Bioorganic & Medicinal Chemistry 20 (2012) 4390–4396

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Modified tetrahalogenated benzimidazoles with CK2 inhibitory activity are active against human prostate cancer cells LNCaP in vitro

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ARTICLE INFO

Article history:
Received 24 November 2011
Revised 9 May 2012
Accepted 16 May 2012
Available online 24 May 2012

Keywords:
CK2
Tetrahalogenobenzimidazoles
Kinase inhibitors
Anticancer activity
Human prostate cancer cell line LCNaP

ABSTRACT

A series of novel CK2 inhibitors, tetrahalogenated benzimidazoles carrying an aminoalkylamino group at position 2, has been prepared by nucleophilic substitution of the respective 2,4,5,6,7-pentabromobenzimidazoles and 2-bromo-4,5,6,7-tetraiodobenzimidazoles. The new derivatives as well as some previously obtained tetrahalogenobenzimidazoles, including 4,5,6,7-tetrabromobenzimidazole (TBI) and 4,5,6,7-tetraiodobenzimidazole (TIBI), were evaluated for activity against the hormone-sensitive human prostate cancer cell line LNCaP. The activity of 2-aminoalkylamino derivatives was notably higher (LD_{50} 4.75–9.37 μM) than that of TBI and TIBI ($LD_{50} \approx 20$ μM). The determination of the LD_{50} value identified the 2-aminoethylamino-4,5,6,7-tetraiodobenzimidazole with an additional methyl group at position 1 (**6**) as the most efficient compound (LD_{50}: 4.75 ± 1.02 μM). Interestingly, there was no clear correlation between cell viability and apoptosis induction indicating additional cell death mechanisms.

1. Introduction

Protein kinase CK2 (previously known as casein kinase 2/casein kinase II) has traditionally been viewed as the most pleiotropic serine/threonine-specific kinase. However, there is evidence that CK2 can also phosphorylate tyrosine residues in vitro as well as in yeast and in mammalian cells.[1,2] The list of endogenous substrates of CK2, which includes important regulatory proteins and tumor suppressor gene products, comprises almost 400 proteins and continues to grow.[3] CK2 is one of the most consistently elevated protein kinases associated with oncogenic phenotype across various cancer types.[4,5] CK2 overexpression has been invariably observed in transformed tissues and is considered an unfavorable prognostic marker in some malignancies.[6,7] The nucleotide pocket of CK2 can accept both ATP and GTP as phosphate donor. The structures of CK2–substrate complexes indicate that water molecules are critical for switching the active site of the enzyme from ATP- to GTP-compatible state.[8,9] The specific environment of the highly conserved ATP-binding site in the CK2 polypeptide chain, which includes a number of bulky amino acid residues, has been the focus of CK2 inhibitor designing.[10] The hydrophobic pocket adjacent to the ATP-binding site is known to be important for the inhibitory activity of polyhalogenated benzimidazoles and their related benzotriazoles. This is because of specific interactions of apolar side chains of some amino acid residues that form this structure, particularly those of Val 66 and Ile 174,[11,12] with the polyhalogenated part of the inhibitors.

The majority of known CK2 inhibitors are small molecules that are not retained in the hydrophobic pocket of most kinases, but are easily entrapped in that of CK2, resulting in efficient inhibition. In the last years, numerous small molecules have been developed as CK2 inhibitors, including halogenated benzimidazoles and benzotriazoles, coumarins, pyrrazoletriazines, anthraquinones and a few more classes of chemicals.[13–18]

CK2 inhibitors induce apoptosis and effectively reduce cell viability in many human cancer cell lines in vitro.[19–26] Oral CK2 inhibitor CX-4945 (Cylene Pharmaceuticals, San Diego, CA, USA) is currently undergoing phase I clinical evaluation in patients with a variety of solid tumors, including prostate, pancreatic and breast cancers together with inflammatory breast cancer, as well as in patients with multiple myeloma and Castleman's disease.[27] There are also a few reports on the use of small molecule CK2 inhibitors in animal models of cancer.[28,29]

The first hint that derivatives of halogenated benzimidazoles may be effective inhibitors of CK2 was provided by the observation that 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB), which is an inhibitor of initiation of eukaryotic mRNA transcription by RNA polymerase II, shows also considerable activity as CK2

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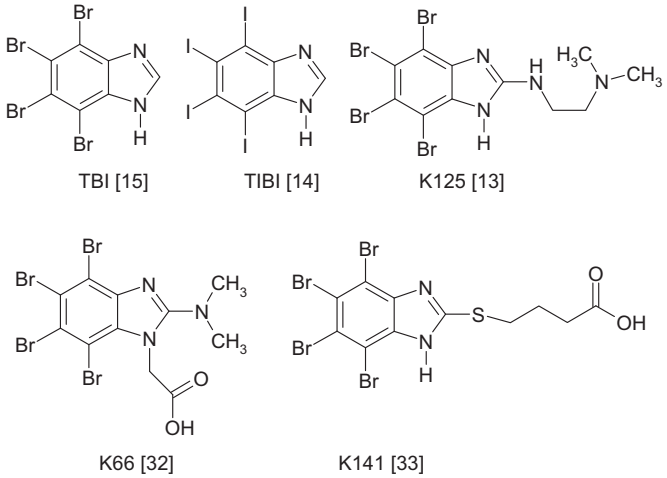
http://dx.doi.org/10.1016/j.bmc.2012.05.038

inhibitor. It has also been found in that study that the 5,6-dibromo analog of DRB is almost fivefold more effective in this respect than the 'parental' DRB.[30] Further studies on polybrominated benzotriazoles and benzimidazoles provided more valuable structures that hamper ATP binding by CK2, and 4,5,6,7-tetrabromobenzotriazole (TBB) was found one of the most powerful CK2 inhibitors that are capable of cell membrane crossing.[31] Our previous synthetic studies concerning these heterocycles resulted in the development of several highly effective CK2 inhibitors including 2-dimethylamino-4,5,6,7-tetrabromobenzimidazole (DMAT; K_i = 0.040 μM) and 4,5,6,7-tetraiodobenzimidazole (TIBI) (K_i = 0.023 μM).[13,14]

