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Tetraiodobenzimidazoles are potent inhibitors of protein kinase CK2

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ABSTRACT

A series of novel iodinated benzimidazoles have been prepared by iodination of respective benzimidazole with iodine and periodic acid in sulfuric acid solution. Additionally several 2-substituted- and *N*-1-carboxymethyl-substituted derivatives of 4,5,6,7-tetraiodobenzimidazole (TIBI) were obtained. For sake of comparison, some new 4,5,6,7-tetrabromobenzimidazoles were also synthesized. The ability of the new compounds to inhibit protein kinase CK2 has been evaluated. The results show that 4,5,6,7-tetraiodobenzimidazoles are more powerful inhibitors of CK2 than their tetrabrominated analogs. Molecular modeling supports the experimental data showing that tetraiodobenzimidazole moiety fills better the binding pocket than respective tetrabromo and tetrachlorocompounds. To note that 4,5,6,7-tetraiodobenzimidazole (TIBI) is one of the most efficient CK2 inhibitors (K_i = 23 nM) described to date.

1. Introduction

The crucial role of protein kinases in nearly all cellular processes and the growing list of kinases whose dysregulation underlies pathological conditions (www.cellsignal.com/reference/kinase_disease.html) highlight the importance of kinases as drug targets.[1,2] Protein kinases have been actually implicated not only in oncology but also in a number of non-oncology indications including central nervous system disorders, autoimmune disease, post-transplant immunosuppression, osteoporosis, and several metabolic disorders like diabetes. Of special interest, among the large family of protein kinases, appears the regulation of kinases implicated in fundamental processes of cell life like proliferation, survival and apoptosis owing to the crucial commitment with the development and progression of cancer. The search of small molecules capable of down-regulating kinase activity was primarily addressed toward the nucleotide pocket of the kinase[3] exploiting structural differences in this region for molecular recognition.[4] However, due to the high conservation of the ATP-binding site, development of absolutely specific ATP-competitive inhibitors is hard to attain and the possibility to develop allosteric inhibitors is being explored.[4]

Protein kinase CK2, formerly known as casein kinase-2 or II, is a multifunctional serine/threonine protein kinase that specifically phosphorylates residues located in acidic sequences of more than 300 substrates most of which are phosphorylated also in vivo.[5] It is ubiquitously distributed, constitutively active and participates in a wide variety of cellular processes including gene expression, cell differentiation and proliferation and its activity has been associated with a number of pathologies including inflammation, infection, and tumorigenesis.[6–10] CK2 overexpression has been invariably observed in transformed tissues and it appears to represent an unfavorable prognostic marker in some types of cancer (e.g., prostate and lung carcinomas and in acute myeloid leukemia).[11,12] The nucleotide pocket of CK2, which can utilize both ATP and GTP as phosphate donors, displays unique features for being smaller than it is in most other kinases due to the presence of some unique bulky residues hampering the entrance of large molecules like staurosporine, a very powerful, highly promiscuous inhibitor of most protein kinases but weakly effective on CK2.[13,14] By contrast, smaller molecules which are not retained in the hydrophobic pocket of most kinases are firmly entrapped in that of CK2, resulting in efficient inhibition. The molecular structure of CK2 in mammals invariably consists of a heterotetrameric holoenzyme composed of two catalytic (α and/or α′) and two regulatory (β) subunits although emerging evidence would indicate specific roles also for the isolated subunits under certain circumstances.[15] Searching for CK2 inhibitors, just in the late 1980s polyhalogenated benzimidazoles were found to represent a valuable scaffold to effectively compete with ATP binding[16] and 4,5,6,7-tetrabromobenzotriazole (TBB) was later demonstrated to be one of the most powerful and selective cell permeable inhibitors of CK2.[17,18] The presence of four bromine atoms on the benzene ring of TBB is critical to fill the CK2 hydrophobic pocket adjacent to the ATP-binding site and to assure optimal apolar interactions with

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some bulky side chains, in particular those of Val66 and Ile174.[13,14] A direct correlation was also recently established for TBB derivatives between the log K_i and the variation in the accessible surface area upon binding on CK2.[13] From this point of view, it was known since longtime that the reduction of the number of bromine atoms or their replacement by less bulky halogens like chlorine and fluorine severely impairs the inhibitory efficiency.[16,19] However, the replacement of all four bromine atoms of the benzoimidazole or benzotriazole moiety by bulkier iodines was hard to attain and iodo-bromo or iodo-chloro benzimidazoles were unknown. Recently, structurally related tetraiodinated-isoindole derivatives were synthesized and found to inhibit protein kinase CK2 in an ATP-competitive manner with IC_{50} values ranging between 0.15 and 1.5 μM.[20] The highest efficiency documented in the literature for a CK2 inhibitor belongs to a class of pyrazolo-triazine derivatives reported to affect CK2 with K_i values in the nanomolar and subnanomolar range.[21,22] Regrettably, however, the experimental conditions for these assays were not detailed; this hampers reliable comparison with other inhibitors. In this paper the synthesis of a number of tetraiodinatedbenzimidazoles is described for the first time together with a structure–activity analysis in comparison with the corresponding tetrabrominated derivatives. In most cases the replacement of bromines by iodines increases the potency of the inhibitor reaching with some derivatives IC_{50} values in the low nanomolar range.

2. Results and discussion

2.1. Chemical syntheses

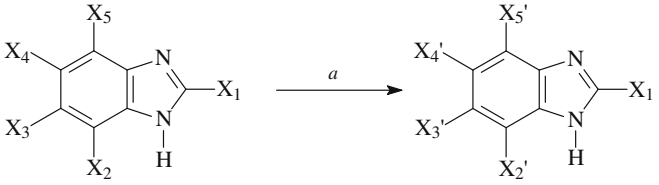
In this study, we made an effort to use an iodine-periodic acid system to obtain three series of iodinated heterocyclic compounds, namely iodinated benzimidazoles, benzotriazoles, and indazoles. We succeeded only in the case of benzimidazoles, while the other heterocycles tested, that is, benzotriazoles and indazoles, decomposed under the reaction conditions. The series of variously substituted benzimidazoles **1**–**13** that are commercially available or were obtained according to the previously described methods (e.g., see[23–28]) were used for the synthesis of the respective tetraiodinated derivatives **1a**–**13a** using, with minor modifications, the procedure described for aromatic compounds[29] (Scheme 1).

Of the obtained tetraiodinatedbenzimidazoles, 2-bromo-4,5,6,7-tetraiodobenzimidazole (**13a**) was found particularly useful for further modifications. This compound was converted to 2-mercaptoderivative **15** in the reaction with thiourea, whereas substitution of its bromine atom with ethanoloamine provided 2-ethanoloamino-4,5,6,7-tetraiodobenzimidazole (**14**), and heating of the compound with sodium acetate in acetic acid yielded the 2-oxoderivative (**17**) The sulfur atom in **15** can be easily alkylated by halogenated compounds; for example, we used bromoacetic acid to obtain the respective 2-carboxymethylthioderivative **16** (Scheme 2).

The ethyl esters of 1-carboxymethyl-substituted tetrahalogenated benzimidazoles (**18** and **19**) were obtained from 4,5,6,7-tetrabromo- (TBI) and 4,5,6,7-tetraiodobenzimidazole **1a** (TIBI) in the reaction with ethyl bromoacetate, using potassium carbonate as base catalyst. The esters were then subject to further modifications, such as alkaline hydrolysis—to provide the respective carboxylic acids **20** and **21.** Acetamido derivatives of 4,5,6,7-tetrahalogeneobenzimidazoles **22** and **23** were obtained by two synthetic procedures: (a) by alkylation of the respective benzimidazole with iodoacetamide, or (b) by amidation with methanolic ammonia. A nucleophilic substitution of above mentioned esters by methylamine or hydrazine yielded the respective *N*-methylamides **24** and **25** or hydrazides, **26** and **27** (Scheme 3).

