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# New inhibitors of protein kinase CK2, analogues of benzimidazole and benzotriazole

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**Abstract** Derivatives of 4,5,6,7-tetrabromobenzotriazole (TBBt) and 4,5,6,7-tetrabromobenzimidazole (TBBi) with $IC_{50}$ in the low micromolar range and with high selectivity belong to the most promising inhibitors of protein kinase CK2 (casein kinase 2). Treatment of various cell lines with TBBt, TBBi or 2-dimethylamino-4,5,6,7-tetrabromo-1*H*-benzimidazole (DMAT) affected cell viability with simultaneous induction of apoptosis. The inhibitory activity of newly synthesized hydroxyalkyl derivatives of TBBi and TBBt depends on the length of the alkyl chain. The hydroxypropyl substituted derivatives show higher or similar inhibitory activity than the parent compounds when tested with human protein kinase CK2. To test the distribution of this class of compounds in mammals, [$^{14}$C] TBBi was synthesized.

**Keywords** Analogues of 4,5,6,7-tetrabromo-1*H*-benzimidazole · Analogues of 4,5,6,7-tetrabromo-1*H*-benzotriazole · Inhibitors of CK2

**Abbreviations**

| | |
|---|---|
| TBBi | 4,5,6,7-tetrabromo-1*H*-benzimidazole |
| TBBt | 4,5,6,7-tetrabromo-1*H*-benzotriazole |
| DMAT | 4,5,6,7-tetrabromo-1*H*-benzimidazol-2-N, N-dimethylamine |
| $N^1$HETBBt | 2-(4,5,6,7-tetrabromo-1*H*-benzotriazol-1-yl)ethan-1-ol |
| MB001 | 3-(4,5,6,7-tetrabromo-1*H*-benzimidazol-1-yl) propan-1-ol |
| MB002 | 3-(4,5,6,7-tetrabromo-1*H*-benzotriazol-1-yl) propan-1-ol |
| MB003 | 3-(4,5,6,7-tetrabromo-2*H*-benzotriazol-2-yl) propan-1-ol |
| BK005 | 4-(4,5,6,7-tetrabromo-2*H*-benzotriazol-2-yl) butan-1-ol |

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## Introduction

Protein kinase CK2, an ubiquitous serine/threonine kinase, plays a significant role in many cellular functions, including the regulation of cell cycle progression [1], malignant transformation, cell survival, angiogenesis [2] and apoptosis. Nowadays, more than 300 substrates of CK2 are known, and identification of others may be expected since approximately 20% of the isolated phosphopeptides conform to the consensus for phosphorylation by CK2 [3]. Dysfunctions of this enzyme are frequently associated with various pathological states [4], and major efforts are currently devoted to development of specific inhibitors.

A number of CK2 inhibitors effective in the low micromolar ranges have been synthesized. Those are derivatives of benzotriazole, benzimidazole [5, 6], flavonoids, antraquinones, quinazolones, quinolines, polypeptides and polysaccharides. The most specific inhibitor of CK2, 4,5,6,7-tetrabromo-1*H*-benzotriazole (TBBt) [7] is used very widely to elucidate the role of phosphorylation by

CK2 of a variety of proteins of important metabolic functions. Much structural information has been accumulated on *Zea mays* CK2 alfa subunit in complex with various inhibitors [8], indicating that TBBt and derivatives of 4,5,6,7-tetrabromo-1*H*-benzimidazole (TBBi) may have an effect on the conformation of the Gly-rich loop at the active site of CK2. The ATP-binding site holds conserved water molecules [9], so it seemed interesting to explore the possibility of replacing them with substituents with a hydroxyl group. To test this idea for finding more potent inhibitors, new derivatives of TBBt and TBBi with hydroxyl group were designed and synthesized, and their influence on the activity of human CK2 alfa subunit and holoenzyme was determined.

Bearing in mind the role of CK2 in regulation of cell proliferation and knowing that TBBt, TBBi and DMAT can induce apoptosis [9, 10], we undertook the study of the distribution of this class of compounds in mammals. The [$^{14}$C]TBBi was synthesized and this compound was used to study its organ distribution in rats.

## Materials and methods

The starting compounds- 4,5,6,7-tetrabromo-1*H*-benzimidazole (TBBi) and 4,5,6,7-tetrabromo-1*H*-benzotriazole (TBBt) were synthesized by bromination of 1*H*-benzimidazole or 1*H*-benzotriazole according to published method [5].

### Expression of subunits of CK2 and determination of CK2 activity

The pT7-7 vector carrying human CK2$\alpha$ subunit (hCK2$\alpha$) cDNA and the pT7-7 vector carrying human CK2$\beta$ subunit (hCK2$\beta$) cDNA were a kind gift of Dr. Stefania Sarno, University of Padova, Italy. Expression and purification of rhCK2$\alpha$ and rhCK2$\beta$ subunits was according to published methods [11, 12].