In this study we have addressed the relationship between the structure of 2-substituted tetrahalogenobenzimidazoles and their anticancer activity in vitro by seeking answers to two questions: (i) what is the influence of the kind of halogen atoms at the aromatic ring and (ii) what is the influence of the 2-aminoalkylamino side chain. Preliminary testing included **TIBI**, 4,5,6,7-tetrabromo-1*H*-benzimidazole (**TBI**), two **TBI** derivatives with a carboxy group in the side chain, that is, 2-dimethylamino-1-carboxymethyl-4,5,6,7-tetrabromo-1*H*-benzimidazole (**K66**) and 2-carboxybutylsulfanyl-4,5,6,7-tetrabromo-1*H*-benzimidazole (**K141**), and a single TBI derivative with basic dimethylaminoethyleneamine substituent in position 2 (**K125**); see Scheme 1.[14,32,33] Promising results of the initial phase prompted us to extend the panel of TBI and TIBI derivatives with those carrying a 2-aminoalkylamino substituent.

We tested anticancer activity of the new compounds using a human prostate cancer cell line. Prostate cancer is the most common cancer in elderly men in the Western world.[34] Its growth is dependent on androgens and androgen receptor signalling. Androgen ablation by orchiectomy and/or treatment with LHRH-analogs or anti-androgens are the most common therapeutical strategies. Chemical castration often leads to a selection of hormone-refractory cells, which are mostly characterized by activation of anti-apoptotic signalling pathways,[35] for example, that relying on elevated CK2 activity. Treatment with apoptosis-inducing agents seems to be a promising approach to treat the resulting hormone-independent malignancy. CK2, which has potent anti-apoptotic action, is usually elevated in tumor cells compared to their normal counterparts. CK2 is a global regulator of apoptotic pathways. Its activity modulates apoptotic pathways which may contribute in promoting cancer cells survival.[36] Phosphorylated CK2α localizes to the mitotic spindle where it might target mitotic specific targets.[37] Additional effects of CK2 however, cannot be excluded. It has already been shown that inhibition of CK2 kinase in prostate cancer cells results in the generation of reactive oxygen species that may be responsible for mitochondrial damage and the consequential cytochrome *c* release-related activation of the intrinsic apoptotic pathway.[38–42] Below we present a number of new CK2 inhibitors of the 2-aminoalkylamino-4,5,6,7-tetrabromo-(iodo)benzimidazole group, which show high activity against the androgen-responsive human prostate cancer cell line LNCaP.



Scheme 1. The chemical structures of previously obtained CK2 inhibitors.

2. Results and discussion

2.1. Chemical synthesis

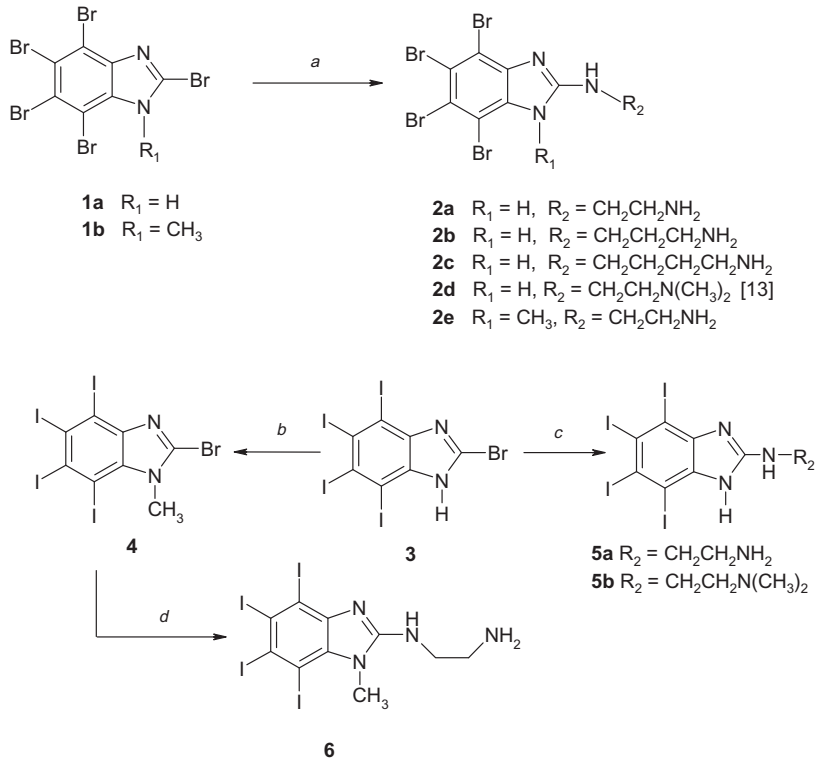
In the preliminary phase of the study we have tested anticancer activity of the following previously described tetrahalogenobenzimidazoles: TBI, TIBI, 2-dimethylamino-4,5,6,7-tetrabromo-1-carboxymethyl-1*H*-benzimidazole (**K66**), 4,5,6,7-tetrabromo-1*H*-benzimidazole-2-sulfanylbutyric acid (**K141**) and N^1,N^1-dimethyl-N^2-(4,5,6,7-tetrabromo-1*H*-benzimidazol-2-yl)-ethane-1,2-diamine (**K125**).[13,14,32,33] The obtained results indicated that the diamine derivative K125 was the most prospective structure for further studies. Hence, we decided to extend this subclass of tetrahalogenobenzimidazole derivatives by introducing various aminoalkylamino groups into position 2 of the core benzimidazole. With this aim, 2,4,5,6,7-pentabromo-1*H*-benzimidazole (**1a**) and its 1-methyl derivative (**1b**) were subject to reaction with 1,2-ethylenediamine to give compounds **2a** and **2e**, respectively, and compound **1a** was also subject to reactions with 1,3-diaminopropane, 1,4-diaminobutane and *N,N*-dimethylethylenediamine to give derivatives **2b**, **2c**,and **2d**, respectively. Similarly, the compounds **5a** and **5b** were obtained from 2-bromo-4,5,6,7-tetraiodo-1*H*-benzimidazole (**3**) by reactions with 1,2-ethylenediamine and *N,N*-dimethylethylenediamine, respectively. Methylation of compound **3** with methyl iodide solution in DMSO gave the expected 1-methyl derivative **4**. Bromine atom in position 2 of compound **4** was substituted with ethylenediamine to give **6** (see Scheme 2). Structures of the new compounds were confirmed by ^{1}H NMR, mass spectroscopy and elemental analyses.

