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CK2α and CK2α′ subunits differ in their sensitivity to 4,5,6,7-tetrabromo- and 4,5,6,7-tetraiodo-*1H*-benzimidazole derivatives

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ABSTRACT

The goal of this study was to test the inhibitory activity of a series of tetrahalogenobenzimidazoles, including a number of novel derivatives, on individual catalytic subunits of human CK2. 4,5,6,7-tetrabromo- and 4,5,6,7-tetraiodo-*1H*-benzimidazoles and their newly obtained N^1- and 2-S-carboxyalkyl derivatives showed potent inhibitory activity against both these subunits. CK2α′ was up to 6 times more sensitive to the studied compounds than CK2α. The investigated iododerivatives showed, in most cases, stronger inhibitory properties than the respective brominated congeners, but the differences showed considerable dependence on the protein substrate used.

1. Introduction

Protein kinases, which are key regulators of many cellular functions, constitute one of the largest human gene families. The human genome encodes some 518 protein kinases that control protein activity by catalyzing the addition of a negatively charged phosphate group to proteins [1]. Protein kinases modulate a wide variety of biological processes, especially those that transfer signals from the cell membrane to intracellular targets and coordinate complex biological functions. A number of severe diseases, including cancer, diabetes, and inflammation, are linked to perturbations of kinase–mediated cell signaling pathways, thus they represent some of the most promising drug targets. Protein kinases share a catalytic domain conserved in the sequence and structure, but are notably different in how their catalysis is regulated. The ATP-binding pocket between the two lobes of the kinase fold, together with less conserved surrounding pockets, has been the focus of inhibitor design that had exploited differences in the kinase structure and adaptability in order to achieve selectivity [2].

CK2 is a highly ubiquitous and multifaceted serine/threonine kinase described as a heterotetrameric holoenzyme generated by the association of two α and/or α′ catalytic subunits with a dimer of β regulatory subunits [3–6]. Although human CK2α and CK2α′ are the products of expression of different genes, their catalytic domains show about 90% identity, which may explain the similarity in their enzymatic properties *in vitro* [5,7]. Notably, the C-termini of CK2α and CK2α′ are completely unrelated [7].

Protein kinase CK2 plays a global role in activities related to cell growth, survival and death, and has a large number of potential substrates localized in various cell compartments. In addition to its involvement in cell growth and proliferation, CK2 is also a potent suppressor of apoptosis, which characteristic underlies its importance for cancer cell phenotype [3–6].

To date, most of the publications on CK2 made no distinction between the different isoenzymic forms of CK2 catalytic subunits. However, knockout studies in mice and yeast have revealed some degree of functional specialization [5]. Mice with knockout of catalytic α and regulatory β subunits die in embryonic stage, which indicates that CK2α and CK2β are essential for viability and that CK2α′ cannot compensate for CK2α loss. The cellular functions of all CK2 isoforms were also studied in human osteosarcoma cell lines expressing active or inactive versions of each CK2 isoform. The examination of these cell lines showed functional specialization of both isoforms at the cellular level in mammals, and indicated that CK2α′ is involved in the control of proliferation and/or cell survival [8].

Abbreviations: TBI, 4,5,6,7-tetrabromo-*1H*-benzimidazole; DMAT, 2-(dimethylamino)-4,5,6,7-tetrabromo-*1H*-benzimidazole; TIBI, 4,5,6,7-tetraiodo-*1H*-benzimidazole.

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CK2 has been found to be deregulated in all cancers examined. Those studies demonstrated increased CK2 expression and enhanced nuclear localization in cancer cells compared to their normal counterparts [9]. Overexpression of protein kinase CK2 is an unfavorable prognostic marker in several cancers [10].

Consequently, CK2 has emerged as a relevant therapeutic target. Several classes of ATP-competitive inhibitors have been identified, showing variable effectiveness. In the beginning of the 1990s, multihalogenated benzimidazoles were described as one of the most powerful CK2 inhibitors that can cross the cell membranes [11,12]. In addition, it was shown that some of these inhibitors can distinguish between different CK2 isoforms both *in vitro* and *in vivo* [13,14]. During last years, numerous small molecules were identified that inhibit CK2 activity by various mechanisms, e.g. allosteric inhibitors, substrate-competitive inhibitors, CK2β-targeting inhibitors, and CK2α/CK2β interaction inhibitors [15–20].

