the S phase with no cells at G2/M. Structure-activity relationship was discussed on the basis of molecular modeling study using Molecular modeling Environment program (MOE).

1. Introduction

Cancer is one of the main causes of death around the world that results from high rate of proliferation for abnormal cells [1,2]. Many factors, as infectious agents, tobacco, chronic inflammation and UV light, can lead to genomic instability which can stimulate cancerous injuries [1,2]. Radiotherapy and chemotherapy are often used in addition to surgery to decrease the probabilities cancer recurrence. The generic mode of chemotherapies, genomic instability results from targeting DNA in cancer treatment, the rapid emergence of drug resistance via development of chemo-resistance by cancer cells, have emphasized the urgent need to solve their shortcomings and find new strategy for cancer treatment [3,4]. The key components of the DNA damage response pathway are two kinases named as ataxia-telangiectasia mutated (ATM) and Rad3-related (ATR). They subsequently phosphorylate several substrates essential for DNA repair, cell cycle arrest, transcription, and apoptosis [5–9]. Cell cycle checkpoints represent the restriction points between each phase of the cell cycle whereby the entire process can be delayed/stopped its progress to enable the proper sequential of the process ensuring that each stage occurs in an imperative system or to allow time for DNA repair [10–12]. Downstream phosphorylation targets of ATM and ATR are the effectors serine/threonine checkpoint kinases 1 and 2 (Chk1& Chk2) [13], which in turn phosphorylate

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partially overlapping residues in other target proteins to induce cell cycle arrest and facilitate DNA repair. Chk1 is activated by ATR phosphorylation on Ser-317 and Ser-345 whereas Chk2 is activated by ATM phosphorylation on Thr-68 [14]. Downstream targets of Chk2 include Cdc25A and Cdc25C, which, on phosphorylation, go through degradation and cytoplasmic relocalization, respectively, and induce cell arrest at G1, S, and G2-M phases [15]. Another important target of Chk2 is p53, the phosphorylation of which on Ser20 regulates p53 transcriptional activation. Moreover, Chk2 phosphorylation of Hdmx Ser367, a negative regulator of p53, enhances its degradation [16,17] and endorses the accumulation of p53 and transcriptional induction of p53 responsive genes. Chk2 also phosphorylates the transcription factor E2F-1 on Ser364, thereby enhancing its stability and encourages apoptosis [18,19].

According to the current understanding of Chk2 function in tumor cells, in both biological and genetic context, Chk2 plays an important role in maintaining genomic integrity by acting as a signal transducer of DNA damage. Chk2 is endogenously activated in precancerous lesions with genomic instability and in cancer cells grown in culture. Studies have shown that the activated Chk2 acts as a survival factor for cancer cells. There are several rationales for the development of Chk2 inhibitors. First, wide range of anticancer drugs and ionizing radiation caused activation of Chk2 in tumor cells which seriously limited their effect in these cells [20–26]. These shortcomings of current chemotherapeutic approaches can be addressed by selective inhibition of Chk2. Second, inhibition of Chk2 in normal cells leads to protection normal tissues during chemotherapy or radiation therapy that increase the therapeutic indices of DNA-targeted and ionizing radiation(IR) agents in these cells. Therefore, Chk2 inhibitors would protect healthy tissues as well as sensitize the tumor to chemotherapy. Therefore, inhibition of Chk2 can address limitations of current cancer therapies and rationalize the basis of using Chk2 inhibitors as chemotherapeutic agents [27–30].

2-Arylbenzimidazole derivatives as 2-(4-(4-hydroxyphenylthio) phenyl)-*1H*-benzo[*d*]imidazole-5-carboxamide (1) (Fig. 1A) are defined as potent and selective inhibitors of Chk2 [31–33] and 4-fluoro-2-(4-{[(3S,4R)-4-(2-hydroxypropan-2-yl)pyrrolidin-3-yl] amino}-6,7-dimethoxyquinazolin-2-yl)phenol (XBJ) (Fig. 1B) were described as a new series of potent and selective ATP-competitive inhibitors of Chk2 that was generated on the basis of structure-based design [34]. A series of pyrazole-benzimidazole conjugates were developed by Galal et al. on the basis of structure based design such as *N*-isopropyl-2-(4-(3-methyl-4-nitro-1-phenyl-*1H*-pyrazol-5-yloxy)phenyl)-*1H*-benzo[*d*]imidazole-5-carboxamide (**2**) (Fig. 1C) [35] and 2-(4-(4-((2-carbamoylhydrazono)methyl)-3-methyl-1-phenyl-*1H*-pyrazol-5-yloxy)phenyl)-5-nitro-*1H*-benzo [*d*]imidazole (**3**) [36] exhibiting high potency as Chk2 inhibitors (Fig. 1D).