2.2. Biological activity

LNCaP prostate cancer cells were treated for 24 h with the tested compounds (at 10 μM or 20 μM concentration). After lysis of the cells, CK2 activity was assessed by the incorporation of ^{32}P-phosphate into a CK2-specific synthetic substrate peptide. The results obtained showed that the inhibition of CK2 activity was hardly elevated by doubling the concentration of the most tested compounds. N^1-(4,5,6,7-Tetrabromo-1*H*-benzimidazol-2-yl)-propane-1,3-diamine (**2b**) was the most potent CK2 inhibitor which leads to a residual activity of 20–30%, whereas no inhibitory activity was found for 2-dimethylamino-1-carboxymethyl-4,5,6,7-tetrabromo-1*H*-benzimidazole (**K66**) and hardly for **K141**. The remaining compounds show but a moderate residual activity between 20% and 60% (Fig. 1).

To test the anticancer effect of the different tetrahalogenobenzimidazoles we treated the LNCaP cells for 24 h and 48 h with 10 or 20 μM of the compounds. The MTT assay employed showed that compounds **K66** and **K141** did not affect cell viability (Fig. 2). Prolongation of the incubation time significantly reduced the number of viable LNCaP cells irrespective of the CK2 inhibitor concentration. **TBI**, **TIBI** and **2c** reduced cell viability by 20–80%. The other compounds lead to a nearly complete loss of cell viability after 48 h at the higher tested concentration.

It is interesting to compare the effect of the tested compounds on LNCaP cells' viability (Figs. 1 and 2) against the literature data



Scheme 2. Reagents and conditions: (a) H_2N-X-NR_2, 1-propanol, reflux; (b) CH_3I, DMSO, NaOEt, rt; (c) H_2N-X-NR_2, 1-propanol, reflux; (d) $H_2NCH_2CH_2NH_2$.

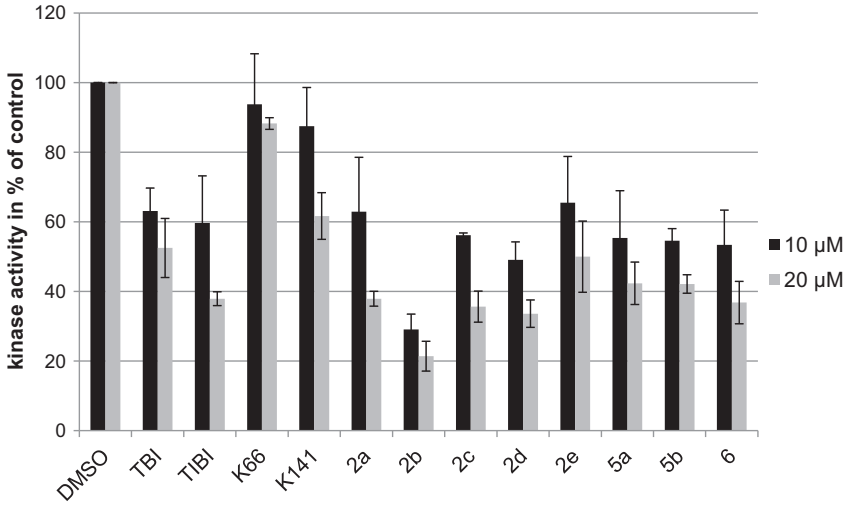


Figure 1. Inhibition of protein kinase CK2 in LNCaP cells. Cells were treated with 10 or 20 μM of the different tetrahalogenated benzimidazoles for 24 h. Proteins were extracted and the CK2 activity was determined by the incorporation of ^{32}P-phosphate into the CK2 specific synthetic substrate RRRDDDSDDD. Cells treated with the solvent DMSO alone were used as a control and the activity set to 100%. The diagram shows the mean of at least three independent experiments. # means not significant.