2.2. Biological activity

Table 1 shows the inhibitory potency of all new derivatives toward protein kinase CK2. Whenever applicable, K_i values of new iodinated inhibitors are reported in comparison with the analogous brominated compounds either newly synthesized and tested here or drawn from the literature. As a main outcome of these determinations we observed firstly that the iodinated inhibitors behave in general more efficiently than the corresponding brominated ones with K_i values for CK2 which, in some cases, reach the low nanomolar range. In particular, the inhibition constants of tetraiodinated compounds **1a**, **7a**, **9a**, and **14** (23, 24, 19, and 27 nM, respectively) are among the lowest values ever reported in literature for CK2 inhibitors. Also these compounds, as it was previously found with similar polyhalogenated derivatives,[28] behave as competitive inhibitors with respect to the phosphodonor nucleotide,

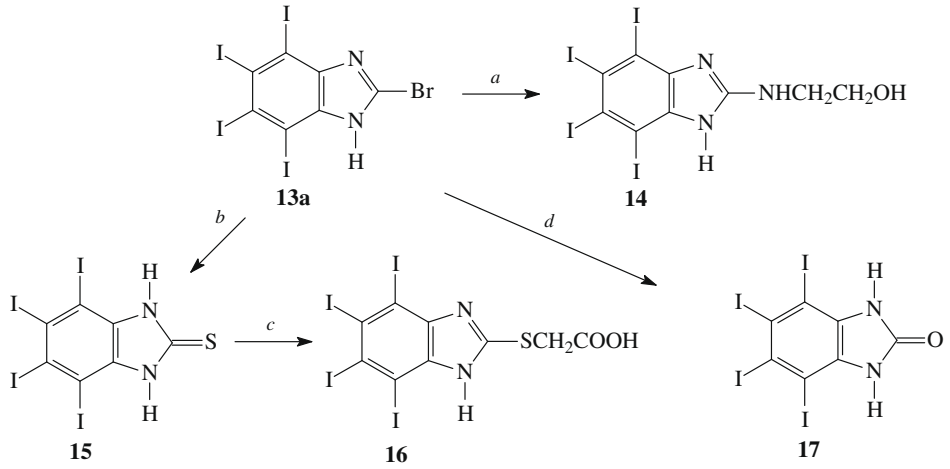


1-13 **1a - 13a**

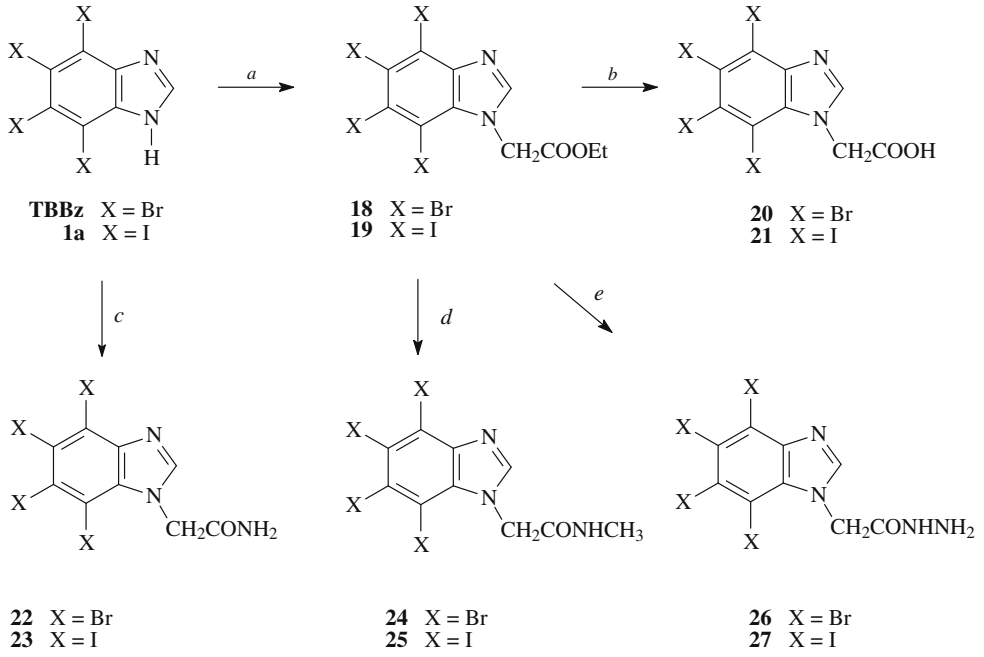
	X_1	X_2	X_3	X_4	X_5
1	H	H	H	H	H
2	H	H	H	Cl	H
3	H	H	H	Br	H
4	H	H	Cl	Cl	H
5	H	H	Cl	H	Cl
6	H	H	Br	H	Br
7	CH_3	H	H	H	H
8	CH_3	H	Cl	Cl	H
9	C_2H_5	H	H	H	H
10	CF_3	H	H	H	H
11	C_2F_5	H	H	H	H
12	Cl	H	H	H	H
13	Br	H	H	H	H

	X_1	X_2'	X_3'	X_4'	X_5'
1a	H	I	I	I	I
2a	H	I	I	Cl	I
3a	H	I	I	Br	I
4a	H	I	Cl	Cl	I
5a	H	I	Cl	I	Cl
6a	H	I	Br	I	Br
7a	CH_3	I	I	I	I
8a	CH_3	I	Cl	Cl	I
9a	C_2H_5	I	I	I	I
10a	CF_3	I	I	I	I
11a	C_2F_5	I	I	I	I
12a	Cl	I	I	I	I
13a	Br	I	I	I	I

Scheme 1. Reagents and conditions: (a) I_2, H_5IO_6, H_2SO_4, 3 h, 5–60 °C.



Scheme 2. Reagents and conditions: (a) 2-aminoethanol, EtOH; (b) thiourea, MeOEtOH; (c) $BrCH_2COOH$, K_2CO_3, 2-butanone, reflux, 3 h; (d) acetic acid, sodium acetate, reflux, 20 h.



Scheme 3. Reagents and conditions: (a) $BrCH_2COOEt$, K_2CO_3, 2-butanone, reflux, 3 h; (b) NaOH, EtOH–H_2O, 50 °C, 3 h; (c) ICH_2CONH_2, K_2CO_3, 2-butanone, reflux, 3 h; (d) H_2NCH_3, EtOH, rt, 2d; (e) $H_2NNH_2{\cdot}H_2O$, EtOH or MeOEtOH.

displaying a classical double-reciprocal plot allowing to calculate, in the case of 4,5,6,7-tetraiodobenzimidazole (**1a** or TIBI), a K_i value of 23 nM (Fig. 1A). Conversely, inhibition is non-competitive with respect to the peptide phosphoacceptor substrate (Fig. 1B) further supporting a mechanism of action implying the occupancy of the nucleotide binding pocket in competition with ATP. Moreover, the performance of compound **1a** allows to definitely rank the efficiency of halogens as substituents of the benzene ring, iodine being the best, followed by bromine (0.023 vs 0.3 μM by comparing, compounds **1a** and K17 (also termed TBI) of Table 1) which in turn proved to be about two orders of magnitude more effective than chlorine, 4,5,6,7-tetrachlorobenzimidazole (TCI) exhibiting a K_i value of 21 μM under comparable conditions.[30] This came not as a surprise considering the data already available for di-substituted benzimidazole derivatives in which fluorine was found to be the least effective.[16,19] However, a deeper analysis of the K_i values of tetraiodinated versus tetrabrominated compounds highlights that the presence of an additional substitution on the imidazole ring may affect not only the absolute potency of the inhibitor but also its relative efficacy with respect to the brominated homologue. In fact, all the four compounds bearing a substitution at position 1 of the imidazole ring (compounds **21**, **23**, **25** and **27** of Table 1) display a K_i value about one order of magnitude higher than that of derivative **1a** having no substitution at that position. Nevertheless, they are still 10-fold more potent than the correspondent brominated derivatives as it was observed in the case of the un-substituted compound **1a**. On the contrary, the added value of the presence of four iodine atoms on the benzene moiety appears to be variably affected by substitutions at position 2 of the imidazole ring. These latter compounds, with very few exceptions, display inhibition constants significantly higher and close to those of the corresponding brominated derivatives.

