Optimization of Protein Kinase CK2 Inhibitors Derived from 4,5,6,7-Tetrabromobenzimidazole

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Casein kinase 2 (CK2) is a ubiquitous, essential, and highly pleiotropic protein kinase whose abnormally high constitutive activity is suspected to underlie its pathogenic potential in neoplasia and infective diseases. Thus, CK2 inhibitors designed to dissect the signaling pathways affected by this kinase, in perspective, may give rise to pharmacological tools. One of the most successful CK2 inhibitors is TBB (4,5,6,7-tetrabromobenzotriazole). Here we show that its inhibitory properties can be markedly improved by generating adducts in which N^2 is replaced by a carbon atom bound to a variety of polar functions. The most efficient inhibitor is 4,5,6,7-tetrabromo-2-(dimethylamino)benzimidazole (**2c**) followed by the methylsulfanyl (**8**), isopropylamino (**2e**), and amino (**2a**) congeners. All these compounds display K_i values <100 nM (40 nM in the case of **2c**). **2c** induces apoptosis of Jurkat cells more readily than TBB (DC_{50} value 2.7 vs 17 μM) and, unlike TBB, it does not display any side effect on mitochondria polarization up to 10 μM concentration. Molecular modeling of the CK2−**2c** complex, based on the crystal structure of the CK2−TBB complex suggests that a number of additional apolar contacts between its two methyl groups and hydrophobic residues nearby could account for its superior inhibitory properties. Consequently, **2c** is even more susceptible than TBB to mutations of the unique hydrophobic residues V66 and/or I174 to alanine. We propose to adopt **2c** as first choice CK2 inhibitor instead of TBB, especially for in cell studies.

Introduction

Protein kinases, catalyzing the transfer of the γ phosphate of ATP (and eventually GTP) to serine, threonine, and tyrosine residues of protein substrates, make up one of the largest superfamilies of enzymes, with more than 500 members encoded by the human genome.[1] They play a central role in controlling nearly all cellular functions, with special reference to signal transduction pathways. Protein kinases generally are silent enzymes, which are turned on in response to specific stimuli. Often unscheduled and/or constitutive activation of protein kinases, resulting from mutations and genetic rearrangements, underlies pathologies, with special reference to neoplasia.[2] Consequently, the development of efficient and selective inhibitors of protein kinases not only represents a powerful tool for unraveling the functional implication of individual kinases, but in many cases, it may also provide compounds with therapeutic potential. Nowadays protein kinases represent the second most important targets for therapeutical agents.[3] Several protein kinase inhibitors are already in advanced clinical trials or even in clinical practice to cure tumors and other diseases.[3,4] Especially telling in this respect is the example of imatinib (also termed STI-571 or Gleevec), an inhibitor of Brc-Abl tyrosine kinase fusion protein that is proving extremely successful to cure chronic myelogenous leukaemia.[5]

Given these premises, one might argue that those few pleiotropic protein kinases that are constitutively active under normal conditions are not useful targets like the majority of the other members of the family. This may be not the case, however, as illustrated by the example of casein kinase 2 (CK2).[6] CK2 probably is the most pleiotropic protein kinase known, with more than 300 protein substrates already identified,[7] a feature which might, at least partly, account for its lack of strict control over catalytic activity. Its catalytic subunits (α and/or α′) are in fact constitutively active either with or without the regulatory β-subunits, which appear to play a role in targeting and substrate recruiting, rather than controlling catalytic activity. Although constitutively active CK2 is ubiquitous, essential, and implicated in a wide variety of important cell functions,[8] evidence has been accumulating that its catalytic subunits may behave as oncogenes,[9−12] consistent with the observation that they display an antiapoptotic effect in prostate cancer cell lines.[13] Actually, they are invariably more abundant in tumors as compared to normal tissues and their overexpression is causative of neoplastic growth in animal and cellular models presenting alterations in the expression of cellular oncogenes or tumor suppressor genes.[14] These data, in conjunction with the observation that many viruses exploit CK2 as phospho-

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rylation agent of proteins essential to their life cycle, are raising interest in CK2 as a potential target for antineoplastic and/or anti-infectious drugs.

Consequently, an increasing number of reports dealing with the development and usage of CK2 inhibitors recently appeared in the literature. In the case of five inhibitors, all competitive with respect to ATP [emodin, 1,8-dihydroxy-4-nitro-xanthen-9-one (MNX), 1,4-diamino-5,8-dihydroxyanthraquinone (DAA), TBB (4,5,6,7-tetrabromobenzotriazole), and 5-oxo-5,6-dihydroxyindolo-[1,2-*a*]quinazolin-7-ylacetic acid (IQA)], the structure in complex with the catalytic subunit of maize CK2 (whose ATP binding site is nearly identical to that of its human counterpart) has been solved.[15−18] Although these inhibitors belong to different classes of chemical compounds (three are related to hydroxyantraquinones, one is a brominated benzotriazole, and one is a quinazoline derivative), all are accommodated in a hydrophobic pocket which in CK2 is smaller than in the majority of protein kinases, owing to the presence of unique bulky residues, notably Val/Ile66, Ile174, and Met 163. This may well account for the selectivity of these inhibitors, which, with the partial exception of emodin, are more effective on CK2 than they are on a panel of >30 different protein kinases.[18−20] Among these inhibitors, TBB proved especially successful for in cell and in vivo studies, notably to highlight the antiapoptotic role of CK2[21] and its implication in the potentiation of Bcr/Abl mediated cell proliferation.[22]

While the selectivity of TBB is remarkable,[19] its potency, though higher than that of most other CK2 inhibitors, is not outstanding: in vitro it inhibits purified CK2 with IC_{50} values around 1 μM, while the concentration required to induce half-maximal apoptosis of Jurkat cells is 17 μM.

It is possible that the relatively low potency of TBB as well as of other CK2 inhibitors, as opposed to their fairly high specificity, is due to their mode of binding, which is almost exclusively based on apolar contacts with unique hydrophobic side chains, while polar interactions, quite common with potent inhibitors of other kinases, are nearly absent.