on the CK2 inhibitory properties of these compounds. **TIBI** is about six times more potent than **TBI** as the inhibitor of rat liver CK2 (K_i = 0.023 and μM K_i = 0.139 μM, respectively),[14] whereas **K66** and **2d** have similar K_i values (K_i = 0.25 μM and K_i = 0.16 μM, respectively).[13,32] However, the effect of some compounds on CK2 activity in the lysates of cells that were cultured with these compounds does not correspond with the CK2 inhibitory activity of these compounds against isolated CK2. Compounds such as **K66** and **K141** (IC_{50}: 0.27 μM, L.A. Pinna, priv. comm.) being good inhibitors of isolated CK2 form zwitterions at physiological pH, hence their transport through the cell membrane is greatly hindered. However, introduction of a hydrophilic aminoalkylamino residue might facilitate the influx of even bulky tetraiodobenzimidazoles (e.g., **5a**, **5b** and **6**) into cells. The presence of a 2-aminoalkylamino group in the tested tetrahalogenobenzimidazoles clearly increased their activity against LNCaP cells. Elongation of the alkyl linker did not improve this activity. Compound **2c** was about twice less active than **2a** and **2b**; interestingly, in contrast to what was seen for the other compounds studied, doubling the incubation time did not enhance anticancer effect of **2c** (Fig. 2). Introduction of the methyl group in the position N^1 of the benzimidazole nucleus did not affect the activity of the resulting deriv-

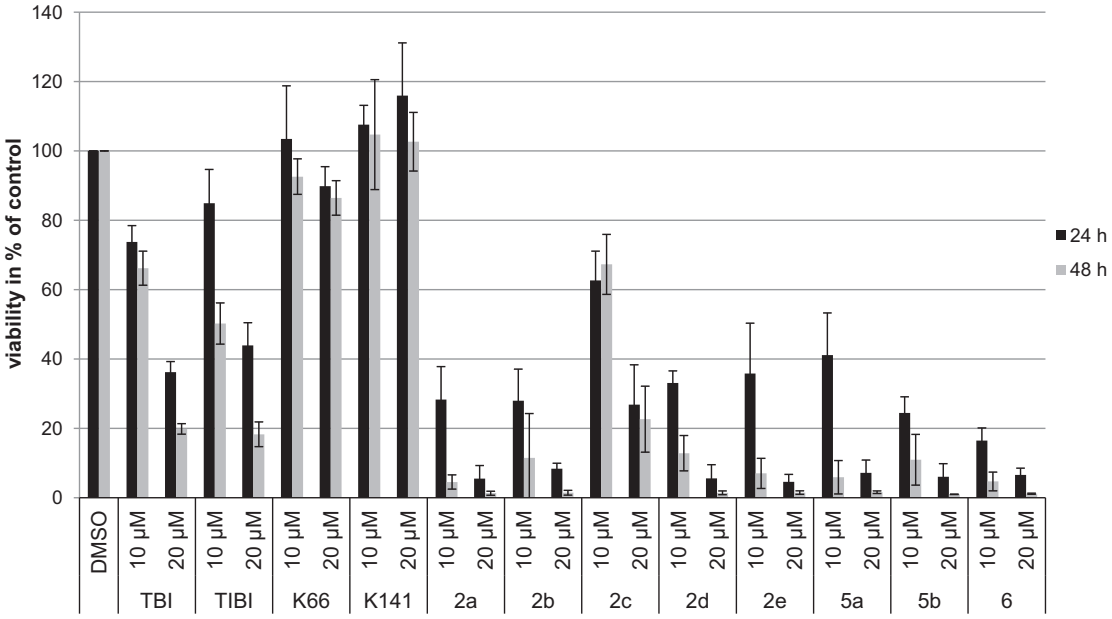


Figure 2. Viability of LNCaP cells after CK2 inhibition. 2×10^4 cells were seeded in a 24 well tissue culture plate. Growth of mock treated and cells treated with 10 or 20 μM of the different tetrahalogenated benzimidazoles was measured after 24 and 48 h by the MTT assay. The histogram shows the percentage of viable cells compared to the DMSO treated control cells. The diagram shows the mean of at least three independent experiments. # means not significant.

atives against LNCaP cells (cf. **2e,** and **6** and **5a**, Fig. 2). There was no considerable difference between the effect of methylated and the respective nonmethylated derivatives. It should be stressed that almost all tested 2-aminoalkylamino derivatives showed LD_{50} <10 μM; hence, they belong to the most effective drugs against human prostate cancer cells in vitro. Additionally, we compared the four selected compounds **2a, 2d, 5b** and **6** in concentrations lower than 10 μM to analyze whether the compounds might differ in their LD_{50} values. Therefore we performed a MTT assay with the compounds in a concentration range between 3 and 10 μM. After an incubation time of 24 h we found the results shown in Figure 3. Using the Graph Pad Prism 5 software the LD_{50} value for **2a** was calculated as 7.22 ± 1.05 μM, for **2d** 9.37 ± 1.05 μM, for **5b** 7.37 ± 1.02 μM and for **6** 4.75 ± 1.02 μM. Therefore, we have to conclude that **6** is the most promising anticancer compound due to the lowest LD_{50} value.