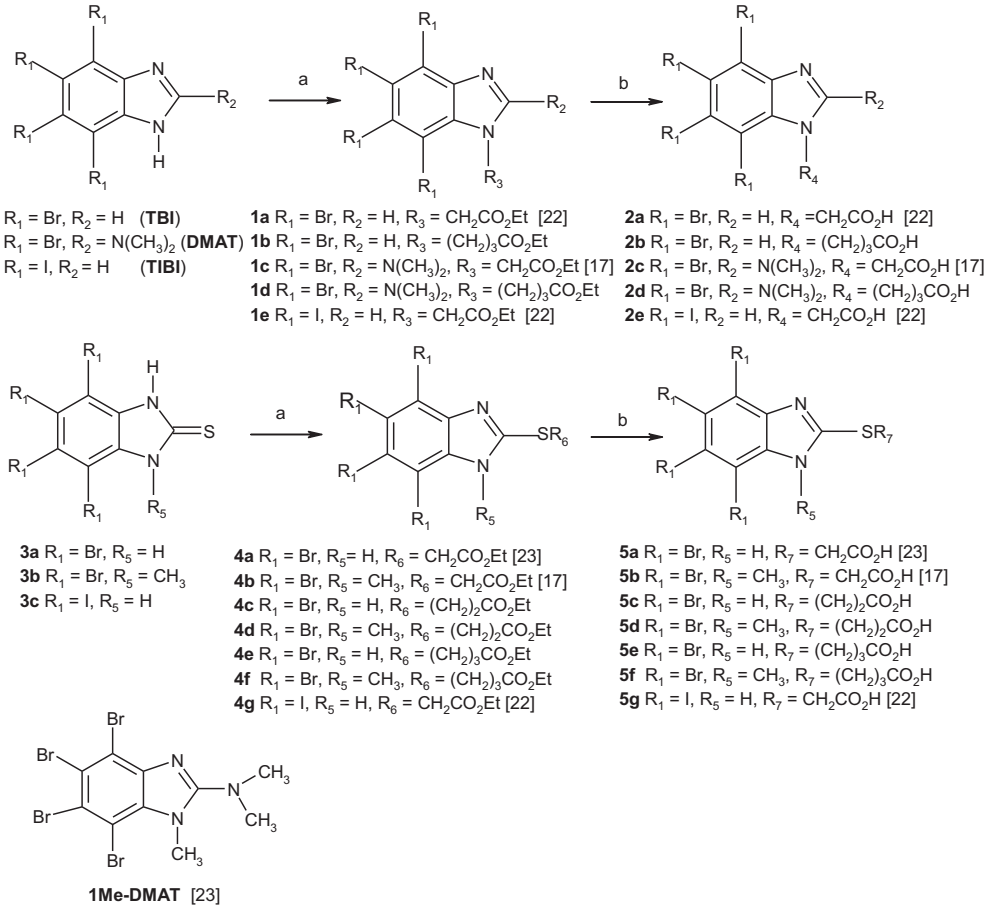
In this report we describe the effects of a series of halogenated benzimidazoles on the activity of both α and α′ catalytic subunits of human CK2.

2. Results and discussion

For this study, besides from the most representative benzimidazole-derived CK2 inhibitors like 4,5,6,7-tetrabromo-*1H*-benzimidazole (TBI), 4,5,6,7-tetraiodo-*1H*-benzimidazole (TIBI), 2-(dimethylamino)-4,5,6,7-tetrabromo-*1H*-benzimidazole (DMAT) and their previously synthesized analogs, some new N^1-carboxyalkyl and 2-S-carboxyalkyl derivatives with or without N^1-methyl group were synthesized. For the syntheses, 4,5,6,7-tetrabromo- and 4,5,6,7-tetraiodo-benzimidazoles and their respective 2-mercaptoderivatives (**3a**–**c**) were alkylated with ω-bromoalkyl esters in the presence of potassium carbonate in aprotic solvents to yield the desired compounds **1a**–**e** and **4a**–**g**. The obtained esters were hydrolyzed in alkaline medium to yield the corresponding carboxyalkyl compounds **2a**–**e** and **5a**–**g**, respectively (Scheme 1). The structures of the new compounds were confirmed by ^{1}H NMR and UV spectra and elemental analyses.

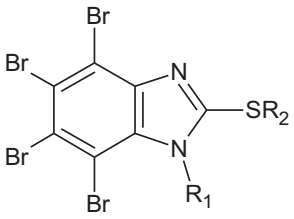
Recently we examined 4,5,6,7-tetrabromo-*1H*-benzotriazole (TBB) and TBI as inhibitors of five CK2 isoforms from *Saccharomyces cerevisiae* [21]. Interestingly, we have not only observed differences between free catalytic subunits (CK2α and CK2α′) and holoenzymes ($\alpha_2\beta\beta'$, $\alpha'_2\beta\beta'$ and $\alpha\alpha'\beta\beta'$), but also between both catalytic subunits. Furthermore, we have shown the difference in K_i values using as phosphoacceptor the well-known CK2 substrates, including mRNA polyadenylation factor Fip1, survival factor Svf1, transcription elongation factor Elf1 and acidic ribosomal protein P2B, or the synthetic peptide RRRADDSDDDDD.

In the present study we investigated 16 halogenated benzimidazole derivatives for their activity against human CK2α and CK2α′ subunits. Both these subunits were over expressed and purified to homogeneity by affinity chromatography as described in the experimental section. The inhibitory effect was examined by increasing concentrations of a given compound. All results obtained using the synthetic peptide RRRADDSDDDDD or P2B as a substrate are summarized in Tables 1–3. The introduction of S-carboxymethyl (**5a**), S-carboxyethyl (**5c**) or S-carboxypropyl (**5e**)



Scheme 1. Synthesis of new 4,5,6,7-tetrabromo and 4,5,6,7-tetraiodo-1H-benzimidazole derivatives. a) K_2CO_3, butanone or acetone, ω-bromoalkylcarboxylic acid ethyl ester, b) EtOH/H_2O, NaOH, H^+.

Table 1
Derivatives of TBI and their K_i values (nM) for CK2α and CK2α′.