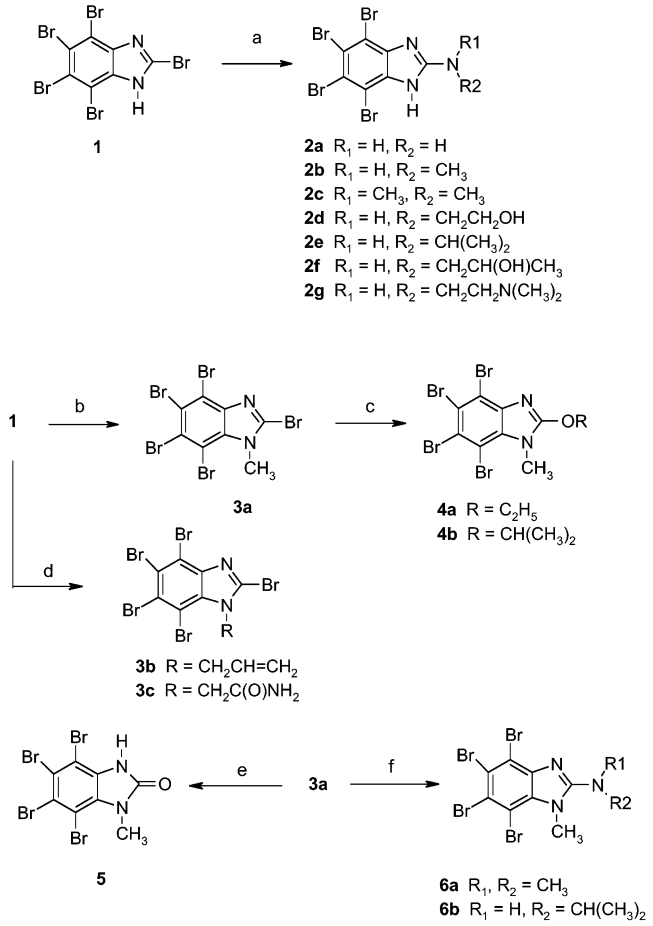
On the basis of this assumption, we started a study aimed at developing TBB derivatives in which the tetrabromobenzene moiety responsible for selectivity is conserved, while the triazole ring is replaced by an imidazole one derivatized with substituents that could provide interactions with polar side chains of the kinase. Here we report on such a study and we describe a TBB analogue whose potency, both in vitro and in vivo, is markedly higher than that of TBB.

Results and Discussion

Chemistry. A series of congeners of the tetrabrominated benzimidazole has been synthesized using 2,4,5,6,7-pentabromo-1*H*-benzimidazole (**1**) as a starting compound.[23]

The reaction of **1** with ammonia and a variety of amines, including monomethyl-, dimethyl-, ethanol-, isopropyl-, 2-hydroxypropyl-, and dimethylaminoethylamine, provided the corresponding 2-modified 4,5,6,7-tetrabromo-1*H*-benzimidazoles **2a**−**g** (Scheme 1). The substitution of **1** with either alkoxylates or hydroxide anion was unsuccessful even under more harsh conditions (autoclave, 110−120 °C). Methyl, allyl, and acetamidyl groups were introduced in position N^1 of **1** by alkylation with dimethyl sulfate, allyl bromide, or iodoacetamide in alkaline medium to give **3a**−**c**, respectively. The *N*-methylation allowed the substitution of bromine in position 2 by heating **3a** with ethanolic ethanolate and 2-propanolic 2-propanolate solutions, producing 2-alkoxy derivatives **4a** and **4b**, respectively. Alkaline hydrolysis of **3a** yielded 1-methyl-4,5,6,7-tetrabromo-1*H*,3*H*-benzimidazol-2-one (**5**). The *N*-methyl-substituted benzimidazole **3a** was also aminated with dimethylamine and isopropylamine to give, correspondingly, **6a** and **6b**. Of the here synthesized tetrabromobenzimidazoles, only **2a** has been mentioned in the literature as a destruction product of benomyl in fruits.[24]

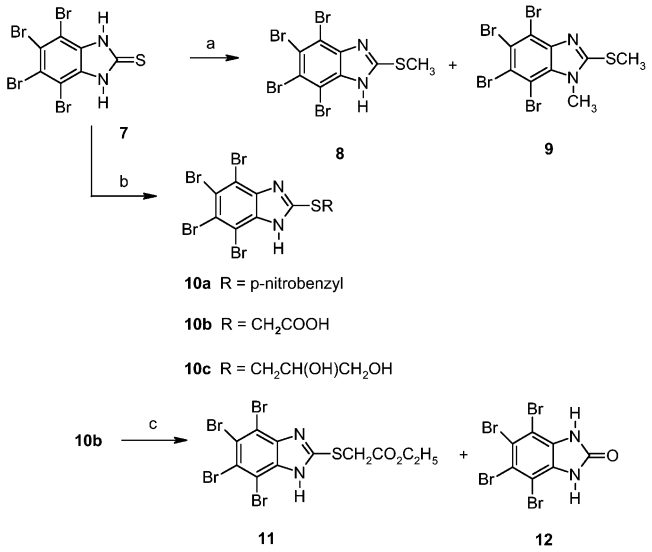
Scheme 1. Synthesis of 2-Substituted Benzimidazoles **2**−**6**[a]



[a] Reagents: (a) ammonia or corresponding amine **2a**−**g**. (b) EtOH/H_2O, $(CH_3)_2SO_4$, KOH. (c) EtOH, EtONa (**4a**), ^{i}PrOH, ^{i}PrONa (**4b**). (d) Allyl bromide (**3b**) or iodoacetamide (**3c**), EtOH/H_2O, K_2CO_3. (e) EtOH/H_2O, KOH. (f) Dimethylamine (**6a**) or isopropylamine (**6b**).

Another group of 2-modified derivatives of 4,5,6,7-tetrabromo-1*H*-benzimidazole was obtained by the nucleophilic substitution of 4,5,6,7-tetrabromo-1*H*,3*H*-benzimidazol-2-thione (**7**).[23] (Scheme 2). Methylation of **7** with dimethyl sulfate in an alkaline medium provided *S*-methyl (**8**) and *N*,*S*-dimethyl (**9**) derivatives of 4,5,6,7-tetrabromo-1*H*-benzimidazole. A similar procedure was exploited for preparation of **10a**−**c** using *p*-nitrobenzyl bromide, bromoacetic acid, and glycidol as alkylating agents. Prolonged heating of 2-*S*-carboxymethyl compound **10b** in a mixture of ethanol and hydrochloric acid

Scheme 2. Synthesis of 2-Substituted Benzimidazoles 8−12[a]



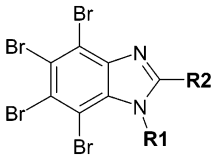
[a] Reagents: (a) EtOH/H_2O, $(CH_3)_2SO_4$, KOH. (b) EtOH/H_2O, KOH, *p*-nitrobenzyl bromide (**10a**), bromoacetic acid (**10b**), glycidol (**10c**). (c) EtOH/H_2O, H^+.

yielded the ethyl ester of the carboxymethyl derivative (**11**), and 4,5,6,7-tetrabromo-1*H*,3*H*-benzimidazol-2-one (**12**). The structures of all the products were determined by elemental analysis, ^{1}H NMR, UV, and mass spectroscopy.