As the assessment of the number of surviving cells using the MTT assay cannot discriminate between the effect on cell viability and on the cell cycle arrest, we investigated the cleavage of PARP as an indication of apoptosis, using LNCaP cells treated for 24 h with 10 or 20 μM of the different compounds. After lysing the cells, the protein extracts obtained were electrophoresed on SDS–polyacrylamide gels and transferred onto a PVDF membrane. The respective Western blots (Fig. 4) showed that compounds **K66** and **K141** did not induce any PARP cleavage. Compound **2a** showed the strongest induction of apoptosis of all the tested compounds both at 10 μM

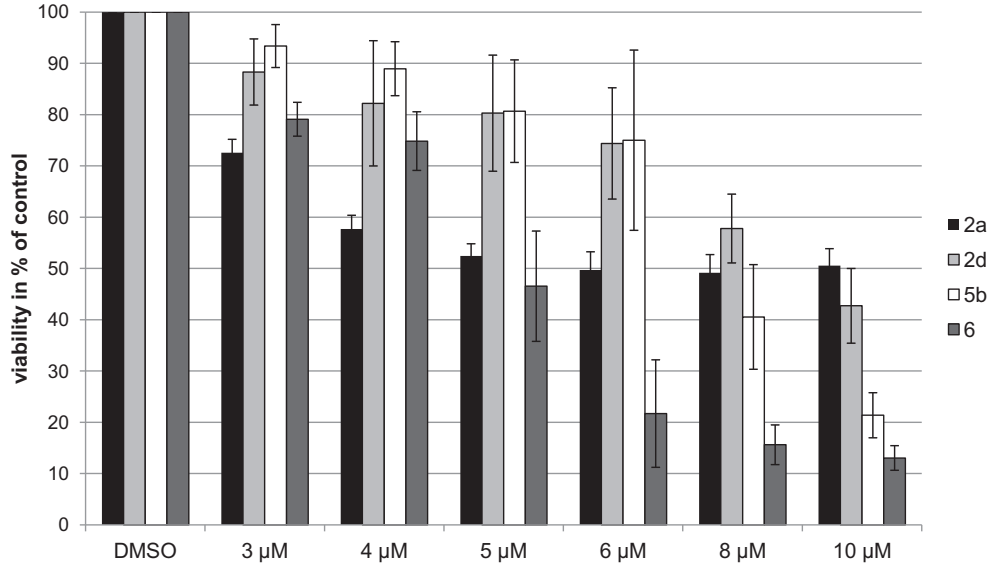


Figure 3. Viability of LNCaP cell after CK2 inhibition. 2×10^4 cells were seeded in a 24 well tissue culture plate. Growth of mock treated and cells treated with 3–10 μM of the tetrahalogenated benzimidazoles **2a**, **2d**, **5b** and **6** was measured after 24 by the MTT assay. The histogram shows the percentage of viable cells compared to the DMSO treated control cells. The diagram shows the mean of at least three independent experiments. # means not significant.

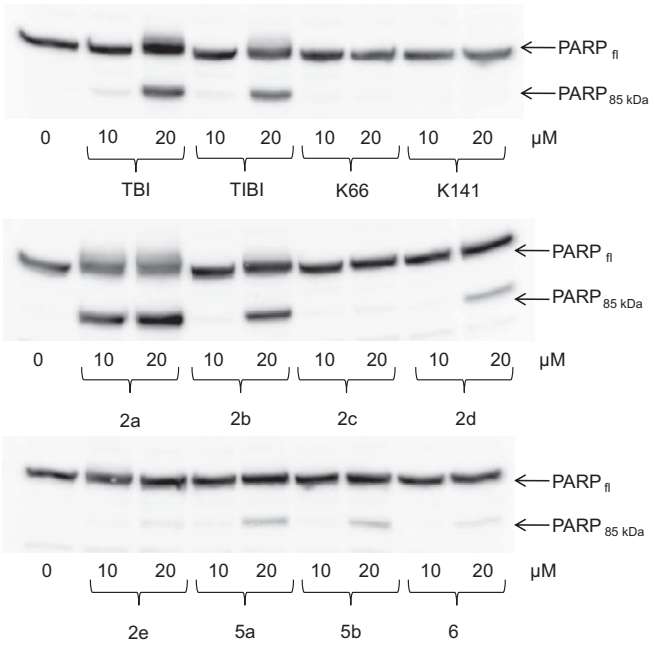


Figure 4. Detection of PARP cleavage in LNCaP cells after CK2 inhibition. Cell extracts (150 µg) of mock treated and LNCaP cells treated with the different tetrahalogenated benzimidazoles (10 and 20 µM for 24 h) were separated by electrophoresis through a 7.5% SDS–polyacrylamide gel and transferred onto a PVDF membrane. PARP full length ($PARP_{fl}$) and PARP fragments ($PARP_{85\ kDa}$) were detected with a polyclonal rabbit antibody.

and 20 µM. PARP cleavage was concentration-dependent (Fig. 4). Compounds **TBI**, **TIBI**, **2b**, **5a**, **2d** and **5b** only lead to a PARP cleavage at the higher tested concentration. Compounds **2c**, **2e** and **6** induced nearly no PARP cleavage although, they caused a nearly complete loss of cell viability. Thus, at least these two compounds carrying an *N*-methyl group reduced cell viability without inducing apoptosis. An alternative explanation might be that the cells dye by necrosis and/or by mitotic catastrophe.