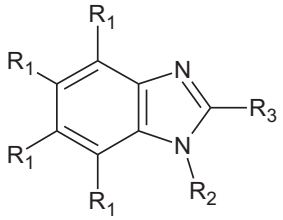
Compound	R_1	R_2	CK2α		CK2α′	
			P2B	Peptide	P2B	Peptide
TBI	H	H	462 ± 44	315 ± 32	270 ± 25	343 ± 37
5a	H	CH_2CO_2H	106 ± 10	170 ± 18	37 ± 3	49 ± 4
5c	H	$(CH_2)_2CO_2H$	116 ± 12	172 ± 16	35 ± 3	31 ± 3
5e	H	$(CH_2)_3CO_2H$	77 ± 6	210 ± 19	88 ± 7	44 ± 3
5b	CH_3	CH_2CO_2H	143 ± 14	13 ± 2	200 ± 17	24 ± 2
5d	CH_3	$(CH_2)_2CO_2H$	112 ± 11	137 ± 12	100 ± 11	55 ± 5
5f	CH_3	$(CH_2)_3CO_2H$	88 ± 8	501 ± 48	83 ± 7	768 ± 64

residue at position 2 of TBI resulted in an increase in the inhibitory activity against CK2α′ for up to 8-fold and up to 11-fold, using the synthetic peptide and P2B as a substrate, respectively, whereas the respective increases in inhibitory activity of these derivatives against CK2α were markedly lower. Additional introduction of the methyl group at position 1, which led to compounds **5b**, **5d** and **5f**, did not significantly changed the inhibitory activity (as compared to those of **5a**, **5c** and **5e**) using P2B as the protein substrate. However, this substitution has brought mixed results when the synthetic peptide was used for substrate (see Table 1). Compound **5c** showed significant selectivity towards CK2α′ subunit, as its inhibitory potency against CK2α was 3–5 times lower (Fig. 1A). Noteworthy, the inhibitory activity of **5b** toward both CK2 subunits studied was much higher than that of the parental compound TBI when using the synthetic peptide as the substrate (Fig. 1B). Interestingly, **5f** showed much weaker inhibitory effect using the synthetic peptide as the substrate than that using P2B as the substrate (Fig. 1C).

It has been previously reported that iodinated inhibitors are in general more active than their brominated counterparts toward the $\alpha_2\beta_2$ holoenzyme [22]. We examined if this holds true also for different free catalytic subunits, and we found that this is, in general, the case (Table 2). This is particularly clear for the compounds bearing carboxymethyl group at position 1 (compounds **2a** and **2e**). However, the compounds carrying S-carboxymethyl moiety at position 2 (e.g. **5a** and **5g**) did not follow this rule (see Table 2).

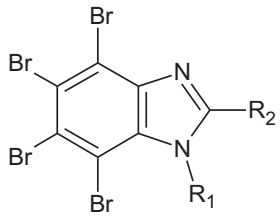
Next, we tested DMAT, an analog of TBI with a dimethylamino residue at position 2, and its derivatives as potential inhibitors of free CK2 catalytic subunits (Table 3). DMAT was already shown to exert higher inhibitory activity than TBI on CK2 holoenzyme [17]. The same was found for both free catalytic subunits in this study. Introduction of a methyl group into position 1 of DMAT yielded 1Me-DMAT that was similarly or somewhat more effective against CK2α′, and was similarly or less potent towards CK2α, depending on the substrate used. To examine the influence of the length of the residue at position 1, we have synthesized a 1Me-DMAT congener with the carboxymethyl residue at this position (**2c**). This compound showed a considerable inhibitory activity toward both catalytic CK2 subunits irrespective of the substrate employed. In case of CK2α′, its K_i value was 2 and 6 times lower than that of

Table 2
Derivatives of TBI and TIBI and their K_i values (nM) for CK2α and CK2α′.



Compound	R_1	R_2	R_3	CK2α		CK2α′	
				P2B	Peptide	P2B	Peptide
TBI	Br	H	H	462 ± 44	315 ± 32	270 ± 25	343 ± 37
TIBI	I	H	H	266 ± 26	43 ± 4	71 ± 8	21 ± 2
2a	Br	CH_2CO_2H	H	798 ± 70	670 ± 65	609 ± 58	342 ± 32
5a	Br	H	SCH_2CO_2H	106 ± 10	170 ± 18	37 ± 3	49 ± 4
2e	I	CH_2CO_2H	H	378 ± 36	261 ± 24	62 ± 5	324 ± 32
5g	I	H	SCH_2CO_2H	110 ± 10	80 ± 7	78 ± 6	106 ± 11

Table 3
Derivatives of TBI and their K_i values (nM) for CK2α and CK2α′.



Compound	R_1	R_2	CK2α		CK2α′	
			P2B	Peptide	P2B	Peptide
TBI	H	H	462 ± 44	315 ± 32	270 ± 25	343 ± 37
DMAT	H	$N(CH_3)_2$	97 ± 8	139 ± 12	112 ± 10	133 ± 11
1Me-DMAT	CH_3	$N(CH_3)_2$	212 ± 17	154 ± 14	109 ± 8	35 ± 3
2b	$(CH_2)_3CO_2H$	H	472 ± 44	368 ± 34	188 ± 15	195 ± 18
2c	CH_2CO_2H	$N(CH_3)_2$	88 ± 7	83 ± 9	66 ± 6	21 ± 2
2d	$(CH_2)_3CO_2H$	$N(CH_3)_2$	860 ± 75	456 ± 46	139 ± 12	93 ± 8