In Vitro Biological Data. It has been already unambiguously shown that the efficacy of halogenated benzimidazole CK2 inhibitors critically depends on the number and the size of the halogen atoms present on the benzene ring, with bromine being more effective than chlorine, which in turn is more effective than fluorine.[23−26] This makes the 4,5,6,7-tetrabromobenzene scaffold the most effective one to inhibit CK2, irrespective of the rest of the molecule. The structural basis underlying optimal inhibition by the tetrabromobenzene scaffold was provided by the crystal structure of the complex between maize CK2α (>70% identical to its human homologue) and TBB, showing that, thanks to the size of the bromine atoms, the ligand almost perfectly fills a hydrophobic cavity which in CK2 is smaller than in the majority of protein kinases due to a number of unique bulky residues.[17] Attempts to assess the effect of replacing the four bromine atoms with even larger iodines failed, due to the impossibility of synthesizing the 4,5,6,7-tetraiodo derivative. The observation, however, that the 4,7-diiodo derivative is less inhibitory than its 5,6-dibromo counterpart (IC_{50} 14 vs 5 μM) (not shown) suggested that iodine is too large and cannot be accommodated in the hydrophobic pocket of CK2 as easily as bromine.

We therefore decided to keep the optimal "thickness" of the tetrabrominated benzene ring unchanged and to try to improve the inhibitory efficiency by making substitutions on the imidazole ring, with special reference to the carbon atom at position 2, which is more amenable than the two nitrogen atoms to chemical modifications. This led to the synthesis of a series of new 4,5,6,7-tetrabromobenzimidazole (TBI) derivatives variably substituted at position 2 and to some derivatives substituted at position 1, which is equivalent to position 3 owing to the symmetry of the molecule (see Methods, Schemes 1 and 2). The new inhibitors are listed in Table 1 with their IC_{50} values for CK2 inhibition determined with the specific CK2 peptide substrate RRRADDSDDDDD and 20 μM ATP as phosphate donor. An even cursory examination of these values highlights the fact that the nature of the substituents can deeply affect the inhibitory efficiency of the parent compound, 4,5,6,7-tetrabromobenzimidazole (TBI), which in turn is very similar to that of its 2-azo counterpart, TBB.

Table 1. Derivatives of 4,5,6,7-Tetrabromobenzimidazole and Their Efficacy as CK2 Inhibitors



compd[a]	R1	R2	IC_{50} (μM)	K_i (μM)
TBI	H	H	0.50	0.30
1	H	Br	0.74	0.23
2a	H	NH_2	0.30	0.09
2b	H	$NHCH_3$	0.21	0.09
2c	H	$N(CH_3)_2$	0.14	0.04
2d	H	$NHCH_2CH_2OH$	0.27	0.13
2e	H	$NHCH(CH_3)_2$	0.16	0.06
2f	H	$NHCH_2CH(OH)CH_3$	0.28	0.14
2g	H	$NHCH_2CH_2N(CH_3)_2$	0.25	0.16
3a	CH_3	Br	0.43	0.54
3b	$CH_2CH{=}CH_2$	Br	0.88	0.63
3c	$CH_2C(O)NH_2$	Br	1.57	1.90
4a	CH_3	OC_2H_5	>40.00	nd
4b	CH_3	$OCH(CH_3)_2$	20.39	nd
5	CH_3	=O	0.39	0.20
6a	CH_3	$N(CH_3)_2$	0.13	0.20
6b	CH_3	$NHCH(CH_3)_2$	0.18	0.19
8	H	SCH_3	0.25	0.07
9	CH_3	SCH_3	0.60	0.36
10a	H	$SCH_2(C_6H_4)NO_2$	3.48	nd
10b	H	SCH_2COOH	0.25	0.18
10c	H	$SCH_2CH(OH)CH_2OH$	0.15	0.14
11	H	$SCH_2COOC_2H_5$	1.55	−
12	H	=O	0.36	0.15

[a] Nomenclature used during chemical synthesis (see Methods, Schemes 1 and 2). nd= not determined.

Kinetic analyses run with most of the compounds listed in Table 1 were consistent with a reversible, competitive mechanism of inhibition, where the K_M but not the V_{max} values were affected by the inhibitors. On the basis of these experiments the inhibition constants (K_i) were calculated (see Table 1). These values, reflecting the affinity for CK2 regardless of ATP concentration, show that the most efficient ligand is the 2-dimethylamino derivative (**2c**) with a K_i value (40 nM) 1 order of magnitude below those of TBI and of TBB. Four other derivatives display K_i values below 100 nM, namely the 2-methylamino (**2b**) (90 nM), the 2-thiomethyl (**8**) (70 nM), 2-isopropylamino (**2e**) (60 nM), and 2-amino (**2a**) (90 nM) ones. The finding that in a few cases the K_i values are not lower but even slightly higher (notably with compounds **3a**, **3c**, **6a**, and **6b**) than IC_{50} values calculated with 20 μM ATP may be accounted for by a not purely competitive mechanism of inhibition. It may be worthy to note that such a behavior mainly occurs with compounds substituted at position 1, suggesting that for some reason these compounds are less readily displaced by increasing the ATP concentration.

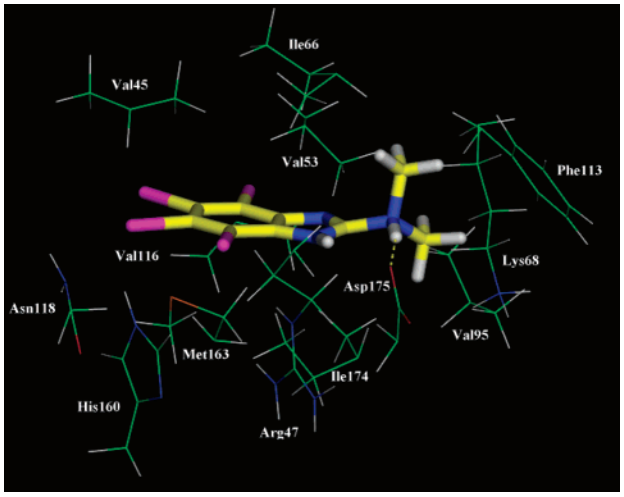


Figure 1. Molecular modeling of **2c** bound to the active site of the CK2 catalytic subunit. The structure of the **2c**−CK2α complex was generated on an Indy R4000 (SGI) workstation using the Builder module of Insight II package (Accelrys Inc., San Diego, CA 92121) and minimized using the Discover module until the energy reached a minimum. Modeling has been performed by manually superimposing the structure of **2c** to the structure of TBB inhibitor as in the crystal structure of maize CK2α−TBB binary complex (PDB code 1J91). The TBB structure was removed and an energy minimization of the complex CK2α−**2c** was performed until the energy reached a minimum. The figure is drawn using the Pymol program (DeLano, W. L. The PyMOL Molecular Graphics System (2002) on World Wide Web http://www.pymol.org). Residues at distance <4.5 Å from the inhibitor are shown.