On the other hand, there are great numbers of antitumor active agents possessing the pyrimidine nucleus [37–41] such as pyrimidinylmethyl benzimidazole derivatives (**I**) (Fig. 2) which exhibited potent antitumor effect against panel of cancer cell lines [38] and AZD6738 (Fig. 1E) is orally active anti-tumor single agent acting as selective ATR inhibitor in phase I clinical trial and used in cancer treatment as a monotherapy or in combination with chemo/radiation therapy in patients with solid tumors [40,41].

The present study aimed to synthesize new candidates of 2-arylbenzimidazoles, on the basis of molecular hybridization of 2-arylbenzomidazoles and pyrimidines, guided by molecular docking studies, while assessing their biological activities not only as Chk2 inhibitors and antitumor agents, but also in combination with cisplatin or doxorubicin.

2. Results and discussion

2.1. Rational design

Although, 2-arylbenzimidazoles represent one of the most potent selective class of Chk2 inhibitors and showed effective radioprotection of human T-cells subjected to ionizing radiation during radiotherapy [31–33], their antitumor effect is not reported and so they cannot be used either as single agent in monotherapy treatment or in combined chemotherapies where they do not potentiate the antitumor effect of the genotoxic anticancer drugs [42]. To address their shortage and following the same strategy used in the formation of pyrazole-benzimidazole conjugates [35,36] which succeeded in the synthesis of new candidates of 2-arylbenzimidazoles with effective anticancer properties and potentiated antitumor effect of genotoxic drugs [35,36] that formed on the basis of hybridization two different bioactive molecules with complementary pharmacophoric functions or with different mechanisms of action often exhibited enhanced effects [43–45].

A small library 212 of pyrimidine-benzimidazole conjugates were designed by replacing the lateral aryl group of 2-arylbenzimidazoles **(II)** with pyrimidine moieties in formula I (Fig. 2). Most of the designed pyrimidine-benzimidazole conjugates of formula **III** (Fig. 2) revealed high binding affinities towards Chk2 receptor (PDB code: 2XBJ) [34] according to MOE program (supplementary material: Table 2S). Twelve synthetically feasible compounds were chosen among the best fifty candidates that exhibiting the highest binding affinities into Chk2 for chemical synthesis. Variation of the substituents permitted the deduction of structure-activity relationship. Finally, their possible interactions with Chk2 were investigated by docking using the MOE program into the crystal structure of Chk2 in complex with the potent and selective 2-(quinazolin-2-yl)phenol inhibitor (PDB code: 2XBJ) [34].

2.2. Chemistry

The target pyrimidine-benzimidazole conjugates were synthesized via straight forward synthetic routes as shown in Schemes 1–3. They were classified into three series according to the linker (X) between 2-phenylbenzimidazoles and pyrimidines moieties (Fig. 2). The desired pyrimidines derivatives **7a-g** have been achieved by synthesis of 6-aryl-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitriles **(4a-f)** that were prepared following a previously described protocol [38,39]. The intermediate compounds, ethyl 2-(5-cyano-4-(4-florophenyl)-6-oxo-1,6-dihydropyrimidin-2-ylthio)acetate (**5**) and ethyl 2-(5-cyano-4-(4-chlorophenyl)-6-oxo-1,6-dihydropyrimidin-2-ylthio)acetate **(6)**, were obtained respectively by alkylation of compounds **4b** and **4c** with ethyl bromoacetate in benzene and potassium carbonate. Chloropyrimidine derivatives **7a-g** were obtained through treatment of hydroxypyrimidine derivatives **4a, 5, 4c, 4d, 4e, 4f** and **6**, respectively, with phosphorus oxychloride (Scheme 1).

The first series of target compounds **9a-c** and **10a-c** with ether linker (X = O, Fig. 2) was obtained by reaction of **7a-c** with 4-hydroxybenzaldehyde in DMF, potassium hydroxide and copper (I) iodide that yielded phenyl-pyrimidinyl ether derivatives **8a-c** in good yield. Subsequently, reaction of 3,4-diaminobenzoic with compounds **8a-c** in DMF and sodium thiosulphate afforded acid derivatives of 2-arylbenzimidazoles (**9a-c**). Finally, coupling of 2-arylbenzimidazoles **9a-c** with ammonium carbonate in DMF and CDI yielded amides of analogues of 2-arylbenzimidazoles **10a-c** (Scheme 2).

The second series of pyrimidine-benzimidazole conjugates