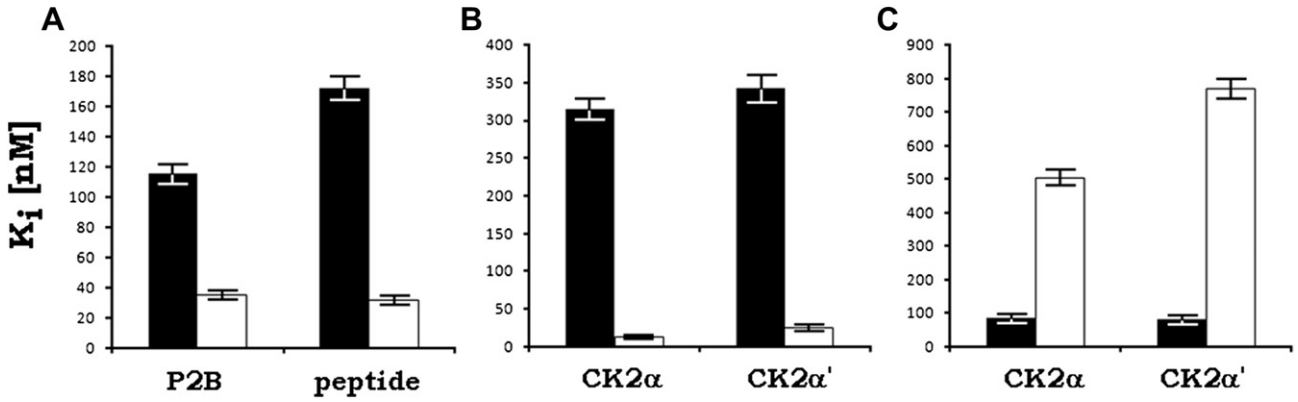


Fig. 1. Comparison of the results of phosphorylation assays. (A) Inhibition by **5c** in relation to the choice of CK2 isoform and substrate; CK2α – black bars, CK2α′ – white bars; (B) Inhibition of peptide phosphorylation in relation to the choice of CK2 isoform and inhibitor; TBI – black bars, compound **5b** – white bars; (C) Inhibition by **5f** in relation to the choice of CK2 isoform and substrate, P2B – black bars, synthetic peptide – white bars.

DMAT, using P2B or peptide as phosphate acceptor, respectively. The effect on CK2α was similar but weaker (Table 3). Further elongation of the carbon chain of the substituent at position 1 led to a large reduction of the inhibitory effect, especially in case of CK2α (see compound **2d** data). The opposite effects were observed for the respective TBI derivative (**2b**), i.e. while it inhibited the phosphorylation reaction mediated by CK2α similarly to the parent compound, its inhibitory activity against CK2α′ was much stronger. It seems reasonable to assume that the differences described above are related to the zwitterionic nature of the compounds **2c** and **2d**.

3. Conclusions

In most cases, the human CK2α′ subunit compared to its CK2α counterpart is more sensitive to the inhibition by tetrahalogenobenzimidazole derivatives. Although comparisons to the results of other scientific groups are difficult and not entirely reliable, it is obvious that the inhibitory activity of these compounds against CK2α, CK2α′ or holoenzyme can differ [17,22,23]. Small differences between our values and from other research groups may also result from different reaction conditions, like buffer composition, reaction time and source of enzyme. As shown in this study, the effect of modification of tetrahalogenobenzimidazoles on CK2 inhibitory activity may yield different results depending on the substrate and subunit employed. The structural feature that underlies these differences needs to be further investigated. Deduced from our present and former studies [22], the most prominent characteristics of effective human CK2 benzimidazole inhibitors include the following: (1) iodination at the benzene ring of the benzimidazole core, and (2) the presence of a polar substituent at position 2.

Crystallography studies may help to clarify the stronger inhibition of CK2α′. It is highly probable that the inhibitor/enzyme complex differs between both catalytic subunits.

Our aim should be to analyze in more details the differences in inhibition of individual catalytic subunits and the holoenzyme. It is intriguing whether selectivity with respect to the subunits translates into differences in anti-cancer activity of human CK2 inhibitors.

4. Experimental

4.1. Chemistry

The following investigated compounds were prepared according to previously described procedures: 4,5,6,7-tetraiodobenzimidazole TIBI, (4,5,6,7-tetrabromo-1H-benzimidazol-1-yl)-acetic acid (**2a**), (4,5,6,7-tetraiodo-1H-benzimidazol-1-yl)-acetic acid (**2e**), (4,5,6,7-tetraiodo-1H-benzimidazol-2-ylsulfanyl)-acetic acid (**5g**) [22]; 2-

dimethylamino-4,5,6,7-tetrabromobenzimidazol-1-yl)-acetic acid (**2c**), (4,5,6,7-tetrabromo-1-methyl-1H-benzimidazol-2-ylsulfanyl)-acetic acid (**5b**) [17]; 2-(dimethylamino)-4,5,6,7-tetrabromo-1H-benzimidazole (DMAT), 2-(dimethylamino)-1-methyl-4,5,6,7-tetrabromo-1H-benzimidazole (1Me-DMAT), (4,5,6,7-tetrabromo-1H-benzimidazol-2-ylsulfanyl)-acetic acid **5a** [23].

Melting points were determined on a Gallenkamp Melting Point Apparatus, Mod. MFB 595 030G, in open capillary tubes. The ^{1}H NMR spectra were recorded on a Bruker AMX instrument (400 MHz ^{1}H frequency) at 25 °C. Chemical shifts are reported in ppm from internal tetramethylsilane standard are given in δ-units. The solvent used for NMR spectra was deuteriodimethylsulfoxide. The UV spectra were determined on Techcomp UV8500 spectrophotometer. Elemental analyses were performed at the Faculty of Chemistry, Warsaw Technical University using a Heraeus CHN Rapid Analyzer.