To gain information about the role of the dimethylamino group in optimizing the binding of **2c**, a molecular modeling study was performed on the basis of the crystal structure of the CK2−TBB complex. This was done by assuming that the dimethylamino group was either protonated or not. The nonprotonated form is expected to be largely predominant in solution at physiological pH; the protonated one, however, could be stabilized through electrostatic interactions with charged side chains in the kinase−inhibitor complex. Modeling is consistent with the latter possibility, since the interactions observed with nonprotonated **2c** are quite similar to those occurring with TBB, [17] thus failing to account for the higher affinity of **2c**. In contrast, modeling with protonated **2c** (Figure 1) reveals that the amino group is now at 1.67 Å distance from the caboxylate of Asp175, while in the crystal structure of the TBB−CK2 complex N-2 of TBB (structurally homologue to C-2 of **2c**) is far away from Asp175 (about 4 Å distance).[17] This implies the displacement of both the inhibitor and Asp175 from the positions occupied in the TBB−CK2 complex, with the planar molecule of the inhibitor changing its inclination and rotating laterally with respect to the position occupied by TBB. Due to this displacement and to the two methyl groups, the apolar contacts between **2c** and CK2 are more numerous than in the case of TBB (see Table 2). The closer fit of **2c** to the hydrophobic cavity of CK2 is also demonstrated by the molecular surfaces that are buried: in the TBB−CK2 complex total buried surface is 596 Å^2, while this figure rises to 631Å^2 in the **2c**−CK2 complex. The crucial role of apolar interactions for the binding of **2c** has been validated by mutating two hydrophobic residues, Val66 (the homologue of Ile66 in human CK2α) and Ile174 (both generally replaced by smaller residues in the other kinases), to Ala. The single mutants display 50% of the activity of the wild type and the double mutant is as active as wild type. As shown in Table 3, all the mutants are less susceptible to inhibition by **2c**, with the double mutant being especially refractory.

Unlike other compounds marketed as inhibitors of CK2, notably DRB (5,6-dichloro-1-β-D-ribofuranosylbenzimidazole), quercetin, and apigenin, which inhibit several protein kinases as efficiently as CK2,[27,28] TBB is quite selective, as it inhibits only one kinase, DYRK1a, among >30 tested, as efficiently as CK2.[18,19] To check whether a comparable specificity is also displayed by **2c**, this compound has been tested on the same panel of kinases assayed for TBB inhibition (Table 4). The results show that also in the case of **2c** DYRK1a is the only kinase inhibited to the same extent as CK2. GSK3, which is 60% inhibited by 10 μM TBB, is instead unaffected by **2c**; in contrast, the active CDK2−cyclinA complex is more susceptible to **2c** than it is to TBB. Although the data presented in Table 4, obtained at constant inhibitor concentrations (10 μM), may give the

Table 2. Catalytic Pocket and TBB/**2c** Inhibitor Atoms[a]

van der Waals contact s (<4.4 Å)			
TBB atom	residue (no. of contacts)	**2c** atom	residue (no. of contacts)
Br4	Ile66 (3), Val95 (1), Ile174 (2)	Br4	Ile66 (3), Val116 (6), Met163 (2)
Br5	Ile66 (3), Val116 (1)	Br5	Val116 (1), Asn118 (1), Met163 (2)
Br6	Val45 (2), His160 (2), Met163 (1)	Br6	Val45 (3), His160 (2), Met163 (1)
Br7	Gly46 (1), Arg47 (6), Val53 (2), His160 (2)	Br7	Val45 (1), Arg47 (5), His160 (2)
N1	Val53 (1), Lys68 (3), Asp175 (3)	N1	Arg47 (1), Val53 (2), Ile174 (1), Asp175 (3)
N2	Val53 (1), Lys68 (3), Asp175 (6)	N2	Phe113 (1), Asp175 (4)
N3	Lys68 (3), Asp175 (3)	N3	Ile66 (2), Ile174 (3)
		C1	Val53 (1), Ile66 (1), Ile174 (1), Asp175 (3)
C2	Val53 (3), Ile174 (1)	C2	Val53 (1), Ile66 (1), Ile174 (1), Asp175 (1)
C3	Val53 (2), Ile174 (1)	C3	Ile66 (1), Ile174 (1)
C4	Val53 (1), Ile66 (1), Ile174 (2)	C4	Ile66 (1), Met163 (3), Ile174 (1)
C5	Val53 (1), Ile166 (1), Ile174 (1)	C5	Met163 (3)
C6	Val53 (3), Ile174 (1)	C6	Val45 (1), Met163 (2)
C7	Val53 (3), His160 (1), Ile174 (1)	C7	Val45 (1), Val53 (1), Met163 (1), Ile174 (1)
		C8	Val53 (2), Ile66 (4), Lys68 (1), Phe113 (4), Asp175 (1)
		C9	Val95 (2), Phe113 (6), Ile174 (1), Asp175 (5)
total	72	total	99

[a] Based on the coordinates of the crystal structures of CK2−TBB binary complex (PDB code 1J91) and on the model where TBB has been replaced by **2c** (see Figure 1).

Table 3. Mutation of Val66 and/or Ile174 to Ala Reduces CK2 Inhibitability by **2c**

	IC_{50} (μM)
wild type	0.14
V66A	1.78 (9.36)[a]
I174A	3.30 (17.36)
V66A, I174A	5.79 (30.47)

[a] Fold increase as compared to the wild type is indicated in parentheses.

Table 4. Inhibition of Protein Kinases by **2c**[a]

kinase	TBB	2c	kinase	TBB	2c
MKK1[a]	94	53	GSK3β	64	103
MAPK2/ERK2	111	77	ROCK-II	91	69
JNK/SAPK1c	93	97	AMPK	96	73
SAPK2a/p38	107	85	CHK1	101	81
SAPK2b/p38β2	103	65	phosph. kinase	49	41
SAPK3/p38γ	89	88	CK2	13	4
SAPK4/p38δ	99	109	CDK2/cyclin A	70	20
MAPKAP-K1a	90	52	CK1	91	87
MAPKAP-K2	108	101	G-CK	95	92
MSK1	111	73	PiD261	98	97
PRAK	87	77	Lck	98	104
PKA	90	80	Lyn	99	91
PKCα	102	95	c-Fgr	98	94
PDK1	97	112	Syk	102	103
PKBα	91	96	CSK	101	109
SGK	97	29	DYRK1a	22	2
S6K1	89	65	PP2A	117	67

[a] Activity assays were linear with respect to time and enzyme concentration and were performed as previously detailed.[18] Residual activity was determined in the presence of 10 μM inhibitor and expressed as a percentage of the control without inhibitor. **2c** was also tested on the lipid kinase PI3K (isoforms α and γ) without any significant effect up to 20 μM. The IC_{50} values of **2c** were also calculated with respect to protein kinases inhibited 50% or more at 10 μM concentration. The following values (μM) were determined: CK2, 0.14; DYRK1a, 0.12; MKK, 10.50; MAPKAP-K1a, 10.28; SGK, 3.57; phosph. kinase, 7.04; CDK2/cyclinA, 2.38. From these data the selectivity index of **2c** for CK2 (expressed as percentage of kinases of the panel inhibited with IC_{50} values >10-fold higher than the IC_{50} value for CK2) was calculated to be 97%.

impression that **2c** is somewhat less selective than TBB, it should be considered that CK2 is more readily inhibited by **2c** than it is by TBB. Consequently, the IC_{50} value for the inhibition of CK2 by **2c** is 1−2 orders of magnitude lower than those calculated for the few other "**2c**-sensitive" kinases (see footnote *a* of Table 4), with the only exception being DYRK1a, which is inhibited with the same potency as CK2 not only by **2c** but also by TBB.[18] TBB also inhibits phosphorylase kinase with a IC_{50} value less than 10-fold higher than its IC_{50} value with CK2.[19] It can be concluded, therefore, that the selectivity index of **2c** for CK2 (97%, see legend of Table 4) is slightly higher than that of TBB tested toward the same panel of protein kinases,[18,19] whose value is 94%.