Table 1
Inhibition of protein kinase CK2 by iodinated benzimidazole derivatives (A) in comparison to respective brominated compounds (B)

A Iodinated compounds	K_i (μM)	B Brominated compounds	K_i (μM)	Ratio B/A	Imidazole substitution
1a (K88, TIBI)	0.023	K17[a,b] (TBI)	0.30	13.04	None
2a (K105)	0.16	—	—	—	None
3a (K104)	0.10	—	—	—	None
4a (K98)	0.46	—	—	—	None
5a (K106)	0.59	—	—	—	None
6a (K95)	0.075	—	—	—	None
7a (K92)	0.024	—	—	—	2-CH_3
8a (K99)	0.33	—	—	—	2-CH_3
9a (K93)	0.019	—	—	—	2-C_2H_5
10a (K89)	0.12	K10[a]	0.37	3.08	2-CF_3
11a (K94)	0.07	K11[a]	0.20	2.87	2-C_2F_5
12a (K103)	0.12	K21[a]	0.25	2.08	2-Cl
13a (K96)	0.09	K20[a]	0.37	4.11	2-Br
14 (K100)	0.027	K30[b]	0.14	5.18	2-$NH(CH_2)_2OH$
15 (K97)	0.05	K22[a]	0.20	4.00	2-SH
16 (K101)	0.054	K33[b]	0.12	2.22	2-SCH_2COOH
17 (K112)	0.05	K32[b]	0.18	3.60	2=O
21 (K107)	0.14	**20** (K68)	1.34	9.57	1-CH_2COOH
23 (K108)	0.13	**22** (K83)	1.45	11.15	1-CH_2CONH_2
25 (K109)	0.18	**24** (K84)	1.91	10.60	1-$CH_2CONHCH_3$
27 (K110)	0.16	**26** (K85)	1.51	9.40	1-$CH_2CONHNH_2$

Bold labels refer to compounds whose synthesis is described in this paper. The corresponding nomenclature (K) adopted in previous publications is also reported as a reference.
[a] Drawn from Andrzejewska et al.[27]
[b] Drawn from Pagano et al.[28]

2.3. Molecular modeling studies

Searching for a plausible structural explanation of the superiority of tetraiodinated derivatives as CK2 inhibitors, a molecular modeling approach was undertaken. Starting from the crystal structure of K17 in complex with *Zm*CK2α subunit (PDB code 2OXY)[13] we determined the binding mode of compound **1a** by a molecular docking strategy, in comparison with that of the tetrabromo (TBI, K17) and tetrachloro (TCI) homologues, using human CK2 as target protein (PDB code: 1JWH)[31] (Fig. 2). All three inhibitors lay inside the CK2 binding cleft exactly at the same position as K17 in *Zm*CK2α, revealing identical binding modes. However, compound **1a** is able to fill better the CK2 binding cleft due to the increased dimensions of iodine substituents with respect to bromine and chlorine. To better validate this hypothesis we calculated the percentage of human CK2 ATP-binding cleft occupancy (occupancy factor, OF) by the three analogs. The CK2 ATP-binding pocket volume was calculated as described in Section 3 by using the apo crystal structure 1JWH plus one conserved water molecule.[13] The OF values were calculated relative to the binding cleft and the inhibitor's volume. As also shown in Fig. 2, TCI shows a low binding cleft OF value (69%), and tetrabromine substitution is responsible of an increased occupancy up to 75% while the most potent inhibitor **1a** (TIBI), bearing the iodine substitution, fills by 86% the CK2 ATP-binding pocket. Almost identical conclusions can be drawn by comparing the increased hydrophobicity with the increased inhibitory potency of the tetrahalogenated derivatives. Moreover, iodine atoms of derivative **1a** are able to perform stronger halogen bonds with the binding cleft, in particular with the backbone carbonyls of Glu114 and Val116 of the hinge region.[13] In fact, even if all four halogen atoms are capable of acting as halogen bond donors, iodine usually forms the strongest interactions, followed by bromine and chlorine atoms, respectively.[32] The same arguments apply also to the other tetraiodobenzimidazole derivatives considered in this paper, all displaying in general an inhibitory efficiency higher than that of their brominated counterparts. Note, however, that a double mutant of CK2 in which two bulky residues of the hydrophobic pocket have been replaced by smaller ones (V66I174AA) undergoes a 70- and 16-fold reduced inhibition by compounds **1a** and TBI, respectively (data not shown). This corroborates the view that, a part from halogen bonds, occupancy and hydrophobic interactions play a crucial role in optimizing the performance of tetraiododerivatives.

3. Experimental

3.1. Chemical synthesis

3.1.1. General procedure

All chemicals and solvents were purchased from Sigma–Aldrich. Melting points (uncorr.) were measured in open capillary tubes on a Gallenkamp-5 melting point apparatus. Ultraviolet absorption spectra were recorded on Kontron Uvikon 940 spectrophotometer. ^{1}H NMR spectra (in ppm) were measured with Varian Gemini 200 MHz (or Varian UNITYplus 500 MHz) spectrometer at 298 K in DMSO-d_6 using tetramethylsilane as internal standard. Mass-spectra (70 eV) were obtained with AMD-604 (Intectra) spectrometer. Flash chromatography was performed with Merck silica gel 60 (200–400 mesh). Elemental analyses of the new compounds were within ±0.4% of the respective theoretical values.

3.1.2. General procedure for iodination of benzimidazole derivatives

The amounts of reagents used for the preparation of tetraiodobenzimidazoles were according below described procedure. For diiodo- or triiodo-derivatives amounts of iodine and periodic acid were, respectively, reduced.

Periodic acid (2.28 g, 10 mmol) was dissolved in concd H_2SO_4 (50 mL). Finely powdered iodine (10.1 g, 40 mmol) was added to the solution. After 20 min of stirring the homogenous mixture was placed in ice bath. Next, the respective benzimidazole (10 mmol) was slowly added (exothermic reaction). The reaction mixture was stirred at room temperature for 1 h and then has been heated to the final (60 °C) temperature. After next 2 h the mixture was poured onto crushed ice. The resulting dark solid was filtered off, added to the solution of sodium dithionite (25 mL, 10%) and