Whereas at present the first choice treatment for prostate cancer is surgery, the novel tetrahalogenobenzimidazoles we present here show considerable promise for adjuvant therapy of this malignancy. The anticancer activity of the tested compounds appears related to their inhibitory activity against CK2, but their anti-LNCaP activity likely involves other metabolic pathways as well.

3. Experimental

3.1. Chemical synthesis

3.1.1. General procedure

Melting points were determined in open capillary tubes using model MFB 595 030G Gallenkamp melting point apparatus. ^{1}H NMR spectra were recorded on a Bruker Avance II instrument (400 MHz ^{1}H frequency) at 25 °C. Chemical shifts are given in ppm relative to tetramethylsilane as an internal standard. The solvent used for NMR spectra was deuteriodimethylsulfoxide. UV spectra were determined on a Techcomp UV8500 spectrophotometer. Classical mass spectra (MS) (70 eV) and electrospray ionization mass spectra (ES-MS) were obtained with an AMD-604 (Intecta) spectrometer. Elemental analyses were performed at the Faculty of Chemistry, Warsaw Technical University.

3.1.2. N^1-(4,5,6,7-Tetrabromo-1*H*-benzimidazol-2-yl)-ethane-1,2-diamine (2a)

A mixture of **1a**[43] (930 mg, 1.8 mmol), 1,2-ethylenediamine (2.2 g, 37 mmol) in 1-PrOH (35 ml) was stirred and refluxed for 24 h. The mixture was evaporated to oil and deposited on silica gel column (2.5 × 12 cm). Column chromatography was performed with $CHCl_3$–MeOH (100 ml, 90:10, v/v) and then with $CHCl_3$–MeOH–triethylamine (80:20:2). The product containing fractions were evaporated to dryness and the residue was crystallized from EtOH to give white powder (yield: 460 mg, 52%). Mp 248–250 °C, ^{1}H NMR (Me_2SO-d_6) δ (ppm): 2.90 (t, 2H, H_2C, J = 5.4 Hz), 3.36 (t, 2H, H_2C, J = 4.9 Hz), 6.0 (br s, 2H, H_2N), 6.78 (s, 1H, H–N), 10.8 (br s, 1H, H-benzim.). UV (MeOH): 240 (20,700), 267 (10,600), 312 (10 100). MS (*m/z*): 496 (3, M^++4), 494 (7, M^++2), 492 (12, M^+), 490 (8, M^+−2), 488 (3, M^+−4), 462 (100, M^+−30). Anal. Calcd for $C_9H_8Br_4N_4$ (491.81): C, 21.98; H 1.64; N, 11.39. Found: C, 21, 85; H 1.71; N, 11.49.

3.1.3. N^1-(4,5,6,7-Tetrabromo-1*H*-benzimidazol-2-yl)-propane-1,3-diamine (2b)

Similarly to described above *n*-propanolic 1,3-propanediamine (30 ml, 3 g, 36 mmol) was used (mp >295 °C, with decomp, 51% ^{1}H NMR (Me_2SO-d_6) δ (ppm): 1.86 (m, 2H, H_2C), 2.87 (t, 2H, H_2C, J = 7.2 Hz), 3.43 (q, 2H, H_2C, J = 5.9 Hz), 6.87 (t, 1H, H–N, J = 4.8 Hz), 8.5 (br s, 2H, H_2N), 10.7 (br s, 1H, H-benzim.). UV (MeOH): 238 (23,500), 265 (9900), 311 (7700). MS (*m/z*): 510 (5, M^++4), 508 (13, M^++2), 506 (21, M^+), 504 (14, M^+−2), 502 (6, M^+−4), 476 (43, M^+−30), 463 (77, M^+−43), 40 (100). Anal. Calcd for $C_{10}H_{10}Br_4N_4$ (505.83): C, 23.75; H, 1.99; N, 11.08. Found: C, 23.78; H, 1.83; N, 10.97.

3.1.4. N^1-(4,5,6,7-Tetrabromo-1*H*-benzimidazol-2-yl)-butane-1,4-diamine (2c)

Similarly to described above *n*-propanolic 1,4-butanediamine (30 ml, 3.5 g, 36 mmol) was used. Mp 287–298 °C (sint. >240 °C), 57%. ^{1}H NMR (Me_2SO-d_6) δ (ppm): 1.61 (m, 4H, 2 × H_2C), 2.80 (t, 2H, H_2C, J = 6.8 Hz), 3.33 (m, 2H, H_2C), 7.6–7.8 (br s, 3H, H_2N and H–N), 10.7 (br s, 1H, H-benzim.). UV (MeOH): 239 (24,500), 267 (9700), 312 (7400). MS (*m/z*): 524 (6, M^++4), 522 (21, M^++2), 520 (34, M^+), 518 (20, M^+−2), 516 (8, M^+−4), 476 (28, M^+−44), 449 (100, M^+−71). Anal. Calcd for $C_{11}H_{12}Br_4N_4$ (519.86): C, 25.41; H, 2.33; N, 10.78. Found: C, 25.38; H, 2.22; N, 10.67.

3.1.5. N^1,N^1-Dimethyl-N^2-(4,5,6,7-tetrabromo-1*H*-benzimidazol-2-yl)-ethane-1,2-diamine (2d)

Compound **2d** was prepared according to[13].