4.1.1. 4-(4,5,6,7-Tetrabromobenzimidazol-1-yl)-butyric acid ethyl ester (**1b**)

To the refluxed and stirred mixture of 4,5,6,7-tetrabromobenzimidazole (1.3 g, 3 mmol) and potassium carbonate 900 mg (6.5 mmol) in butanone (30 ml) 4-bromobutyric acid ethyl ester (800 mg, 4.1 mmol) was added. The reflux was continued for 24 h. The mixture was filtered and filter cake washed with acetone. The filtrate was evaporated to dryness and the residue crystallized from EtOH-H_2O (2:1, v/v) to give 1.4 g (83%) of colorless needles. m.p. 138–140 °C ^{1}H NMR (DMSO-d_6): 1.12 (t, 3H, CH_3, J = 6.3 Hz), 2.10 (m, 2H, CH_2), 2.38 (t, 2H, CH_2, J = 7.3 Hz), 3.93 (q, 2H, CH_2, J = 7.1 Hz), 4.53 (t, 2H, CH_2, J = 7.1 Hz). 8.48 (s, 1H, CH). Anal. calcd for $C_{13}H_{12}Br_4N_2O_2$ (547.87): C, 28.50; H, 2.21; N, 5.11. Found: C, 28.43; H, 2.25; N, 4.99.

4.1.2. 4-(2-Dimethylamino-4,5,6,7-tetrabromobenzimidazol-1-yl)-butyric acid ethyl ester (**1d**)

To the refluxed and stirred mixture of 2-dimethylamino-4,5,6,7-tetrabromobenzimidazole (720 mg, 1.5 mmol) and potassium carbonate 1.38 g (10 mmol) in butanone (30 ml) 4-bromobutyric acid ethyl ester (1.56 g, 8 mmol) was added. The reflux was continued for 20 h. The mixture was filtered and filter cake washed with acetone. The filtrate was evaporated and the residue was chromatographed on silica gel column (3 × 10 cm) with $CHCl_3$ (200 ml) and $CHCl_3$-MeOH (98:2). The product containing fractions were evaporated to a viscous oil (740 mg, 84%). ^{1}H NMR (DMSO-d_6): 1.14 (t, 3H, CH_3, J = 7.1 Hz), 1.80 (m, 2H, CH_2), 2.09 (t, 2H, CH_2, J = 6.9 Hz), 2.98 (s, 6H, 2 X CH3), 3.98 (q, 2H, CH2, J = 7.1 Hz), 4,33 (t, 2H, J = 7.1 Hz). Anal. calcd for $C_{15}H_{17}Br_4N_3O_2$ (590.94): C, 30.49; H, 2.90; N, 7.11. Found: C, 30.39; H, 2.95; N, 7.00.

4.1.3. 4-(4,5,6,7-Tetrabromobenzimidazol-1-yl)-butyric acid (**2b**)

The mixture containing EtOH (20 ml), water (10 ml), NaOH (200 mg, 5 mmol), and **1b** (580 mg, 1.05 mmol) was stirred at room temperature for 4 h. Next it was brought to the reflux and after cooling adjusted to pH 4 with acetic acid and concentrated to 15 ml. The white chromatographic pure precipitate was formed (495 mg, 91%). m.p. 194–196 °C (from butanone-water; 3:1, v/v). ^{1}H NMR (DMSO-d_6): 2.03 (m, 2H, CH_2), 2.25 (t, 2H, CH_2, J = 7.3 Hz), 4.51 (t, 2H, CH2, J = 7.1 Hz), 8.47 (s, 1H, CH), 12.2 (bs, 1H, COOH). UV (MeOH): 230 (28 500), 269 (9700), 274 (9600), 303 (3500). Anal. calcd for $C_{11}H_8Br_4N_2O_2$ (519.81): C, 25.42; H, 1.55; N, 5.39. Found: C, 25.38; H, 1.60; N, 5.27.

4.1.4. 4-(2-Dimethylamino-4,5,6,7-tetrabromobenzimidazol-1-yl)-butyric acid (**2d**)

Analogously as described for **2b** from **1d**. Yield 96%, m.p. 220–222 °C ^{1}H NMR (DMSO-d_6): 1.77 (m, 2H, CH_2), 2.04 (t, 2H, CH_2, J = 7.1 Hz), 2.98 (s, 6H, 2 X CH_3), 4,33 (t, 2H, J = 7.0 Hz). 12.1 (bs, 1H, COOH). UV (MeOH): 240 (29 600), 274 (12 700), 313 (7700). Anal. calcd for $C_{13}H_{13}Br_4N_3O_2$ (562.88): C, 27.24; H, 2.33; N, 7.47. Found: C, 27.16; H, 2.40; N, 7.39.