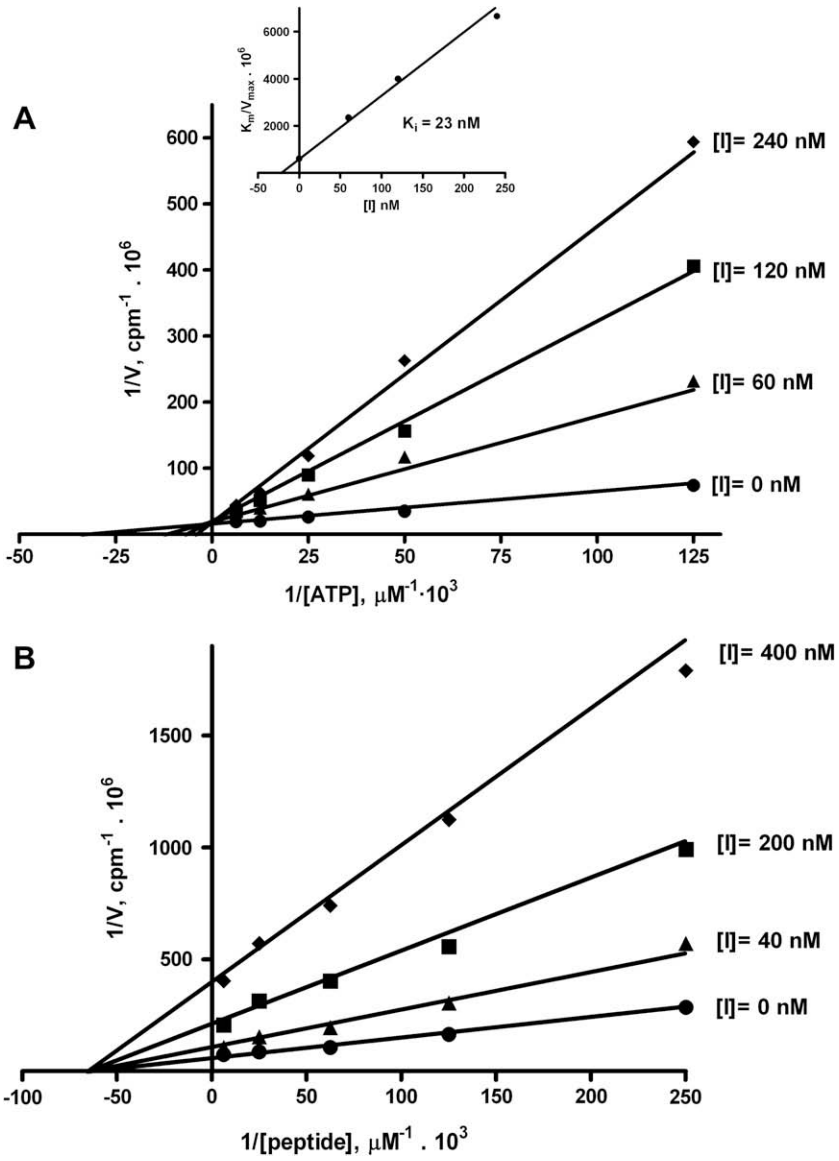


Figure 1. Double-reciprocal plot analysis of CK2 inhibition by compound **1a** (TIBI). CK2 activity was determined as described in Section 3 either in the absence or in the presence of the indicated fixed concentrations of inhibitor. In A the concentration of the peptide substrate was constant (100 μM) at increasing concentrations of ATP while in B the ATP concentration was constant (80 μM) at increasing concentrations of peptide substrate. The data represent the mean of triplicate experiments with SEM never exceeding 15%. The K_i of the inhibitor competitive with respect to ATP was then calculated by linear regression analysis of K_m/V_{max} versus inhibitor concentration plot shown in the inset of panel A.

stirred for 10 min. The crude product was dissolved in EtOH/2 M NaOH (1:1) and filtered. The pale yellow or green filtrate was brought to pH 4–5 with acetic acid to yield respective iodinated benzimidazole as yellow or yellowish chromatographically pure precipitate. For analysis and biological investigation small amounts were crystallized from EtOH, MeOEtOH, dioxane, DMF/dioxane or purified by alkalization and precipitation with acetic acid from organic solvent–water mixtures.

3.1.3. 4,5,6,7-Tetraiodo-*1H*-benzimidazole (1a)

From benzimidazole as described above: (mp 296–299 °C (decomp.) I_2 evol. >250 °C, 77%. A sample for analysis was crystallized from DMF/dioxane. UV (MeOH): 233 (28,600), 275 (9900), 295 (10,300). ^{1}H NMR (Me_2SO-d_6) δ (ppm): 8.21 (s, 1H, H–C), 12.85 (br s, 1H, H–N). MS *m/z*: 623 (9, M^++1), 622 (100, M^+), 495 (16, M^+−127), 368 (22, M^+−254). Anal. Calcd for $C_7H_2I_4N_2$ (621.72): C, 13.52; H, 0.32; N, 4.51. Found: C, 13.67; H, 0.44; N, 4.40.

3.1.4. 5-Chloro-4,6,7-triiodo-*1H*-benzimidazole (2a)

As described above starting from 5-chloro-benzimidazole. Mp 256–259 °C (with decomp.), 65%. A sample for analysis was crystallized from EtOH. UV (MeOH): 230 (23,300), 281 (14,300), 305 (sh, 5800). ^{1}H NMR (Me_2SO-d_6) δ (ppm): 8.32 (s, H–C), 13.05 (br s, H–N). MS *m/z*: 532 (33, M^++2), 530 (100, M^+), 440 (19, M^+−70), 438 (22, M^+−22), 403 (M^+−127). Anal. Calcd for $C_7H_2ClI_3N_2$ (530.27): C, 15.86; H, 0.38; N, 5.28. Found: C, 15.88; H, 0.40; N, 5.32.

3.1.5. 5-Bromo-4,6,7-triiodo-*1H*-benzimidazole (3a)

As described above starting from 5-bromo-benzimidazole. Mp 253–255 °C (with decomp.), 65%. A sample for analysis was crystallized from methoxyethanol. UV (MeOH): 236 (19,500), 282 (12,900), 305 (sh, 5600). ^{1}H NMR (Me_2SO-d_6) δ (ppm): 8.28 (s, 1H, H–C), 13.0 (br s, 1H, H–N). MS *m/z*: 576 (M^++1), 574 (M^+−1), 482 (32, M^+−93), 449 (15, M^+−126), 447 (M^+−128). Anal. Calcd

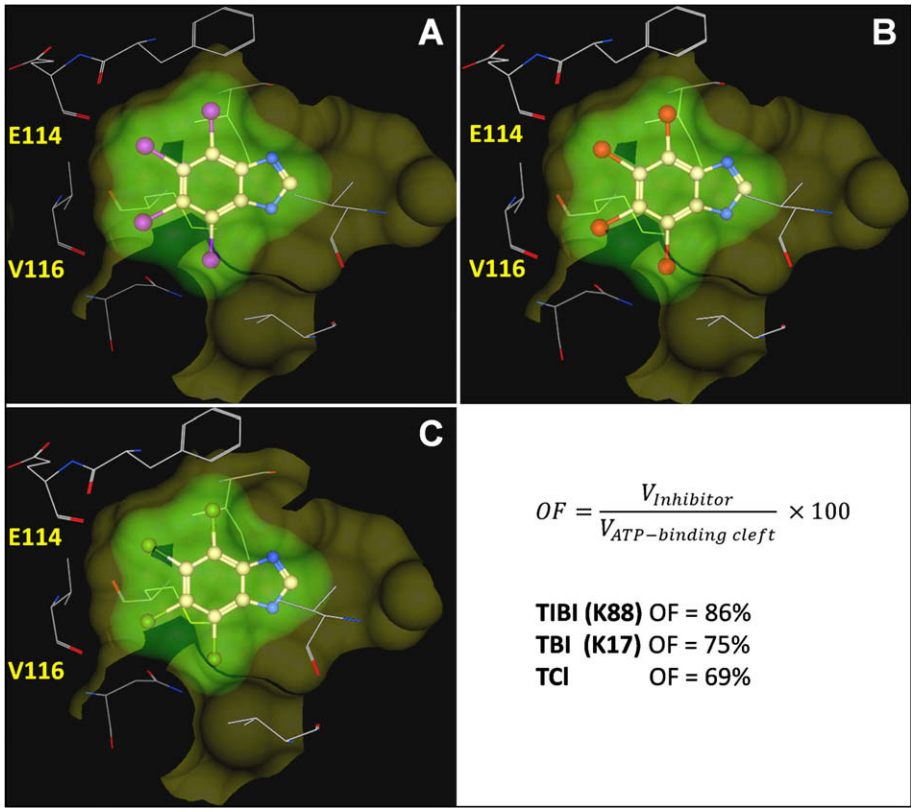


Figure 2. Molecular docking of the tetraiodinated (A), tetrabrominated (B), and tetrachlorinated (C) benzimidazole derivatives bound to the active site of protein kinase CK2. Computational analysis was performed as described in Section 3. Connolly's distribution surfaces of the three inhibitors inside the nucleotide pocket are represented in light green. The occupancy factor (OF) values, discussed in the text, are also indicated.

for $C_7H_2BrI_3N_2$ (574.72): C, 14.63; H, 0.35; N, 4.87. Found: C, 14.50; H, 0.42; N, 4.76.