In Vivo Biological Data. We also wanted to compare the in vivo efficacy of **2c** to that of TBB. This latter compound proved useful to block endogenous CK2, thereby inducing apoptosis of Jurkat cells.[21] The same protocol was used to see if **2c** is cell permeable and can be used to inhibit CK2 in living cells. Treatment of Jurkat cells with 5 μM **2c** was found to drastically inhibit endogenous CK2, as judged from its negligible activity in the lysates of treated cells (not shown). It also induces cell death with an efficiency higher than that of TBB, as illustrated in Figure 2A, showing that the concentration of **2c** required to reduce cell viability

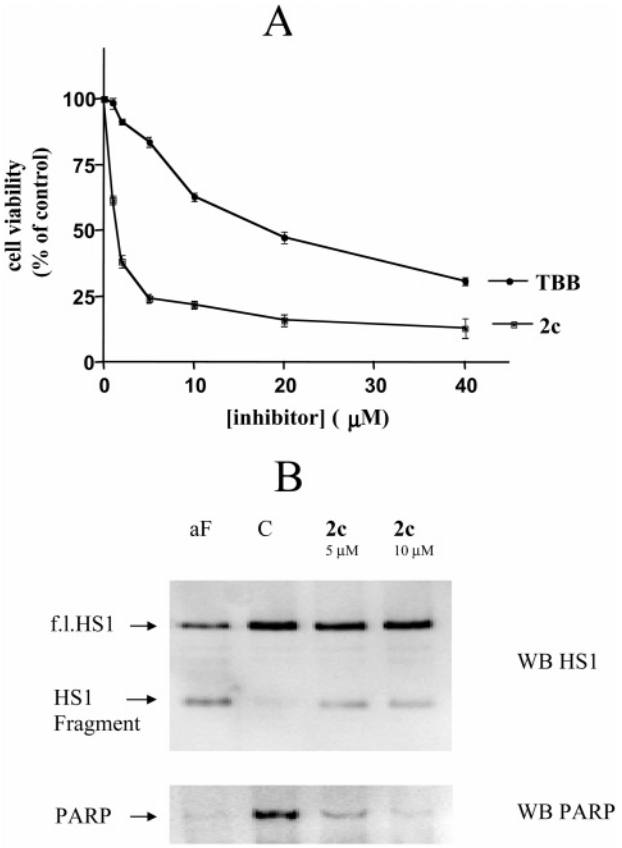


Figure 2. Effect of **2c** on cell viability. (A) Jurkat cells were treated for 24 h with an increasing concentration of TBB or **2c**, as indicated. Cell viability was assessed by the MTT method. Control cells were assigned 100% viability, having been incubated with the solvent DMSO (0.5% v/v). Reported values represent the means ± SEM from three separate experiments. (B) Jurkat cells were treated for 14 h with nothing (C), 5 or 10 μM **2c**, or 50 ng/mL antiFas, as an apoptotic control. Caspase activation was assessed following the degradation of the caspase substrates HS1 protein (upper part) or PARP (lower part), by means of Western blot (WB) with specific antibodies, as indicated. The anti-HS1 Western blot shows the full length (fl) HS1 protein as well as its major fragment generated upon caspase activation.

by 50% is 6-fold lower than that of TBB (DC_{50} values of 2.7 and 17 μM, respectively). The higher concentration of inhibitors required for in vivo effects, as compared to in vitro experiments, is, at least in part, accounted for by the competitive mechanism of inhibition, which in living cells is counteracted by the high concentration of endogenous ATP (in the millimolar range). As in the case of TBB, cell death is due, at least in part, to apoptosis, since it is accompanied by caspase activation monitored by the cleavage of the caspase substrates PARP and HS1 protein (Figure 2B).

Another potential advantage of **2c** as compared to TBB is its lack of side effects on isolated mitochondria. While in fact 1 μM TBB causes the depolarization of mitochondria, **2c** does not display any comparable effect up to 10 μM concentration (not shown), i.e., well beyond the concentration required to inhibit endogenous CK2 and to induce apoptosis.

Conclusions

The data presented show that it is possible to further improve the properties of TBB, one of the most effective

and selective inhibitors of CK2 described so far, by keeping unaltered the tetrabrominated benzene moiety, which apparently already has an optimal size to fit in the hydrophobic cavity adjacent to the ATP binding site, while properly derivatizing the edge of the triazole ring facing the inner part of the active site. This has been successfully done first by replacing N^2 with a carbon atom, which per se has no significant consequences on CK2 inhibition but makes easier the synthesis of a variety of adducts at position 2, and, second, by replacing the hydrogen of C^2 with polar groups, which make possible new interactions with polar side chains of the kinase. A number of adducts bearing amino groups were proved particularly effective. The most effective among the newly synthesized inhibitors is **2c** [2-(dimethylamino)-4,5,6,7-tetrabromobenzimidazole], whose K_i value (40 nM) is the lowest reported so far of a CK2 inhibitor, being 1 order of magnitude below that of TBB. The selectivity of **2c** is also remarkable and comparable to that of TBB, with only one kinase, DYRK1a, out of a panel of >30, inhibited to the same extent as CK2. More importantly, however, **2c** is effective on cultured cells at concentrations severalfold lower than TBB and, unlike TBB, it does not promote depolarization of isolated mitochondria. While it is conceivable that the inhibitory properties of TBB/TBI derivatives can be further optimized in the future, for the time being **2c** represents the first choice CK2 inhibitor available, with the possible exception of IQA, which is somewhat more specific but slightly less potent and rather unstable.[17] Given its properties and the availability of a CK2 mutant which is 30-fold less susceptible than wild type to inhibition, **2c** is definitely superior to TBB for in cell studies. It may be also worthy to note in this respect that the consequences of pharmacological inhibition of CK2 corroborate the conclusions drawn from previous studies indicating that overexpression of CK2 catalytic subunits protects against apoptosis.[13]

Experimental Section

Chemistry. All chemicals and solvents were purchased from Sigma-Aldrich. Melting points (uncorrected) were measured in open capillary tubes on a Gallenkamp-5 melting point apparatus. Ultraviolet absorption spectra were recorded on Kontron Uvikon 940 spectrometer. ^{1}H NMR spectra (in ppm) were measured with Varian Gemini 200 MHz and Varian UNITYplus spectrophotometers at 298 K in DMSO-d_6. Using tetramethylsilane as the internal standard, mass spectra (70 eV) were obtained with AMD-604 (Intectra) spectrometer. Flash chromatography was performed on silica gel (Merck) (230−400 mesh). Analytical thin-layer chromatography (TLC) was carried out on precoated silica gel F_{254} (Merck) plates (0.25 mm thickness). Elemental analyses of the new compounds were within ±0.4% of the respective theoretical values.