The reaction mixture (final volume of 50 µl) for the determination of CK2 activity (CK2$\alpha$ or holoenzyme CK2$\alpha_2\beta_2$) contained: protein substrate casein (75 µg) or peptide substrate (20 µM RRRDDDSDDD, Biosyntan, Germany), Tris–HCl pH 7.5 (20 mM), $MgCl_2$ (20 mM), $\gamma$[$^{32}$P]ATP (10 µM, 100–200 cpm pmol$^{-1}$) and appropriate concentrations of inhibitor in 1 µl DMSO. After 20 min incubation at 30°C, 40 µl of the assay mixture was spotted onto a square (2 cm × 2 cm) of Whatman 3MM (for casein) or P81 paper (for peptide substrate), which was immediately immersed in cold 5% (w/v) trichloroacetic acid containing 0.3% *o*-phosphoric acid (10 ml per square), and washed with $H_2O$ five times for 10 min. Then the squares were washed in 96% ethanol and allowed to dry. The radioactivity was quantified using a LKB Flexi-Vial liquid scintillation spectrometer.

## Results

*N*-hydroxyalkyl derivatives were prepared by alkylation of TBBt or TBBi with the use of NaH or DBU as bases and appropriate hydroxyalkyl halide as previously reported [13, 14]. The ratio of $N^1$ to $N^2$ substituted anomers of TBBt depends on the reaction conditions and on the length of the alkyl chain.

The influence of the synthesized compounds on human CK2 alfa subunit and on the holoenzyme activity was determined as described in the section "Materials and methods". The results are shown in Table 1. Some of the new analogues showed $IC_{50}$ similar to that of the parent compounds, and their inhibitory activity depended on the length of the alkyl substituents. The 3-(4,5,6,7-tetrabromo-1*H*-benzimidazol-1-yl)propan-1-ol (MB001) exerted a higher potency ($IC_{50}$ 0.5 µM) than the parent compound TBBi ($IC_{50}$ 1.5 µM). The 3-(4,5,6,7-tetrabromo-1*H*-benzotriazol-1-yl)propan-1-ol (MB002) exerted only a slightly higher potency against the catalytic subunit of CK2 $\alpha$, with $IC_{50}$ 0.3 µM compared to 0.5 µM for TBBt. Derivatives with shorter or longer chains exerted lower inhibitory activity.

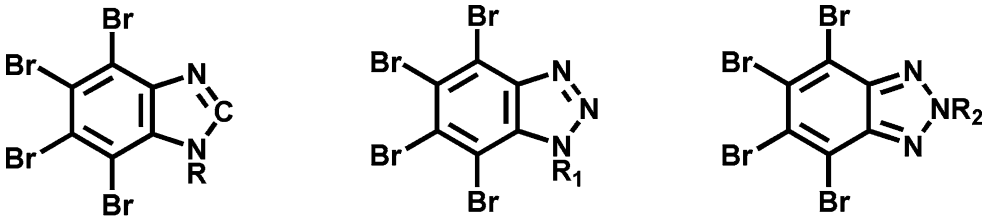
[$^{14}$C]TBBi was synthesized starting from 2,4-dibromo-6-nitroaniline, through reduction to 2,4-dibromophenylene-1,6-diamine, condensation with $^{14}$C formic acid and subsequent bromination. This compound was used to study its organ distribution in rats during 0–72 h after a single per os administration of a single dose of 25 mg/kg body weight; these results will be published separately.

## Discussion

To improve the inhibitory activity versus CK2, TBBt and TBBi have been subjected to chemical modifications (to be published elsewhere). Most modifications either partially

**Table 1** Inhibition of human protein kinase CK2 by TBBi, TBBt and their derivatives

| Inhibitor | $IC_{50}$[µM] CK2$\alpha$ | $IC_{50}$[µM] CK2$\alpha_2\beta_2$ |
|---|---|---|
| TBBi | 1.50 ± 0.14 | 1.53 ± 0.08 |
| TBBt | 0.50 ± 0.07 | 0.5 ± 0.12 |
| $N^1$HETBBt | 9.25 ± 1.09 | 2.61 ± 0.92 |
| MB 001 | 0.54 ± 0.15 | 0.51 ± 0.04 |
| MB 002 | 0.32 ± 0.15 | 0.48 ± 0.14 |
| MB 003 | 0.34 ± 0.12 | 0.82 ± 0.19 |
| BK 005 | 1.73 ± 0.15 | 1.52 ± 0.20 |




**TBBi, R = H**
**MB 001, R = $C_3H_6OH$**

**TBBt, $R_1$ = H**
**$N^1$HETBBt, $R_1 = C_2H_4OH$**
**MB 002, $R_1 = C_3H_6OH$**

**MB 003, $R_2 = C_3H_6OH$**
**BK 005, $R_2 = C_4H_8OH$**

**Fig. 1** Structures of 4,5,6,7-terabromo-1*H*-benzimidazole (TBBi) and 4,5,6,7-terabromo-1*H*-benzotriazole (TBBt) derivatives

or completely abrogated the ability of the new derivatives to inhibit CK2. However, selected modifications with the hydroxypropyl substituent have led to compounds, with comparable or better inhibitory activity than the parent compounds. Two derivatives in particular, 3-(4,5,6,7-tetrabromo-1*H*-benzimidazol-1-yl)propan-1-ol (MB 001, Fig. 1) and 3-(4,5,6,7-tetrabromo-1*H*-benzotriazol-1-yl)propan-1-ol (MB 002, Fig. 1), showed a small improvement in CK2 inhibitory activity.

The newly synthesized compound [$^{14}$C]TBBi proved to be very useful for study of the distribution of TBBt/TBBi derivatives in mammalian tissues. Although the solubility of this type of compounds in water is low, the [$^{14}$C] radioactivity was observed in all the examined organs. It is clear that these compounds can cross the blood/brain barrier, which makes them promising candidates for further modifications as potential therapeutic agents.

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