3.1.6. 5,6-Dichloro-4,7-diiodo-*1H*-benzimidazole (4a)

As described above from 5,6-dichloro-benzimidazole. Mp 271–273 °C (with decomp.), 70%. A sample for analysis was crystallized from EtOH. UV (MeOH): 229 (23,000), 278 (19,000), 302 (9500). ^{1}H NMR (Me_2SO-d_6) δ (ppm): 8.40 (s, 1H, H–C), 13.2 (br s, 1H, H–N). MS *m/z*: 442 (10, M^++4), 440 (63, M^++2), 438 (100, M^+), 311 (M^+−127). Anal. Calcd for $C_7H_2Cl_2I_2N_2$ (438.82): C, 19.16; H, 0.46; N, 6.38. Found: C, 19.19; H, 0.55; N, 6.23.

3.1.7. 4,6-Dichloro-5,7-diiodo-*1H*-benzimidazole (5a)

As described above from 4,6-dichloro-benzimidazole. Mp 254–256 °C, 42%. A sample for analysis was crystallized from EtOH. UV (MeOH): 236 (22,500), 273 (8700), 302 (4400). ^{1}H NMR (Me_2SO-d_6) δ (ppm): 8.39 (s, 1H, H–C), 13.08 and 13.48 (br s, 1H, H–N). MS *m/z*: 440 (28, M^++1), 438 (100, M^+−1), 313 (12, M^+−126), 311 (13, M^+−128). Anal. Calcd for $C_7H_2Cl_2I_2N_2$ (438.82): C, 19.16; H, 0.46; N, 6.38: Found: C, 19.14; H, 0.44; N, 6.34.

3.1.8. 4,6-Dibromo-5,7-diiodo-*1H*-benzimidazole (6a)

As described above from 4,6-dibromo-benzimidazole. Mp 309–311 °C (with decomp.), 70%. A sample for analysis was crystallized from dioxane. UV (MeOH): 236 (21,300), 277 (11,700), 303 (sh, 4200). ^{1}H NMR (Me_2SO-d_6) δ (ppm): 8.34 (s, 1H, H–C), 13.09 and 13.23 (br s, 1H, H–N). MS *m/z*: 529 (47 (M^++2), 527 (100, M^+), 525 (48, M^+−2), 400 (M^+−127). Anal. Calcd for $C_7H_2Br_2I_2N_2$ (527.72): C, 15.93; H, 0.38; N, 5.31. Found: C, 15.90; H, 0.47; N, 5.22.

3.1.9. 2-Methyl-4,5,6,7,-tetraiodo-*1H*-benzimidazole (7a)

As described above from 2-methyl-benzimidazole: Mp 289–291 °C, 69%. A sample for analysis was crystallized from dioxane. UV (MeOH): 234 (29,300), 277 (10,300), 292 (10,500). ^{1}H NMR (Me_2SO-d_6) δ (ppm): 2.47 (s, 3H, H_3C), 12.5 (br s, 1H, H–N). MS *m/z*: 637 (10, M^++1), 636 (100, M^+), 509 (17 (M^+−127), 382 (M^+−254). Anal. Calcd for $C_8H_4I_4N_2$ (635.75): C, 15.11; H, 0.63; N, 4.41. Found: C, 15.09; H, 0.73; N, 4.27.

3.1.10. 5,6-Dichloro-4,7-diiodo-2-methyl-*1H*-benzimidazole (8a)

As described above from 5,6-dichloro-2-methylbenzimidazole. Mp 288–290 °C (with decomp.), 52%. A sample for analysis was crystallized from EtOH. UV (MeOH): 234 (23,500), 275 (18,300), 303 (8500). ^{1}H NMR (Me_2SO-d_6) δ (ppm): 2.52 (s, 3H, H_3C), 12.8 (br s, 1H, H–N). MS *m/z*: 456 (11, M^++4), 454 (56, M^++2), 452 (100, M^+), 311 (M^+−127). Anal. Calcd for $C_8H_4Cl_2I_2N_2$ (452.85): C, 21.22; H, 0.89; N, 6.19. Found: C, 21.15; H, 0.95; N, 6.08.

3.1.11. 2-Ethyl-4,5,6,7-tetraiodo-*1H*-benzimidazole (9a)

As described above from 2-ethyl-benzimidazole. Mp 261–262 °C (from EtOH), 68%. UV (MeOH): 237 (28,500), 277 (9700), 293 (11,100). ^{1}H NMR (Me_2SO-d_6) δ (ppm): 1.28 (t, 3H, H_3C, J = 7.6 Hz), 2.82 (q, 2H, H_2C, J = 7.6 Hz), 12.6 (br s, 1H, H–N). MS *m/z*: 637 (10, M^++1), 636 (100, M^+), 509 (17 (M^+−127), 382 (M^+−254). Anal. Calcd for $C_9H_6I_4N_2$ (649.76): C, 16.64; H, 0.93; N, 4.31. Found: C, 16.59; H, 0.99; N, 4.20.

3.1.12. 4,5,6,7-Tetraiodo-2-trifluoromethyl-*1H*-benzimidazole (10a)

As described above from 2-trifluoromethylbenzimidazole Mp 240–242 °C (with decomp.) (from MeOEtOH), 38%. UV (MeOH): 237 (35,200), 283 (10,000), 303 (9900). ^{1}H NMR (Me_2SO-d_6) δ (ppm): 14.4 (br s, H–N). MS *m/z*: 690 (5, M^++1), 689 (100, M^+), 563 (16, M^+−126), 436 (20, M^+−253). Anal. Calcd for $C_8HF_3I_4N_2$ (689.72): C, 13.93; H, 0.15; N, 4.31. Found: C, 13.78; H, 0.24; N, 4.20.

3.1.13. 2-Pentafluoroethyl-4,5,6,7-tetraiodo-*1H*-benzimidazole (11a)

As described above from 2-pentafluoroethylbenzimidazole. Mp 247–249 °C (with decomp.), 48% (from EtOH). UV (MeOH): 237 (36,100), 284 (11,000), 304 (11,200). ^{1}H NMR (Me_2SO-d_6) δ (ppm): 14.3 (br s, 1H, H–N). MS *m/z*: 741 (10, M^++1), 740 (100, M^+), 613 (16, M^+−127), 486 (20, M^+−254). Anal. Calcd for $C_9HF_5I_4N_2$ (739.73): C, 14.61; H, 0.14; N, 3.79. Found: C, 14.51; H, 0.22; N, 3.64.

3.1.14. 2-Chloro-4,5,6,7-tetraiodo-*1H*-benzimidazole (12a)

As described above from 2-chlorobenzimidazole. Mp 303–306 °C (with decomp., >250 °C I_2 evol.), 37%. A sample for analysis was crystallized from DMF/dioxane. UV (MeOH): 235 (35,200), 275 (12,200) 295 (12,000), 315 (sh). ^{1}H NMR (Me_2SO-d_6) δ (ppm): 13.7 (br s, 1H, H–N). MS *m/z*: 658 (42, M^++2), 656 (100, M^+), 529 (24, M^+−127), 402 (35, M^+−254). Anal. Calcd for $C_7HClI_4N_2$ (656.17): C, 12.81; H, 0.15; N, 4.00. Found: C, 12.75; H, 0.20; N, 3.92.

3.1.15. 2-Bromo-4,5,6,7-tetraiodo-*1H*-benzimidazole (13a)

As described above from 2-bromobenzimidazole: Mp 296–299 °C, (I_2 evol. >230 °C), 88%. A sample for analysis was crystallized from DMF/dioxane. UV (MeOH): 239 (28,100), 273 (12,100), 300 (12,700). ^{1}H NMR (Me_2SO-d_6) δ (ppm): 13.6 (br s, 1H, H–N). MS *m/z*: 701 (96, M^+), 699 (M^+−2), 574 (19, M^+−127), 572 (17, M^+−129). Anal. Calcd for $C_7HBrI_4N_2$ (700.62): C, 12.00; H, 0.14; N, 4.00. Found: C, 12.00; H, 0.19; N, 3.84.