2-Amino-4,5,6,7-tetrabromo-1*H*-benzimidazole (2a). A mixture of 2,4,5,6,7-pentabromo-1*H*-benzimidazole (**1**) (1.54 g, 3 mmol) and ammonia (methanolic ammonia, 40 mL, sat. at 5 °C) was heated in a steel autoclave at 140−145 °C. The deep-pink solution was adsorbed on silica gel and placed on the top of silica gel column (4 × 15 cm). Chromatography was performed with $CHCl_3$ (100 mL), followed by $CHCl_3$/MeOH (200 mL, 90:10) and $CHCl_3$/MeOH (80:20). The product-containing fractions were evaporated to dryness, and the residue was crystallized from 80% EtOH to give colorless crystals (0.485 g, 36%): mp 269−271 C; ^{1}H NMR (DMSO-d_6) 6.68 (s, NH_2), 11.6 (bs, N*H*-benzim). Anal. ($C_7H_3Br_4N_3$) C, H, N.

2-(Methylamino)-4,5,6,7-tetrabromo-1*H*-benzimidazole (2b). A mixture of **1** (1.02 g, 2 mmol) and ethanolic methylamine (35 mL, 30%) was heated in steel autoclave at 110−115 °C. The reaction mixture was evaporated to dryness and crystallized from 80% EtOH to give colorless crystals (630 mg, 68%): mp 283−285 °C; ^{1}H NMR (DMSO-d_6) 2.94 (d, CH_3), 6.69 (q, N*H*), 11.60 (bs, N*H*-benzim). Anal. ($C_8H_5Br_4N_3$) C, H, N.

2-(Dimethylamino)-4,5,6,7-tetrabromo-1*H*-benzimidazole (2c) was obtained from **1** and ethanolic dimethylamine in a similar manner as that described for the preparation **2b** as colorless crystals (75%): mp >330 °C (dec); ^{1}H NMR (DMSO-d_6) 3.13 (s, 2 × CH_3), 11.5 (bs, N*H*-benzim). Anal. ($C_9H_7Br_4N_3$) C, H, N.

2-((2-Hydroxyethyl)amino)-4,5,6,7-tetrabromo-1*H*-benzimidazole (2d) was obtained from **1** and ethanolic ethanolamine in a similar manner as that described for the preparation **2b** as colorless crystals (62%): mp 216−218 °C; ^{1}H NMR (DMSO-d_6) 1.05 (t, CH_2), 4.36 (t, CH_2), 4.90 (bs, O*H*), 6.68 (t, N*H*), 11.50 (bs N*H*-benzim). Anal. ($C_9H_7Br_4N_3O$) C, H, N.

2-(Isopropylamino)-4,5,6,7-tetrabromo-1*H*-benzimidazole (2e) was obtained from **1** and isopropylamine in a similar manner as that described for the preparation **2b** as colorless crystals (70%): mp 288−290 °C (dec); ^{1}H NMR (DMSO-d_6) 1.22 (d, 2 × CH_3), 4.05 (m, C*H*), 6.60 (d, N*H*), 11.2 (bs, N*H*-benzim). Anal. ($C_{10}H_9Br_4N_3$) C, H, N.

2-(2-Hydroxyprop-1-ylamino)-4,5,6,7-tetrabromo-1*H*-benzimidazole (2f) was obtained from **1** and ethanolic 2-hydroxypropylamine in a similar manner as that described for the preparation **2b** as colorless crystals (61%): mp 258−260 °C; ^{1}H NMR (DMSO-d_6) 1.12 (d, CH_3), 3.30 (m, CH_2), 3.83 (m, C*H*), 4.96 (d, O*H*), 6.62 (t, N*H*), 11.4 (s, N*H*-benzim). Anal. ($C_{10}H_9Br_4N_3O$) C, H, N.

2-((2-(Dimethylamino)ethyl)amino)-4,5,6,7-tetrabromo-1*H*-benzimidazole (2g) was obtained from **1** and ethanolic (dimethylamino)ethylamine in a similar manner as that described for the preparation **2b** as colorless crystals (56%): mp >250 °C (dec); ^{1}H NMR (DMSO-d_6) 2.29 (s, 2 × CH_3), 2.54 (m, CH_2), 3.43 (q, CH_2), 6.82 (t, N*H*), 11.1 (bs, N*H*-benzim). Anal. ($C_{11}H_{12}Br_4N_4$) C, H, N.

1-Methyl-2,4,5,6,7-pentabromo-1*H*-benzimidazole (3a). To a stirred solution of **1** (1.03 g, 2 mmol) in a mixture of EtOH (20 mL) and 5 N KOH (20 mL) was added dimethyl sulfate (0.4 mL, 4.2 mmol) over 30 min at room temperature. A white precipitate was formed after a few minutes of stirring. The stirring was continued for an additional 30 min. The formed precipitate was filtered off and crystallized from EtOH/water to give **3a** (830 mg, 79%): mp 215−217 °C; ^{1}H NMR (DMSO-d_6) 4.04 (s, CH_3). Anal. ($C_8H_3Br_5N_2$) C, H, N.

1-Allyl-2,4,5,6,7-pentabromo-1*H*-benzimidazole (3b). To a stirred solution of **1** (1.03 g, 2 mmol) in a mixture of EtOH/H_2O (1:1, 20 mL) and potassium carbonate (1.38 g, 10 mmol) was added allyl bromide (0.17 mL, 2 mmol) at room temperature. The stirring was continued for 5 days and each day allyl bromide (0.17 mL, 2 mmol) was added. After 3 days of stirring a white precipitate was formed. This was filtered and crystallized from EtOH to give colorless needles (710 mg, 64%): mp 158−159 °C; ^{1}HNMR (DMSO-d_6) 4.66 and 4.74 (2s, =CH_2), 5.18 (s, CH_2), 6.10 (m C*H*). Anal. ($C_{10}H_5Br_5N_2$) C, H, N.

1-Acetamido-2,4,5,6,7-pentabromo-1*H*-benzimidazole (3c) was obtained from **1** and iodoacetamide in a similar manner as that described for **3b** as colorless crystals (17%): mp 235 °C (explosive dec); ^{1}H NMR (DMSO-d_6) 4.93 (s, CH_2), 7.44 and 7.78 (2bs, 2 × NH_2). Anal. ($C_9H_4Br_5N_3O$) C, H, N.