4.1.5. 3-(4,5,6,7-Tetrabromo-1H-benzimidazol-2-ylsulfanyl)-propionic acid ethyl ester (**4c**)

A mixture of **3a** (470 mg, 1 mmol), potassium carbonate (420 mg, 3 mmol) and 3-bromopropionic acid ethyl ester (360 mg, 2 mmol) in acetone (30 ml) was stirred at room temperature for 2 d. Next, water (50 ml) was added and the mixture was left at 5 °C. The white precipitate that formed overnight was separated and crystallized from ethanol to yield small needles (410 mg, 72%); mp. 183–185 °C 1H NMR (DMSO-d_6): 1.18 (t, 3H, J = 7.1 Hz, CH_3) 2.88 (t, 2H, J = 6.8 Hz, CH_2), 3.52 (t, 2H, J = 6.8 Hz, CH_2), 4.10 (q, 2H, J = 7.1 Hz, CH_2), 13.3 (bs, 1H, H–N). Anal. calcd. for $C_{12}H_{10}Br_4N_2O_2S$ (565.91): C, 25.47; H, 1.78; N, 4.95. Found: C, 25.41; H, 1.81; N, 4.86.

4.1.6. 3-(1-Methyl-4,5,6,7-tetrabromobenzimidazol-2-ylsulfanyl)-propionic acid ethyl ester (**4d**)

Analogously as described for **4c**, from **3b**. Yield 84%, mp. 132–134 °C ^{1}H NMR (DMSO-d_6):1.19 (t, 3H, J = 7.1 Hz, CH_3), 2.92 (t, 2H, J = 6.8 Hz, CH_2), 3.56 (t, 2H, J = 6.7 Hz, CH_2), 3.89 (s, 3H, CH_3), 4.09 (q, 2H, J = 7.1 Hz, CH_2). Anal. calcd for $C_{13}H_{12}Br_4N_2O_2S$ (579.93): C, 26.92; H, 2.09; N, 4.83. Found: C, 26.98; H, 2.18; N, 4.71.

4.1.7. 4-(4,5,6,7-Tetrabromo-1H-benzimidazol-2-ylsulfanyl)-butyric acid ethyl ester (**4e**)

Analogously to described above for **4c**; instead of 3-bromopropionic acid ethyl ester 4-bromobutanoic acid ethyl ester was used. m.p. 181–183 °C, 82%. 1H NMR (DMSO-d_6): 1.16 (t, 3H, J = 7.1 Hz, CH_3), 2.00 (m, 2H, CH_2), 2.46 (t, 2H, J = 7.3 Hz, CH_2), 3.38 (t, 2H, J = 7.0 Hz, CH_2), 4.05 (q, 2H, J = 7.1 Hz, CH_2), 13.3 (bs, 1H, H–N). Anal. calcd. for $C_{13}H_{12}Br_4N_2O_2S$ (579.93): C, 26.92; H, 2.09; N, 4.83. Found: C, 26.98; H, 2.14; N, 4.74.

4.1.8. 4-(1-Methyl-4,5,6,7-tetrabromobenzimidazol-2-ylsulfanyl)-butyric acid ethyl ester (**4f**)

Analogously as described for **4c**, from **3b**. Yield 87%, m.p. 118–120 °C (from ethanol). ^{1}H NMR (DMSO-d_6): 1.17 (t, 3H, CH3, J = 7.1 Hz), 2.05 (m, 2H, CH2,), 2.50 (t, 2H, CH2, overlap. DMSO), 3.42 (t, 2H, CH2, J = 7.0), 3.93 (s, 3H, CH3), 4.06 (q, 2H, CH2, J = 7.1 Hz). Anal. calcd for $C_{14}H_{14}Br_4N_2O_2S$ (593.96): C, 28.31; H, 2.38; N, 4.72. Found: C, 28.40; H, 2.33; N, 4.63.

4.1.9. 3-(4,5,6,7-Tetrabromo-1H-benzimidazol-2-ylsulfanyl)-propionic acid (**5c**)

A mixture of ester (**4c**) (570 mg, 1 mmol) and sodium hydroxide (120 mg, 3 mmol) in ethanol-water solution (20 ml, 1:1) was stirred at rt for 2 h. The mixture was brought to reflux and acidified with acetic acid to pH ~3. A white precipitate was filtered and crystallized from ethanol-water to give amorphous powder (340 mg, 63%); m.p. 203–205 °C 1H NMR (DMSO-d_6): 2.81 (t, 2H, J = 6.8 Hz, CH_2), 3.48 (t, 2H, J = 6.8 Hz, CH_2), 12.3–13.4 (bs, 2H, H–N and COOH). UV (MeOH): 236 (36 700), 272 (12 500), 301 (11 800), 312 (14 700). Anal. calcd. for $C_{10}H_6Br_4N_2O_2S$ (537.85): C, 22.33; H, 1.12; N, 5.21. Found: C, 22.40; H, 1.19; N, 5.13.

4.1.10. 3-(1-Methyl-4,5,6,7-tetrabromobenzimidazol-2-ylsulfanyl)-propionic acid (**5d**)

Analogously as described for **5c**, from **4d**. Yield: 82%, m.p. >300 °C (with decomp.) (from butanone-water; 3:1, v/v). ^{1}H NMR (DMSO-d_6): 2.81 (t, 2H, CH_2, J = 6.8 Hz), 3.52 (t, 2H, CH_2, J = 6.7 Hz), 3.91 (s, 3H, CH_3), 12.3 (bs, 1H, COOH). UV (MeOH): 233 (33 500), 277 (11 100), 302 (9200), 314 (10 700). Anal. calcd for $C_{11}H_8Br_4N_2O_2S$ (551.88): C, 23.94; H, 1.46; N, 5.08. Found: C, 23,99; H, 1.36; N 4.97.