3.1.16. 2-[(2-Hydroxyethyl)amino)]-4,5,6,7-tetraiodo-*1H*-benzimidazole (14)

To a mixture of **13a** (600 mg, 0.85 mmol) in MeOEtOH (15 mL), ethanoloamine (2 g, 2 mL, 33 mmol) was added. The reaction mixture was stirred and heated at 110–115 °C (bath temp) for 10 h. The reaction mixture was diluted with water (20 mL) and brought to pH 4–5. The precipitate was filtered and deposited on silica gel column (2 × 12 cm). Chromatography was performed with $CHCl_3$ (200 mL) and $CHCl_3$–MeOH (95:5, v/v). The product containing fractions were evaporated to dryness and the residue crystallized from dioxane–water to give pale yellow powder (320 mg, 55%); mp 249–251 °C. UV (MeOH): 244 (35,200), 274 (13,300), 315 (12,200). ^{1}H NMR (Me_2SO-d_6) δ (ppm): 3.40 (q, 2H, H_2C, J = 5.6 Hz), 3.57 (t, 2H, H_2C, J = 5.5 Hz), 5.01 (br s, 1H, HO), 6.45 (t, 1H, H–N–CH_2, J = 6.6 Hz), 10.9 (br s, 1H, H–N-imid.). MS *m/z*: 681 (5, M^++1), 680 (100, M^+), 662 (19, M^+−18), 637 (100, M^+−43), 510 (60, M^+−170). Anal Calcd for $C_9H_7N_3I_4O$ (680.79): C, 15.88; H, 1.04; N, 6.17. Found: 15.91; H, 1.15; N, 6.06.

3.1.17. 4,5,6,7-Tetraiodo-*1H,3H*-dihydro-*2H*-benzimidazole-2-thione (15)

To the stirred solution of **13a** (700 mg, 1 mmol) in MeOEtOH (25 mL) thiourea (156 mg, 2 mmol) was added. The reaction mixture was stirred and heated at 105–110 °C (bath temp) for 14 h. A precipitate formed was filtered, dissolved in EtOH/ 0.2 M aq. NaOH (1:1, v/v) and precipitated by addition of acetic acid to pH 4–5 to give as pale yellow chromatographically pure precipitate (520 mg, 80%); mp (decomp. >290 °C with I_2 evol.) (for analysis crystallized from MeOEtOH–water). UV (MeOH): 222 (18,600), 264 (35,450), 336 (20,500). ^{1}H NMR (Me_2SO-d_6) δ (ppm): 12.8 (br s, 1H, H–N). MS *m/z*: 655 (5, M^++2), 654 (9, M^++1), 653 (100, M^+), 527 (M^+−126), 400 (M^+−253). Anal Calcd for $C_7H_2N_2I_4S$ (653.78): C, 12.86; H, 0.31; N, 4.28. Found: C, 12.95; H, 0.42; N, 4.13.

3.1.18. (4,5,6,7-Tetraiodo-*1H*-benzimidazol-2-ylsulfanyl)-acetic acid (16)

The mixture of 2-mercapto-4,5,6,7-tetraiodo-1H-benzimidazole (751 mg, 1.15 mmol), K_2CO_3 (1 g), bromoacetic acid (400 mg, 2,87 mmol) in butanone (45 mL) was refluxed for 6 h. The insolubilities were filtered off and washed with 50 mL of hot butanone. The filtrate was evaporated to dryness. The residue was crystallized from dioxane; mp 274–276° (decomp. >240); yield 70% (4.98 g). UV (MeOH: 254 (13,100), 309 (12,300). ^{1}H NMR (Me_2SO-d_6) δ (ppm): 4.16 (s, 2H, H_2C), 12.9 (br s, 1H, HO). TOF MS ES$^+$: 712.6257 (M^++H^+, 712.6251). Anal. Calcd for $C_9H_4I_4N_2O_2S$ (711.83): C, 15.19; H, 0.57; N, 4.50. Found: C, 15.20; H, 0.51; N, 4.43.

3.1.19. 4,5,6,7-Tetraiodo-*1H,3H*-dihydro-*2H*-benzimidazole-2-one (17)

The stirred mixture of **13a** (700 mg, 1 mmol), anhyd sodium acetate (410 mg, 5 mmol) in anhyd acetic acid was refluxed for 20 h. The mixture was cooled, the formed precipitate was filtered and washed few times with EtOH–water (1:1). The product (608 mg, 95%) was chromatographically pure. For analytical and biological investigation small amount was crystallized from EtOH/dioxane; mp >310 °C (decomp.) UV (MeOH): 236 (36,900), 306 (6400). ^{1}H NMR (Me_2SO-d_6) δ (ppm): 11.04 (s, H–N-imid.). MS *m/z*: 639 (9, M^++1), 638 (100, M^+), 512 (25 (M^+−128), 384 (27, M^+−256). Anal Calcd for $C_7H_2N_2I_4O$ (637.72): C, 13.18; H, 0.32; N, 4.39. Found: C, 13.37; H, 0.38; N, 4.26.

3.1.20. (4,5,6,7-Tetrabromo-*1H*-benzimidazol-1-yl)-acetic acid ethyl ester (18)

Similarly to **16** butanone was used as a solvent at reflux condition for 5 h. Instead of bromoacetic acid ethyl ester of this acid was used (mp 199–200 °C, 80%). UV (MeOH): 228 (35,900), 264 (11,900), 272 (11,800), 302 (4100). ^{1}H NMR (Me_2SO-d_6) δ (ppm): 1.23 (t, 3H, H_3C, J = 7.1 Hz), 4.19 (q, 2H, H_2C, J = 7.1 Hz), 5.29 (s, 2H, H_2C–N), 8.32 (s, 1H, H–C). MS *m/z*: 524 (16, M^++4), 522 (65, M^++2), 520 (100, M^+), 518 (67, M^+−2), 516 (17, M^+−4). Anal. Calcd for $C_{11}H_8Br_4N_2O_2$ (519.81): C, 25.42; H, 1.55; N, 5.39. Found: C, 25.44; H, 1.56; N, 5.35.

3.1.21. (4,5,6,7-Tetraiodo-*1H*-benzimidazol-1-yl)-acetic acid ethyl ester (19)

Similarly to **18** butanone and bromoacetic acid ethyl ester were used at reflux condition for 5 h: (mp 224 °C, 77%). UV (MeOH): 236 (32,500), 279 (11,400), 299 (10,800). ^{1}H NMR (Me_2SO-d_6) δ (ppm): 1.23 (t, 3H, H_3C, J = 7.1 Hz), 4.19 (q, 2H, H_2C, J = 7.1 Hz), 5.29 (s, 2H, H_2C–N), 8.32 (s, 1H, H–C). MS *m/z*: 709 (13, M^++1), 708 (100, M^+), 635 (14, M^+−73), 553 (20, M^+−155). Anal. Calcd for $C_{11}H_8I_4N_2O_2$ (707.82): C, 18, 67; H, 1.14; N, 4.52. Found: C, 18.65; H, 1.18; N, 4.39.

3.1.22. (4,5,6,7-Tetrabromo-*1H*-benzimidazol-1-yl)-acetic acid (20)

The suspension of **18** (520 mg, 1 mmol) in solution of KOH (280 mg, 5 mmol) in ethanol/water (3:1, v/v, 16 mL) was stirred at room temperature for 3 h. After two hours the mixture became clear. The solution was brought to pH 2–3 with concd HCl. The white chromatographically pure precipitate was filtered and washed with water (390 mg, 79%). A sample for analysis and biological purposes was crystallized from dioxane–water gave white crystals with no melting point until 300 °C (melt. and decomp. >315 °C). UV (MeOH): 228 (28,500), 265 (9800), 272 (9900), 302 (3500). ^{1}H NMR (DMSO-d_6): 5.36 (s, CH_2), 8.32 (s, H–C), 13.4 (br s, OH). MS *m/z*: 496 (16, M^++4), 494 (64, M^++2), 492 (100, M^+), 490 (66, M^+−2), 488 (M^+−4), 447 (70, M^+−45). Anal. Calcd for $C_9H_4Br_4N_2O_2$ (491.76): C, 21.98; H, 0.82; N, 5.70. Found: C, 22.15; H, 0.95; N, 5.51.