2-Ethoxy-1-methyl-4,5,6,7-tetrabromo-1*H*-benzimidazole (4a). A solution of **3a** (530 mg, 1 mmol) in EtOH (30 mL) containing sodium ethanolate (2 mL, 1 N EtOH solvent) was refluxed for 2 h. The reaction mixture was neutralized with acetic acid and evaporated to dryness, and the residue crystallized from 80% EtOH to give colorless crystals (410 mg, 84%): mp 174−176 °C; ^{1}H NMR (DMSO-d_6) 1.43 (t, CH_3), 3.76 (s, N−CH_3), 4.57 (q, CH_2). Anal. ($C_{10}H_8Br_4N_2O$) C, H, N.

2-Isopropoxy-1-methyl-4,5,6,7-tetrabromo-1*H*-benzimidazole (4b). A solution of **3a** (530 mg, 1 mmol) in ^{i}PrOH (30

mL) containing sodium 2-propanolate (2 mL, 1 N ^{i}PrOH solvent) was refluxed for 2 h. The reaction mixture was neutralized with acetic acid, adsorbed on silica gel, and deposited on the top of silica gel column (3 × 15 cm). The chromatography was performed with $CHCl_3$ (150 mL) and $CHCl_3$/MeOH (95:5). The product containing fractions were evaporated to dryness, and the residue was crystallized from 80% ^{i}PrOH to give colorless crystals (360 mg, 71%): mp 164−166 °C; ^{1}H NMR (DMSO-d_6) 1.42 and 1.45 (2s, 2 × C*H*$_3$), 3.76 (s, N−C*H*$_3$), 5.33 (m, C*H*). Anal. ($C_{11}H_{10}Br_4N_2O$) C, H, N.

1-Methyl-4,5,6,7-tetrabromo-1*H*,3*H*-benzimidazol-2-one (5). A solution of **3a** (300 mg, 0.57 mmol) in a mixture of 10 mL of 5 N KOH and 20 mL of EtOH was stirred and refluxed for 2 h. The reaction mixture was cooled and acidified with HCl. The formed precipitate was separated and crystallized from EtOH to give colorless crystals of **5** (220 mg, 83%): mp 333−335 °C; ^{1}H NMR (DMSO-d_6) 3.56 (s, C*H*$_3$), 11.81 (bs, N*H*). Anal. ($C_8H_4Br_4N_2O$) C, H, N.

2-(Dimethylamino)-1-methyl-4,5,6,7-tetrabromo-1*H*-benzimidazole (6a) was obtained from **3a** and ethanolic dimethylamine in a similar manner as that described for **2b** as colorless crystals (70%): mp 171−173 °C; ^{1}H NMR (DMSO-d_6) 2.99 (s, 2 × C*H*$_3$), 3.74 (s, N−C*H*$_3$). Anal. ($C_{10}H_9Br_4N_3$) C, H, N.

2-(Isopropylamino)-1-methyl-4,5,6,7-tetrabromo-1*H*-benzimidazole (6b) was obtained from **3a** and ethanolic isopropylamine in a similar manner as that described for **2b** as colorless crystals (74%): mp 198−200 °C; ^{1}H NMR (DMSO-d_6) 1.26 (d, 2 × C*H*$_3$), 3.74 (s, N−C*H*$_3$), 4.14 (m, C*H*), 6.98 (d, N*H*). Anal. ($C_{11}H_{11}Br_4N_3$) C, H, N.

2-(Methylsulfanyl)-4,5,6,7-tetrabromo-1H-benzimidazole (8) and 1-methyl-2-(methylsulfanyl)-4,5,6,7-tetrabromo-1*H*-benzimidazole (9). To a stirred solution of 4,5,6,7-tetrabromo-1*H*,3*H*-benzimidazol-2-thione (**7**) (710 mg, 1.5 mmol) in a mixture of EtOH (10 mL) and 2 N KOH (20 mL, 40 mmol) was added dimethyl sulfate (0.4 mL, 4 mmol) in portions over 30 min. The stirring was continued for an additional 2 h. The reaction mixture was brought to pH 5 with acetic acid, and precipitate that formed was filtered, adsorbed on silica gel, and placed on the top of silica gel column (3 × 15 cm). Chromatography was performed with $CHCl_3$ (200 mL) and $CHCl_3$/MeOH (95:5). The faster migrating zone was evaporated to dryness and the residue crystallized from EtOH to give **9** as colorless crystals (530 mg, 72%): mp 186−187 °C; ^{1}H NMR (DMSO-d_6) 2.77 (s, S−C*H*$_3$), 3.91 (s, N−C*H*$_3$). Anal. ($C_9H_6Br_4N_2S$) C, H, N. The slower migrating zone after evaporation and crystallization from EtOH−water gave crystalline **8** (75 mg, 10%): mp 272−274 °C; ^{1}H NMR (DMSO-d_6) 2.72 (s, S−C*H*$_3$), 13.0 (bs, N*H*). Anal. ($C_8H_4Br_4N_2S$) C, H, N.

2-((4-Nitrobenzyl)sulfanyl)-4,5,6,7-tetrabromo-1*H*-benzimidazole (10a). To a stirred solution of **7** (470 mg, 1 mmol) in a mixture of 10 mL of 1 N KOH and 10 mL of EtOH was added *p*-nitrobenzyl bromide (240 mg, 1.1 mmol) portionswise during 30 min. The stirring was continued for an additional 1 h, and the reaction mixture was brought to pH 5 with acetic acid. The formed precipitate was separated and crystallized from 80% EtOH to give the desired product (570 mg, 95%): mp 254−256 °C; ^{1}H NMR (DMSO-d_6) 4.71 (s, C*H*$_2$), 7.83 and 8.15 (2d, *H*-arom.), 13.47 (s, N*H*). Anal. ($C_{14}H_7Br_4N_3O_2S$) C, H, N.

((4,5,6,7-Tetrabromo-1*H*-benzimidazol-2-yl)sulfanyl)-acetic Acid (10b). To a stirred solution of **7** (710 mg, 1.5 mmol) in a mixture of 15 mL of 1 N KOH and 15 mL of EtOH was added bromoacetic acid (420 mg, 3 mmol) portionswise during 1 h. The stirring was continued for an additional 1 h, and the reaction mixture was acidified with HCl. The precipitate that formed was filtered off and crystallized from 80% EtOH to yield the desired product (670 mg, 85%): mp 259−261 °C (dec); ^{1}H NMR (DMSO-d_6) 4.21 (s, C*H*$_2$), 13.14 (bs, N*H*). Anal. ($C_9H_4Br_4N_2O_2S$) C, H, N.