*4.1.11. 4-(4,5,6,7-Tetrabromo-1H-benzimidazol-2-ylsulfanyl)-butyric acid (**5e**)*

Analogously as described for **5c**, from **4e**. m.p.226–228 °C (from EtOH-H_2O), (65%). 1H NMR (DMSO-d_6): 1.99 (m, 2H, CH_2), 2.39 (t, 2H, $J = 7.3$ Hz, CH_2), 3.36 (t, 2H, $J = 7.0$ Hz, CH_2), 12.1 (bs, 1H, COOH), 13.4 (bs, 1H, H–N). UV (MeOH): 238 (32 600), 272 (10 900), 301 (10 600), 313 (13 100). Anal. calcd. for $C_{11}H_8Br_4N_2O_2S$ (551.88): C, 23.94; H, 1.46; N, 5.08. Found: C, 23.98; H, 1.54; N, 5.01.

*4.1.12. 4-(1-Methyl-4,5,6,7-tetrabromobenzimidazol-2-ylsulfanyl)-butyric acid (**5f**)*

Analogously as described for **5c**, from **4f**. Yield 81%, m.p. 153–155 °C (from butanone-water, 3:1, v/v). ^{1}H NMR (DMSO-d_6): 2.00 (q, 2H, CH2, $J = 7.2$ Hz), 2.37 (t, 2H, CH_2, $J = 7.4$ Hz), 3.40 (t, 2H, CH_2, $J = 7.2$ Hz), 3.91 (s, 3H, CH_3), 12.1 (bs, 1H, COOH). UV (MeOH): 235 (32 400), 278 (10 800), 302 (9000), 314 (10 600). Anal. calcd for $C_{12}H_{10}Br_4N_2O_2S$ (565.91): C, 25.47; H, 1.78; N, 4.95. Found: C, 25.41; H, 1.83; N, 4.84.

4.2. Biochemistry

4.2.1. Overexpression and purification of human CK2α and CK2α′ subunits

E.coli BL21(DE3) cells harboring the plasmid pGEX-3X::hsCK2α or pGEX-3X::hsCK2α′ (kind gift from D. Litchfield) for overexpression of human CK2α and CK2α′ proteins were grown until $OD_{600} = 0.6$ at 37 °C. Next, IPTG was added to the final concentration of 0.2 mM and the cultures were continued at room temperature for 4 h and then centrifuged at 5000 × g for 10 min. The obtained bacteria pellets were kept frozen at −80 °C until processed further. Next, the pellets were suspended in 50 mM Tris/HCl buffer pH 7.5 supplemented with 6 mM β-mercaptoethanol, disrupted by sonication, and centrifuged for 15 min at 12 000Xg. The supernatant containing free catalytic subunits CK2α and CK2α′ was mixed with glutathione-sepharose (Pharmacia Biotech) and incubated overnight at 4 °C. The mixture was loaded onto an empty column and washed with the aforementioned buffer. Sepharose-adsorbed protein was eluted with 10 mM reduced glutathione in the same buffer. Fractions containing CK2α or CK2α′ subunit were pooled and dialyzed against 50 mM Tris/HCl buffer pH 7.5 supplemented with 6 mM β-mercaptoethanol and 30% glycerol. The obtained protein preparation were used in enzymatic assays.

4.2.2. Substrates

Yeast ribosomal acidic protein P2B was overexpressed in *E.coli* BL21(DE3) cells containing the plasmid pT7-7::*6xHis-yP2B* (kind gift from J.P. Ballesta) and purified by affinity chromatography using Ni-Affinity Gel (Sigma). The synthetic peptide RRRADDSDDDDD was purchased from Sigma.

4.2.3. Phosphorylation assays

To estimate the inhibitory effect of new synthesized benzimidazole analogs two different assays were employed depending on the substrate.

The reaction mixture containing 20 mM Tris/HCl pH 7.5, 15 mM $MgCl_2$, 2 pmol CK2α or CK2α′ (spec. act. 1 μmol/min/mg), 20 μM [γ-^{32}P]ATP (Hartmann Analytics GmbH) and as substrate 10 μM P2B or 50 μM synthetic peptide RRRADDSDDDDD was incubated at 37 °C for 15 min. Reactions with P2B as substrate were stopped by addition of SDS sample buffer. Samples were subjected to 12.5% SDS/PAGE. After Coomassie Blue staining gels were dried and exposed to Kodak X-ray film at −80 °C overnight. The parts of the gels corresponding to P2B were cut out and the phosphate incorporation was estimated by Cerenkov counting of ^{32}P radioactivity in scintillation counter. Phosphorylation of the peptide substrate was terminated by addition of 10% TCA and aliquots were spotted onto phosphocellulose filters (Whatmann). Filters were washed with 5% TCA three times and dried before counting in scintillation counter.

Inhibition studies were performed at fixed concentrations of substrate and at variable concentrations of ATP in the absence or in the presence of increasing concentrations of inhibitor. Kinetic parameters were determined by non-linear-regression analysis using GraphPad Prism 4.0 (GraphPad Software, Inc San Diego).