3.1.23. (4,5,6,7-Tetraiodo-*1H*-benzimidazol-1-yl)-acetic acid (21)

Similarly to **20** starting from **19**, however stirred at 50 °C: (mp 250–252 °C (decomp. with gas evol.), 70%). UV (MeOH): 238

(32,400), 279 (9100), 298 (8400). ^{1}H NMR (Me_2SO-d_6) δ (ppm): 5.21 (s, H_2C), 8.37 (s, H–C), 13.3 (br s, OH). TOF MS ES$^+$: 680.6526 (M^++H^+, 680.6530) Anal. Calcd for $C_9H_4I_4N_2O_2$ (679.76): C, 15.90; H, 0.59; N, 4.12. Found: C, 15.91; H, 0.58; N, 4.09.

3.1.24. 2-(4,5,6,7-Tetrabromo-*1H*-benzimidazol-1-yl)-acetamide (22)

Method A: The suspension of **19** (260 mg, 0.5 mmol) in methanolic ammonia (10 mL, saturated at 0 °C, diluted with 10 mL MeOH) was stirred at room temperature for one week. The precipitate was filtered off and crystallized from DMF/dioxane gave white powder (195 mg, 80%). Mp >325 °C.

Method B: To the stirred and refluxed mixture of **TBI** (260 mg, 0.5 mmol), anhyd K_2CO_3 (600 mg) in dioxane iodoacetamide (130 mg, 0.7 mmol) was added. The stirring and reflux was continued for 2 h. The mixture was chilled and water (20 mL) was added. The precipitate was filtered off and crystallized from DMF/dioxane gave white powder (180 mg, 73%). Mp >325 °C. UV (MeOH): 230 (30,100), 267 (10,800), 273 (10,400), 302 (3500). ^{1}H NMR (Me_2SO-d_6) δ (ppm): (s, H_2C), 7.37 and 7.72 (2s, H_2N), 8.43 (s, HC). MS *m/z*: 495 (6), 493 (22, M^++4), 491 (36, M^++2), 489 (26, M^+−2), 487 (7, M^+−4), 447 (65, M^+−44), 410 (100, M^+−81). Anal. Calcd for $C_9H_5Br_4N_3O$ (490.78): C, 22.03; H, 1.03; N, 8.58. Found: C, 21.88; H, 1.12; N, 8.43.

3.1.25. 2-(4,5,6,7-Tetraiodo-*1H*-benzimidazol-1-yl)-acetamide (23)

Similarly to **22** (method B) starting from **19**: (mp 291–301 °C (decomp. with I_2 evol.), 65%). UV (MeOH): 236 (29,100), 278 (8100) 300 (8400). ^{1}H NMR (Me_2SO-d_6) δ (ppm): 5.05 (s, H_2C), 7.34 and 7.68 (2s, H_2N), 8.29 (s, H–C). MS *m/z*: 679 (9, M^+), 622 (48, M^+−57), 553 (49, M^+−126), 496 (100, M^+−183). Anal. Calcd for $C_9H_5I_4N_3O$ (678.78): C, 15.93; H, 0.74; N, 6.19. Found: C, 15.99; H, 0.77; N, 6.13.

3.1.26. *N*-Methyl-2-(4,5,6,7-tetrabromo-*1H*-benzimidazol-1-yl)-acetamide (24)

The suspension of **18** (260 mg, 0.5 mmol) in ethanolic methylamine (25 mL, 20%) was stirred at room temperature for 2 d. The precipitate was filtered off and crystallized from dioxane gave pure white crystalline powder (230 mg, 91%); mp 322–325 °C (with dec.). UV (MeOH): 232 (27,900), 267 (10,800), 273 (10,600), 302 (3600). ^{1}H NMR (Me_2SO-d_6) δ (ppm): 2.63 (d, 3H, H_3C, J = 4.6 Hz), 5.16 (s, 2H, H_2C), 8.12 (q, 1H, H–N, J = 4.7 Hz), 8.43 (s, 1H, H–C). MS *m/z*: 507 (9, M^++2), 505 (13, M^+), 503 (10, M^+−2), 426 (100, M^+−79). Anal. Calcd for $C_{10}H_7Br_4N_3O$ (504.80): C, 23.79; H, 1.40; N, 8.32. Found: C, 23.64; H, 1.52; N, 8.29.

3.1.27. *N*-Methyl-2-(4,5,6,7-tetraiodo-*1H*-benzimidazol-1-yl)-acetamide (25)

Similarly to described for **23** however stirred for 3 d: (mp 302–304 °C (decomp. with I_2 evol.) 83%). UV (MeOH): 237 (29,400), 278 (8200), 299 (7500). ^{1}H NMR (Me_2SO-d_6) δ (ppm): 3.29 (d, 3H, H_3C, J = 4.6 Hz), 5.05 (s, 2H, H_2C), 8.06 (q, 1H, H–N, J = 4.7 Hz), 8.28 (s, 1H, H–C). MS *m/z*: 693 (31, M^+), 566 (100, M^+−127), 439 (41, M^+−254). Anal. Calcd for $C_{10}H_7I_4N_3O$ (692.80): C, 17, 34; H, 1.02; N, 6.07. Found: C, 17.30; H, 0.99; N, 6.01.

3.1.28. (4,5,6,7-Tetrabromo-*1H*-benzimidazol-1-yl)-acetic acid hydrazide (26)

The mixture of **18** (320 mg, 0.6 mmol) and hydrazine hydrate (500 mg, 10 mmol) in ethanol (15 mL) was stirred and refluxed for 2 h. The mixture was chilled and left for the crystallization. The white chromatographically pure precipitate was obtained (220 mg, 72%). A sample for analysis was crystallized from dioxane. Mp >305 °C (decomp.). UV (MeOH): 232 (30,600), 267 (11,800), 273 (11,600), 302 (3800). ^{1}H NMR (Me_2SO-d_6) δ (ppm): 4.29 (br s, H_2N), 5.50 (s, H_2C), 8.44 (s, H–N), 9.37 (s, H–C). MS *m/z*: 510 (12, M^++4), 508 (46, M^++2), 506 (71, M^+), 504 (47, M^+−2), 502 (12, M^+−4), 425 (100, M^+−126). Anal. Calcd for $C_9H_6Br_4N_4O$ (505.79): C, 21.37; H, 1.20; N, 11.08. Found: C, 21.44; H, 1.30; N, 11.26.

3.1.29. (4,5,6,7-Tetraiodo-*1H*-benzimidazol-1-yl)-acetic acid hydrazide (27)

Similarly to **26**, however instead ethanol methoxyethanol at 100 °C (bath temp) was used. Mp 264–266 (decomp. with gas evol.), 75%. A sample for analysis was crystallized from dioxane/ethanol. UV (MeOH): 236 (40,100), 282 (10,500), 299 (10,300). ^{1}H NMR (Me_2SO-d_6) δ (ppm):4.23 (br s, 2H, H_2N), 5.05 (s, 2H, H_2C), 8.27 (s, 1H, H–N), 9.31 (s, 1H, H–C). MS *m/z*: 694 (7, M^+), 621 (100, M^+−73), 496 (87, M^+−198). Anal. Calcd for $C_9H_6I_4N_4O$ (693.79): C, 15.58; H, 0.87; N, 8.08. Found: C, 15.55; H, 0.89, 8.05.