3.1.6. N^1-(4,5,6,7-Tetrabromo-1-methyl-1*H*-benzimidazol-2-yl)-ethane-1,2-diamine (2e)

Similarly to described above from **1b**[13] and *n*-propanolic 1,2-etylenediamine, but the refluxing time was 6 h. Mp 224–226 °C, with decomp., 59%, from EtOH). ^{1}H NMR (Me_2SO-d_6) δ (ppm): 2.79 (t, 2H, H_2C, J = 6.4 Hz), 3.41 (t, 2H, H_2C, J = 6.5 Hz), 3.76 (s, 3H, H_3C) 6.70 (br s, 1H, H-N), 7.10 (br s, 2H, H_2N). UV (MeOH): 236 (34,500), 270 (7800), 314 (6000). MS (*m/z*): 510 (2, M^++4), 508 (6, M^++2), 506 (9, M^+), 504 (6, M^+−2), 502 (2, M^+−4), 476 (100, M^+−30), 463 (29, M^+−43). Anal. Calcd for $C_{10}H_{10}Br_4N_4$ (505.83): C, 23.75; H, 1.99; N, 11.08. Found: C, 23.66; H, 2.09; N, 10.90.

3.1.7. 2-Bromo-4,5,6,7-tetraiodo-1-methyl-1*H*-benzimidazole (4)

2-Bromo-4,5,6,7-tetraiodo-1*H*-benzimidazole (**3**)[14] (7.02 g, 10 mmol) was suspended in Me_2SO (20 ml). Then the 1 M ethanolic solution of EtONa (10 mmol, 10 ml) and 12 mmol (1.41 g) of CH_3I was added. The mixture was heated at 40 °C for 1 h. A yellow deposit formed was crystallized from DMF (5.20 g, 72%). Mp 275 °C (decomp.). ^{1}H NMR (Me_2SO-d_6) δ (ppm): 3.95 (s, 3H, H_3C). UV (MeOH): 239 (32,500), 282 (8400), 302 (8500). MS (*m/z*): 715 (M^+), 667 (M^+−48), 587 (M^+−120), 507 (M^+−208). Anal. Calcd for

$C_8H_3BrI_4N_2$ (714.65): C, 13.40; H 0.40; N, 3.90. Found: C, 13.44; H 0.38; N, 3.87.

3.1.8. N^1-(4,5,6,7-Tetraiodo-1*H*-benzimidazol-2-yl)-ethane-1,2-diamine (5a)

A mixture of **3** (1.0 g, 1.4 mmol) and 1,2-ethylenediamine (2.2 g, 37 mmol) in MeOEtOH (15 ml) was stirred and refluxed for 6 h. The mixture was evaporated to oil and 25 ml of methanol was added. A solid precipitate was filtered off and solution was deposited on silica gel column (2.5 × 12 cm). Column chromatography was performed with $CHCl_3$–MeOH (80:20, v/v) and then with $CHCl_3$–MeOH–triethylamine (80:20: 2). The product containing fractions were evaporated to dryness and the residue crystallized from EtOH–dioxane to give pale yellow powder (400 mg, 43%). Mp >240 °C (with decomp.). ^{1}H NMR (Me_2SO-d_6) δ (ppm): 3.11 (t, 2H, H_2C, J = 5.8 Hz), 3.58 (m, 2H, H_2C), 6.59 (t, 1H, H-N, J = 5.5), 7.74 (s, 1H, H-N), 8.58 (br s, 2H, H_2N), 10.7 (br s, 1H, H-benzim.). UV (MeOH): 239 (39,100), 265 (sh, 11,000), 310 (7900). MS (*m/z*): 680 (7, M^+), 650 (100, M^+-30), 523 (M^+-157). Anal. Calcd for $C_9H_8I_4N_4$ (679.8): C, 15.90; H 1.22; N, 8.19. Found: C, 15.87; H 1.21; N, 8.10.

3.1.9. N^1,N^1-Dimethyl-N^2-(4,5,6,7-tetraiodo-1*H*-benzimidazol-2-yl)-etane-1,2-diamine (5b)

The mixture of **4** (400 mg, 0.57 mmol) and *N,N*-dimethylaminoethylamine (1.0 g, 11.6 mmol) in MeOEtOH (12 ml) was stirred and refluxed for 10 h. This was diluted with water (36 ml) and left for 5 °C overnight. The precipitate was separated and crystallized from EtOH–dioxane to yield the yellow desired product (190 mg, 47%). Mp 243–246 °C. ^{1}H NMR (Me_2SO-d_6) δ (ppm): 2.28 (s, 6H, $(H_3C)_2N$), 2.54 (m, 2H, H_2C, overl. Me_2SO), 3.38 (q, 2H, H_2C, J = 5.3 Hz), 6.69 (t, H-N, J = 4.9 Hz), 10.8 (br s, 1H, H-benzim.). UV (MeOH): 239 (48,000), 265 (14,900), 308 (10,600). MS (*m/z*). 708 (6, M^+), 582 (100, M^+-126), 538 (24, M^+-170). Anal. Calcd for $C_{11}H_{12}I_4N_4$ (707.86): C, 18.67; H, 1.71; N, 7.91. Found: C, 18.56; H, 1.80; N, 7.79.