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References

[1] G. Manning, D.B. Whyte, R. Martinez, T. Hunter, S. Sudarsanam, The protein kinase complement of the human genome, Science 298 (2002) 1912–1934.
[2] D. Huang, T. Zhou, K. Lafleur, C. Nevado, Kinase selectivity potential for inhibitors targeting the ATP binding site: a network analysis, Bioinformatics 26 (2010) 198–204.
[3] K. Ahmed, D.A. Gerber, C. Cochet, Joining the cell survival squad: an emerging role for protein kinase CK2, Trends Cell Biol. 12 (2002) 226–230.
[4] B. Guerra, O.-G. Issinger, Protein kinase CK2 and its role in cellular proliferation, development and pathology, Electrophoresis 20 (1999) 391–408.
[5] D.W. Litchfield, Protein kinase CK2: structure, regulation and role in cellular decisions of life and death, Biochem. J. 369 (2003) 1–15.
[6] L.A. Pinna, Protein kinase CK2: a challenge to canons, J. Cell. Sci. 115 (2002) 3873–3878.
[7] L. Bodenbach, J. Fauss, A. Robitzki, A. Krehan, P. Lorenz, F.J. Lozeman, W. Pyerin, Recombinant casein kinase II, Eur. J. Biochem. 220 (1994) 263–273.
[8] D.W. Litchfield, D.G. Bosc, D.A. Canton, R.B. Saulnier, G. Vilk, C. Zhang, Functional specialization of CK2 isoforms and characterization of isoform-specific binding partners, Mol. Cell. Biochem. 227 (2001) 21–29.
[9] G.M. Unger, A.T. Davis, J.W. Slaton, K. Ahmed, Protein kinase CK2 as regulator of cell survival: implications for cancer therapy, Curr. Canc. Drug Targets 4 (2004) 77–84.
[10] J.S. Kim, J.I. Eom, J.-W. Cheong, A.J. Choi, J.K. Lee, W.I. Yang, Y.H. Min, Protein kinase CK2α as an unfavorable prognostic marker and novel therapeutic target in acute myeloid leukemia, Clin. Canc. Res. 13 (2007) 1019–1028.
[11] F. Meggio, D. Shugar, L.A. Pinna, Ribofuranosyl-benzimidazole derivatives as inhibitors of casein kinase-2 and casein kinase-1, Eur. J. Biochem. 187 (1990) 89–94.
[12] R. Szyszka, N. Grankowski, K. Felczak, D. Shugar, Halogenated benzimidazoles and benzotriazoles as selective inhibitors of protein kinases CK I and CK II from *Saccharomyces cerevisiae* and other sources, Biochem. Biophys. Res. Commun. 208 (1995) 418–424.
[13] P. Zień, O. Abramczyk, K. Domańska, M. Bretner, R. Szyszka, TBBz but not TBBt discriminates between two molecular forms of CK2 *in vivo* and its implications, Biochem. Biophys. Res. Commun. 312 (2003) 623–628.
[14] P. Zień, J.S. Duncan, J. Skierski, M. Bretner, D.W. Litchfield, D. Shugar, Tetrabromobenzotriazole (TBBt) and tetrabromobenzimidazole (TBBz) as selective inhibitors of protein kinase CK2: evaluation of their effects on cells and different molecular forms of human CK2, Biochim. Biophys. Acta 1754 (2005) 271–280.
[15] R. Battistutta, Structural bases of protein kinase CK2 inhibition, Cell. Mol. Life Sci. 66 (2009) 1868–1889.
[16] A.G. Golub, V.G. Bdzhola, N.V. Briukhovetska, A.O. Balanda, O.P. Kukharenko, I.M. Kotey, O.V. Ostrynska, S.M. Yarmoluk, Synthesis and biological evaluation of substituted (thieno[2,3-d]pyrimidin-4-ylthio)carboxylic acids as inhibitors of human protein kinase CK2, Eur. J. Med. Chem. 46 (2011) 870–876.
[17] M.A. Pagano, J. Bain, Z. Kazimierczuk, S. Sarno, M. Ruzzene, G. Di Maira, M. Elliott, A. Orzeszko, G. Cozza, F. Meggio, L.A. Pinna, The selectivity of inhibitors of protein kinase CK2: an update, Biochem. J. 415 (2008) 353–365.
[18] R. Prudent, C. Cochet, New protein kinase CK2 inhibitors: Jumping out of the catalytic Box, Chem. Biol. 16 (2009) 112–120.
[19] R. Prudent, C.F. Sautel, C. Cochet, Structure-based discovery of small molecules targeting different surfaces of protein-kinase CK2, Biochim. Biophys. Acta 1804 (2010) 493–498.
[20] S. Sarno, E. Papinutto, C. Franchin, J. Bain, M. Elliott, F. Meggio, Z. Kazimierczuk, A. Orzeszko, G. Zanotti, R. Battistutta, L.A. Pinna, ATP site-directed Inhibitors of protein kinase CK2: an update, Curr. Top. Med. Chem. 11 (2011) 1340–1351.
[21] M. Janeczko, M. Masłyk, R. Szyszka, A. Baier, Interactions between subunits of protein kinase CK2 and their protein substrates influences its sensitivity to specific inhibitors, Mol. Cell. Biochem. 356 (2011) 121–126.
[22] A. Gianoncelli, G. Cozza, A. Orzeszko, F. Meggio, Z. Kazimierczuk, L.A. Pinna, Tetraiodobenzimidazoles are potent inhibitors of protein kinase CK2, Bioorg. Med. Chem. 17 (2009) 7281–7288.
[23] M.A. Pagano, M. Andrzejewska, M. Ruzzene, S. Sarno, L. Cesaro, J. Bain, M. Elliott, F. Meggio, Z. Kazimierczuk, L.A. Pinna, Optimization of protein kinase CK2 inhibitors derived from 4,5,6,7-tetrabromobenzimidazole, J. Med. Chem. 47 (2004) 6239–6247.