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Biological properties and structural study of new aminoalkyl derivatives of benzimidazole and benzotriazole, dual inhibitors of CK2 and PIM1 kinases

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ABSTRACT

The new aminoalkyl-substituted derivatives of known CK2 inhibitors 4,5,6,7-tetrabromo-1*H*-benzimidazole (TBBi) and 4,5,6,7-tetrabromo-1*H*-benzotriazole (TBBt) were synthesized, and their influence on the activity of recombinant human CK2 α, CK2 holoenzyme and PIM1 kinases was evaluated. All derivatives inhibited the activity of studied kinases and the most efficient were aminopropyl-derivatives **8b** and **14b**. These compounds also exerted inhibition of cancer cell lines – CCRF-CEM (acute lymphoblastoid leukemia), MCF-7 (human breast cancer), and PC-3 (prostate cancer) proliferation and their EC_{50} is comparable with the value for clinically studied CK2 inhibitor CX-4945. Preliminary structure activity relationship analysis indicated that the spacer length affected antitumor potency, and two to three methylene units were more favorable. The complex of CK2 $\alpha^{1\text{-}335}$/**8b** was crystallized, both under high-salt conditions and under low-salt conditions giving crystals which diffracted X-rays to about 2.4 Å resolution, what enabled the determination of the corresponding 3D-structures.

1. Introduction

Protein kinases catalyze the transfer of phosphate groups from adenosine triphosphate (ATP) to serine, threonine, or tyrosine residues of target proteins. This phosphorylation is an important stage in regulation of cell growth, cellular signal transduction, cell differentiation, and influences apoptotic mechanisms. Deregulation of protein kinases activity or expression is implicated in a number of diseases, including cancer, diabetes, and inflammation. Thus, targeted inhibition of the deregulated protein kinases has become an attractive therapeutic strategy in cancer therapy. Casein kinase 2 (CK2) and PIM kinase (Proviral Integration site of Moloney Virus) belong to the serine/threonine kinases family and their overexpression is frequently associated with acute myeloid leukemia, and a variety of cancers including prostate, breast or/and lung cancers [1,2]. The high activity of these kinases in cancer cells is associated with inhibition of apoptosis, suggesting a protective role of CK2 and PIM1 in programmed cell death. Recent studies have shown that the contribution of CK2 and PIM1 kinases in regulation of transcription, differentiation, or signaling of DNA damage/repair systems is achieved by regulating survival pathways. For example, both kinases participate in an activation of the transcriptional factor NF-κB, observed in transformed cells [3,4]. Elevated level of CK2 induces abnormal activation of NF-κB, which in turn contributes to the development of breast cancer [5]. Moreover, the tumor transformation of lymphocytes with PIM involvement is dependent on activation of NF-κB [4]. Downregulation of these kinases by chemical methods promotes apoptosis in cells [6], indicating CK2 and PIM1 kinases as molecular targets in the development of new therapeutic agents.

Many PIM inhibitors have been reported to date [7–9] however, none of them has been marketed so far. SGI-1776 is a representative first-generation PIM inhibitor, which had been under clinical trials for leukemia and prostate cancer [10]. While most of the first-generation PIM inhibitors are PIM1-selective, there is currently great interest in the potential of pan-PIM inhibitors to treat cancer because the three PIM kinases are reported to function redundantly [11]. Representative pan-PIM inhibitors are AZD1208 from AstraZeneca, [12] and PIM447 from Novartis [11]. Clinical trials of PIM447 are underway [11].

Among several classes of CK2 inhibitors effectively inhibiting the growth of tumor cells *in vitro* at low micromolar ranges are derivatives of benzotriazole and benzimidazole e.g. 4,5,6,7-tetrabromo-1*H*-benzotriazole (TBBt), 4,5,6,7-tetrabromo-1*H*-benzimidazole (TBBi), 4,5,6,7-tetrabromo-1*H*-benzimidazole-2-*N*,*N*-dimethylamine (DMAT), 4,5,6,7-tetraiodo-1*H*-benzimidazole (TIBI), (Ki 23 nM), and one of the most efficient CK2 and PIM dual inhibitors TDB (K_i 32 nM, and 86 nM respectively) [13,14].