4. Biological evaluation

4.1. Materials

4.1.1. Phosphorylation

CK2 activity was tested in a final volume of 25 μl containing 50 mM Tris–HCl pH 7.5, 100 mM NaCl, 12 mM $MgCl_2$, 100 μM synthetic peptide substrate RRRADDSDDDDD and 0.02 mM [γ-^{33}P-ATP] (500–1000 cpm/pmol), unless otherwise indicated, and incubated for 10 min at 37 °C. Native CK2 purified from rat liver (0.5–1 pmol) was usually the phosphorylating enzyme. Assays were stopped by addition of 5 μl of 0.5 M orthophosphoric acid before spotting aliquots onto phosphocellulose filters. Filters were washed in 75 mM phosphoric acid (5–10 mL/each) four times then once in methanol and dried before counting. Inhibition constants were determined from Lineweaver–Burk double-reciprocal plots of data obtained in experiments run at fixed concentration of peptide substrate and at increasing concentrations of ATP either in the absence or in the presence of variable concentrations of inhibitor. Alternatively inhibition constants were also deduced from the IC_{50}/K_i Cheng–Prusoff relationship.[33]

4.1.2. Molecular modeling

The human CK2α subunit was retrieved from the PDB (code 1JWH)[31] and processed in order to remove the ligands and all water molecules except to the one conserved.[13] Hydrogen atoms were added to the protein structure using standard geometries with the MOE program.[34] To minimize contacts between hydrogens, the structure were subjected to Amber99 force-field minimization until the rms (root mean square) of conjugate gradient was <0.1 kcal mol^{-1} Å^{-1} (1 Å = 0.1 nm) keeping the heavy atoms fixed at their crystallographic positions. To strictly validate the model generated and to calibrate the high-throughput docking protocol, a small database of known CK2 inhibitors was built and a set of docking runs was performed. After the calibration phase, semiflexible ligand-docking steps with three different programs, MOEDOCK,[34] GLIDE,[35] and GOLD,[36] were performed essentially as described previously.[37] Human CK2α ATP-binding pocket volume was calculated starting from the apo crystal structure 1JWH plus one conserved water molecule; firstly a set of MOE dummy atoms defining the active site was created, secondly the volume was calculated using a water molecule probe.[34] On the other hand **1a**, K17 and TCI structures and volumes were calculated with Spartan 08, using a Semi-empirical Quantum Mechanical Methods AM1.[38]

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References and notes

1. Cohen, P. *Nat. Rev. Drug Disc.* **2002**, *1*, 309.
2. Hopkins, A. L.; Groom, C. R. *Nat. Rev. Drug Disc.* **2002**, *1*, 727.
3. Kéri, G.; Őrfi, L.; Erős, D.; Hegymegi-Barakonyi, B.; Szántai-Kis, C.; Horváth, Z.; Wáczek, F.; Marosfalvi, J.; Szabadkai, J.; Pató, J.; Greff, Z.; Hafenbradl, D.; Daub, H.; Müller, G.; Klebl, B.; Ullrich, A. *Curr. Signal Transduct. Ther.* **2006**, *1*, 67.
4. Knight, Z. A.; Shokat, K. M. *Chem. Biol.* **2005**, *12*, 621.
5. Meggio, F.; Pinna, L. A. *FASEB J.* **2003**, *17*, 349–368.
6. Guerra, B.; Issinger, O.-G. *Curr. Med. Chem.* **2008**, *15*, 1871.
7. Singh, N. N.; Ramji, D. P. *J. Mol. Med.* **2008**, *86*, 887–897.
8. Duncan, J. S.; Litchfield, D. W. *Biochim. Biophys. Acta* **2008**, *1784*, 33.
9. Pinna, L. A. *J. Cell Sci.* **2002**, *115*, 3873.
10. Pinna, L. A. *Acc. Chem. Res.* **2003**, *36*, 378.
11. Laramas, M.; Pasquier, D.; Filhol, O.; Ringeisen, F.; Descotes, J. L.; Cochet, C. *Eur. J. Cancer* **2007**, *43*, 928.
12. Kim, J. S.; Eom, J. I.; Cheong, J. W.; Choi, A. J.; Lee, J. K.; Yang, W. I.; Min, Y. H. *Clin. Cancer Res.* **2007**, *13*, 1019.
13. Battistutta, R.; Mazzorana, M.; Cendron, L.; Bortolato, A.; Sarno, S.; Kazimierczuk, Z.; Zanotti, G.; Moro, S.; Pinna, L. A. *ChemBioChem* **2007**, *8*, 1804.
14. Mazzorana, M.; Pinna, L. A.; Battistutta, R. *Mol. Cell. Biochem.* **2008**, *316*, 57–62.
15. Litchfield, D. W. *Biochem. J.* **2003**, *369*, 1–15.
16. Meggio, F.; Shugar, D.; Pinna, L. A. *Eur. J. Biochem.* **1990**, *187*, 89–94.
17. Sarno, S.; Reddy, H.; Meggio, F.; Ruzzene, M.; Davies, S. P.; Donella-Deana, A.; Shugar, D.; Pinna, L. A. *FEBS Lett.* **2001**, *496*, 44.
18. Zien, P.; Duncan, J. S.; Skierski, J.; Bretner, M.; Litchfield, D. W.; Shugar, D. *Biochim. Biophys. Acta* **2005**, *1754*, 271.
19. Szyszka, R.; Grankowski, N.; Felczak, K.; Shugar, D. *Biochem. Biophys. Res. Commun.* **1995**, *208*, 418.
20. Golub, A. G.; Yakovenko, O. Ya.; Prykhod'ko, A. O.; Lukashov, S. S.; Bdzhola, V. G.; Yarmoluk, S. M. *Biochim. Biophys. Acta* **2008**, *1784*, 143.
21. Nie, Z.; Perretta, C.; Erickson, P.; Margosiak, S.; Almassy, R.; Lu, J.; Averill, A.; Yager, K. M.; Chu, S. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4191.
22. Nie, Z.; Perretta, C.; Erickson, P.; Margosiak, S.; Lu, J.; Averill, A.; Almassy, R.; Chu, S. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 619.
23. Phillips, M. A. *J. Chem. Soc.* **1928**, 2393.
24. Smith, W. T.; Steinle, E. C. *J. Am. Chem. Soc.* **1953**, *75*, 1292.
25. Büchel, K. H. *Z. Naturforsch, B* **1970**, *25*, 934.
26. Hoover, J. R. E.; Day, A. R. *J. Am. Chem. Soc.* **1955**, *77*, 4324.
27. Andrzejewska, M.; Pagano, M. A.; Meggio, F.; Brunati, A. M.; Kazimierczuk, Z. *Bioorg. Med. Chem.* **2003**, *11*, 3997.
28. Pagano, M. A.; Andrzejewska, M.; Ruzzene, M.; Sarno, S.; Cesaro, L.; Bain, J.; Elliott, M.; Meggio, F.; Kazimierczuk, Z.; Pinna, L. A. *J. Med. Chem.* **2004**, *47*, 6239.
29. Mattern, D. L. *J. Org. Chem.* **1984**, *49*, 3051.
30. Zien, P.; Bretner, M.; Zastapilo, K.; Szyszka, R.; Shugar, D. *Biochem. Biophys. Res. Commun.* **2003**, *306*, 129.
31. Niefind, K.; Guerra, B.; Ermakowa, I.; Issinger, O.-G. *EMBO J.* **2001**, *20*, 5320.
32. Politzer, P.; Lane, P.; Concha, M. C.; Ma, Y.; Murray, J. S. *J. Mol. Model.* **2007**, *13*, 305.
33. Burlingham, T. B.; Widlanski, T. S. *J. Chem. Ed.* **2003**, *80*, 214.
34. Molecular Operating Environment (MOE 2008.10), C. C. G., 1255 University St., Suite 1600, Montreal, Quebec, Canada, H3B 3X3.
35. Schrodinger, I.; Schrodinger: Portland, OR, 2001.
36. Jones, G.; Willett, P.; Glen, R. C.; Leach, A. R.; Taylor, R. *J. Mol. Biol.* **1997**, *267*, 727.
37. Cozza, G.; Bonvini, P.; Zorzi, E.; Poletto, G.; Pagano, M. A.; Sarno, S.; Donella-Deana, A.; Zagotto, G.; Rosolen, A.; Pinna, L. A.; Meggio, F.; Moro, S. *J. Med. Chem.* **2008**, *49*, 2363.
38. Wavefunction: Irvine, CA USA, 2002.