3.1.10. N^1-(4,5,6,7-Tetraiodo-1-methyl-1*H*-benzimidazol-2-yl)-ethane-1,2-diamine (6)

The mixture of **4** (1 mmol, 715 mg) and of 1,2-ethylenediamine (360 mg, 6 mmol) was stirred in 15 mL of Me_2SO at 70 °C during 45 min. The mixture becomes clear and then 10 ml of water was added. The formed precipitate was filtered off and crystallized from 2-methylbutanol to gave pale yellow crystalline powder (200 mg, 91%). Mp 180 °C. ^{1}H NMR (Me_2SO-d_6) δ (ppm): 2.80 (t, 2H, H_2C, J = 6.2 Hz), 3.38 (t, 2H, H_2C, J = 6.2 Hz), 3.59 (s, 3H, H_3C) 7.1 (br s, 1H, H–N), 7.10 (br s, 2H, H_2N). UV (MeOH): 238 (40,200), 265 (sh, 8500), 314 (8400). ES-MS (*m/z*): 694.7 (100, M^++H^+). Anal. Calcd for $C_{10}H_{10}I_4N_4$ (693.70): C, 17.32; H 1.51; N, 8.09. Found: C, 17.33; H 1.51; N, 8.01.

4. Biological evaluation

4.1. Materials and methods

4.1.1. Cell culture and reagents

The hormone-sensitive prostate cancer cell line LNCaP (ATCC: CRL-1740) was cultured in RPMI1640 (PAA Laboratories GmbH, Cölbe) supplemented with 10% fetal bovine serum at 37 °C in an atmosphere enriched with 5% CO_2.

4.1.2. Extraction of proteins

For harvesting, cells were scraped off the plate with a rubber policeman and sedimented together with floating cells by centrifugation (7 min, 4 °C, 400×*g*). The sedimented cells were washed with cold phosphate buffered saline (PBS) and lysed with two volumes of RIPA buffer (50 mM Tris/HCl, pH 8.0, 150 mM NaCl, 0.5% sodium desoxycholate, 1% Triton X-100, 0.1% sodium dodecylsulfate) supplemented with the protease inhibitor cocktail complete™ according to the instructions of the manufacturer (Roche Diagnostics, Mannheim, Germany). After lysis, cell debris was removed by centrifugation. The protein content was determined according to a modified Bradford method with the BioRad reagent dye (BioRad, München, Germany). Protein extracts were immediately used for Western blot analysis.

4.1.3. CK2 in vitro phosphorylation assay

To determine the activity of CK2, cells were treated with inhibitors or left untreated, lysed and the extracts were used in a kinase filter assay. In this assay we measured the incorporation rate of [γ^{32}P]-phosphate into the synthetic CK2 specific substrate peptide with the sequence RRRDDDSDDD. Twenty microliters of kinase buffer (50 mM Tris/HCl, pH 7.5, 100 mM NaCl, 10 mM MgCl2, 1 mM DTT) containing 30 μg proteins were mixed with 30 μl CK2 mix (25 mM Tris/HCl, pH 8.5, 150 mM NaCl, 5 mM $MgCl_2$, 1 mM DTT, 50 μM ATP, 0.19 mM substrate peptide) containing 10 μCi/500 μl [γ^{32}P]ATP. The mixture was spotted onto a P81 ion exchange paper. The paper was washed three times with 85 mM H_3PO_4. After treatment with ethanol the paper was dried and the Čerenkov-radiation was determined in a scintillation counter.

4.1.4. Cell viability assay

To assess the viability of the treated cells we used the MTT assay. In this assay the tetrazolium salt 3-[4,5-dimethylthiazole-2-yl]-2,5-phenyltetrazolium bromide (MTT) (Sigma–Aldrich Chemie GmbH) is reduced to a formazane that is further solubilized and measured spectrophotometrically. Briefly, the day before treatment 2 × 10^4 cells/500 μl were seeded into the wells of a 24-well plate. The cells were then incubated with the tested CK2 inhibitors. After the incubation, 50 μl aliquots of an MTT solution (5 mg/ml PBS) were added to all the wells. After an additional 1-h incubation the medium was discarded and 500 μl of a lysis solution were added to lyse the cells. Absorption of these lysates was determined at 595 nm after transferring 200 μl aliquots onto a 96-well plate.

4.1.5. SDS–polyacrylamide gel electrophoresis and Western blot analysis

Proteins were separated by SDS–polyacrylamide gel electrophoresis according to the procedure of Laemmli.[42] For Western blot analysis, proteins were transferred to PVDF membranes by tank blotting, using 20 mM Tris/HCl pH 8.3 supplemented with 150 mM glycine as the transfer buffer. The membranes were then blocked for 1 h at room temperature using 5% skimmed milk powder solution in PBS supplemented with 0.1% Tween-20 (PBS-Tween-20). The blocked membranes were incubated overnight at 4 °C with the polyclonal PARP antibody (1:1000) (Cell Signaling Technology, Frankfurt, Germany) in PBS-Tween-20 supplemented with 1% solution of defatted milk powder. Then the membranes were washed twice for 10 min with PBS-Tween-20 supplemented with 1% solution of defatted milk powder. Incubation with the peroxidase-coupled secondary antibody (anti-rabbit 1:30,000) followed for 1 h. Finally, the membranes were washed with PBS-Tween-20 (2 × 10 min). The blots were developed and visualized with the Lumilight system (Roche Diagnostics GmbH).

4.1.6. Statistical analysis

All experiments were repeated at least three times. Statistical analysis was performed with a *t*-test (by using Origin6.1) with $p < 0.05$ considered as significant. All non-significant results were marked with a number sign (#).

Acknowledgments

This study was supported by the Polish Ministry of Science and Higher Education grant #N N209 371439 (Z.K. and A.O.). Authors are indebted to Dr. S. J. Chrapusta of the Mossakowski Medical Research Center for his critical reading of the manuscript.

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