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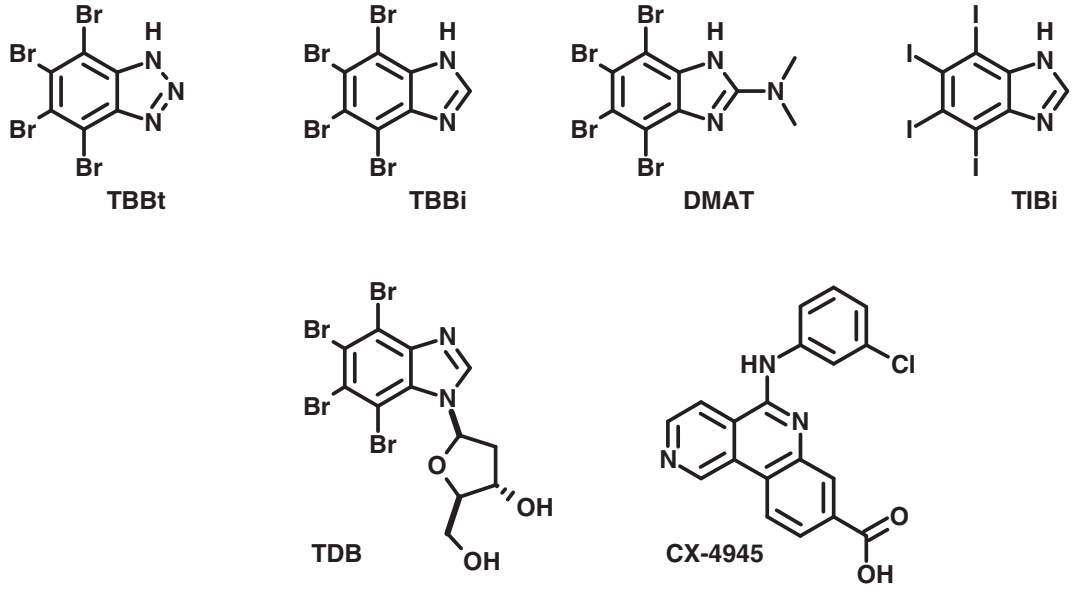
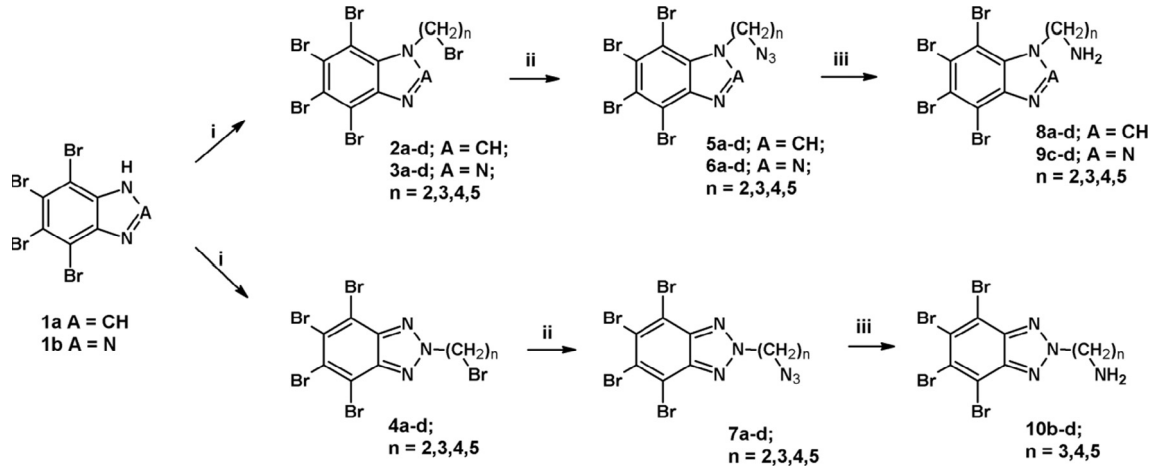


Fig. 1. The known CK2 inhibitors, derivatives of 1H-benzotriazole, 1H-benzimidazole and CX-4945.



Scheme 1. Synthetic route to the TBBi and TBBt derivatives: (i) dibromoalkyl or chlorobromoalkyl, DBU, CH_3CN, rt, 72 h; (ii) NaN_3, DMF, 40 °C, 24 h; (iii) Ph_3P, THF/H_2O, rt, 48 h.

Studies conducted by us [15], as well as others [14] show that CK2 inhibitors frequently exhibit similar inhibitory activity against the PIM1 kinase due to structural similarities within the structures of both kinases [16]. CX-4945 (Silmitasertib), the first ATP-competitive CK2 kinase inhibitor that is currently in Phase I/II clinical trials, also inhibits *in vitro* not only CK2 kinase activity, but also PIM1 and PIM2 kinase with IC_{50} values of 0.048 and 0.186 μM, respectively [7,17]. Recent studies have shown that the use of dual inhibitors (CK2 and PIM inhibition) results in reduced proliferation and induction of apoptosis in human T lymphoblastoid cells (CML) and their multidrug resistant variant (R-CEM), human cervical cancer cells (HeLa) [14] as well as some other cell lines [18].

Continuing our search for CK2 inhibitors we synthesized the new azidoalkyl-, and aminoalkyl-substituted derivatives of known inhibitors TBBi and TBBt. The influence of new derivatives on activity of CK2α, and CK2 holoenzyme was evaluated. We also examined their influence on the kinase PIM1 activity, and on few other kinases to explore the specificity of new compounds. Simultaneous inhibition of the activity of several kinases is a disadvantage when examining the role of different kinases in the cell metabolism or neoplastic transformation, but it can be beneficial in the search for potential anti-cancer drugs.

We previously found that the introduction of a methyl group on the C2 atom of TBBi led to the 4,5,6,7-tetrabromo-2-methyl-1*H*-benzimidazole (2MeTBBi) with a promising inhibitory activity of CK2 and PIM1 and with increased cytotoxicity against CCRF-CEM and MCF-7 cells [19]. Here we report the experimental details of the synthesis of a new series aminoalkyl derivatives of TBBt, TBBi and 2MeTBBi. The inhibitory activity of the synthesized compounds was tested using a standard isoptopic kinase assay and the selectivity of selected compounds against a panel of 8 kinases was determined and compared with selectivity of known inhibitors. The Antiproliferative activity of new compounds was tested against three different cancer cell lines. A crystallographic study of CK2α-inhibitor complexes was performed in order to investigate the enzyme/inhibitor interactions.

2. Results and discussion

2.1. Chemistry

The synthesis of the halogenoalkyl derivatives **2a-d**, **3a-d** and **4b-d** was carried out using as starting materials the 4,5,6,7-tetrabromo-1*H*-benzimidazole **1a**, 4,5,6,7-tetrabromo-1*H*-benzotriazole **1b** (Scheme 1), or