3-((4,5,6,7-Tetrabromo-1*H*-benzimidazol-2-yl)sulfanyl)-propane-1,2-diol (10c). To a stirred solution of **7** (710 mg, 1.5 mmol) in a mixture of 15 mL of 1 N KOH and 15 mL of EtOH was added glycidol (0.25 mL, 4 mmol). The stirring was continued for an additional 1 h, and the reaction mixture was brought to pH 5 with acetic acid. The solution was adsorbed on silica gel and placed on the top of silica gel column (3 × 15 cm). Chromatography was performed with $CHCl_3$ (150 mL) and with $CHCl_3$/MeOH (95:5). The product-containing fractions were evaporated to dryness, and the residue was crystallized from EtOH/Et_2O to yield the desired product (385 mg, 48%) as white powder: mp 254−256 °C (dec); ^{1}H NMR (DMSO-d_6) 1.08 (t, C*H*$_2$O), 3.62 (d, C*H*$_2$), 3.80 (m C*H*), 4.93 and 5.12 (2 bs, 2 × O*H*), 13.41 (bs, N*H*). Anal. ($C_{10}H_8Br_4N_2O_2S$) C, H, N.

4,5,6,7-Tetrabromo-1*H*,3*H*-benzimidazol-2-one (12) and ((4,5,6,7-Tetrabromo-1*H*-benzimidazol-2-yl)sulfanyl)acetic Acid Ethyl Ester (11). A suspension of **10b** (525 mg, 1 mmol) in a mixture of 10 mL of 2 N HCl and 10 mL of EtOH was refluxed for 24 h. The reaction mixture was evaporated to dryness, and the residue was adsorbed on silica gel and placed on the top of silica gel column (4 × 12 cm). Chromatography was performed with $CHCl_3$ (200 mL) and $CHCl_3$/MeOH (9:1). The faster migrating zone was evaporated to dryness and the residue crystallized from ethanol to give **11** (280 mg, 51%): mp 175−177 °C; ^{1}H NMR (DMSO-d_6) 1.19 (t, C*H*$_3$), 4.12 (q, OC*H*$_2$), 4.23 (s, SC*H*$_2$), 13.51 (s, N*H*). Anal. ($C_{11}H_8Br_4N_2O_2S$) C, H, N. The slower migrating zone was evaporated to dryness and the residue crystallized from 80% EtOH to give **12** (70 mg, 16%): mp >330 °C (dec); ^{1}H NMR (DMSO-d_6) 11.60 (s, N*H*). Anal. ($C_7H_2Br_4N_2O$) C, H, N.

Materials. Native CK1 (nCK1) and CK2 (nCK2) were purified from rat liver;[29] Golgi CK (G-CK), purified from rat lactating mammary gland, was provided by Dr. A. M. Brunati (Padova, Italy). Protein tyrosine kinases Lyn, c-Fgr, and Syk (also termed TPK−IIB) were purified from rat spleen as previously referenced.[18] Human recombinant α and β subunits of CK2 were expressed in *Escherichia coli*, and the holoenzyme was reconstituted and purified as previously described.[30] The V66A and I174A CK2 mutants were obtained as described previously.[18] V66AI174A double mutant was obtained with the "QuikChange-SiteDirected Mutagenesis" kit (Stratagene), using human V66A a cDNA inserted in pT7−7 vector as template and two synthetic oligonucleotide primers, 5′-GCACAGAAAGCTACGACTAGCAGACTGGGGTTTGGC-3′ and 5′-GCCAAACCCCAGTCTGCTAGTCGTAGCTTTCTGTGC-3′, each complementary to opposite strands of template. Expression and purification of the mutant was performed as previously described.[18] *Saccharomyces cerevisiae* piD261 was provided by Dr. S. Facchin (Padova, Italy). The source of all the other protein kinases used for specificity assays is as previously described or referenced.[27,31]

Cell Culture, Treatment, and Viability Assay. The human leukemia Jurkat T-cell line was maintained in RPMI-1640, supplemented with 10% (v/v) fetal calf serum, 2 mM L-glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin. For the treatment, cells were suspended at a density of 10^6 cells/mL in a medium containing 1% (v/v) fetal calf serum and then incubated at 37 °C, in the presence of the compounds at the indicated concentrations. Control cells were treated with equal amounts of solvent. At the end of incubations, cells were lysed by the addition of hypo-osmotic buffer, as previously described.[18]

Cell viability was assessed by means of 3-(4,5-dimethylthiazol-2-yl)-3,5-diphenyltriazolium bromide (MTT) reagent, while caspase activation was followed by Western blot monitoring of PARP and HS1 protein degradation, as previously described.[21]

CK2 Phosphorylation Assays. Phosphorylation assays were carried out in the presence of increasing amounts of each inhibitor 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 [^{33}P-ATP] (500−1000 cpm/pmol), unless otherwise indicated, and incubated for 10 min at 37 C. 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 and then once in methanol and dried before counting. Conditions for the

activity assays of all other protein kinases are described or referenced in the legends of the figures.

Kinetic Determination. Initial velocities were determined at each of the substrate concentration tested. K_M values were calculated either in the absence or in the presence of increasing concentrations of inhibitor, from Lineweaver−Burk double-reciprocal plots of the data. Inhibition constants were then calculated by linear regression analysis of K_M/V_{max} versus inhibitor concentration plots. Inhibition constants were also deduced from the IC_{50}/K_i Cheng−Prusoff relationship,[32] by determining IC_{50} for each compound at 1 μM ATP concentration, assuming a competitive mechanism of inhibition.

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Appendix

Abbreviations. MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; MKK, MAPK kinase (also called MEK); JNK, c-Jun N-terminal kinase; SAPK, stress-activated protein kinase; MAPKAP, MAPK-activated protein kinase; MSK, mitogen and stress-activated protein kinase; PRAK, p38-regulated/activated kinase; PKA, cAMP-dependent protein kinase; PKC, protein kinase C; PDK, 3-phosphoinositide-dependent kinase; PKB, protein kinase B (also called Akt); SGK, serum- and glucocorticoid-induced kinase; S6K, ribosomal protein S6 kinase; GSK3, glycogen synthase kinase-3; ROCK, Rho-dependent protein kinase; AMPK, AMP-activated protein kinase; CHK, checkpoint kinase; CDK, cyclin-dependent kinase; G-CK, Golgi casein kinase; CSK, c-Src kinase; DYRK, dual-specificity tyrosine phosphorylation-regulated kinase; PP2A, protein phosphatase type 2A.

Supporting Information Available: Detailed analytical data (TLC, UV, MS, elemental analyses) for newly obtained compounds **2a**−**g**, **3a**−**c**, **4a**,**b**, **5**, **6a**,**b**, **8**, **9**, **10a**−**c**, **11**, **12**. This material is available free of charge via the Internet at http://pubs.asc.org.

References

(1) Manning, G.; Whyte, D. B.; Martinez, R.; Hunter, T.; Sudarsanam, S. The protein kinase complement of the human genome. *Science* **2002**, *298*, 1912−1934.
(2) Blume-Jensen, P.; Hunter, T. Oncogenic kinase signaling. *Nature* **2001**, *411*, 355−65.
(3) Cohen, P. Protein kinases—the major drug targets of the twenty-first century? *Nat. Rev.* **2002**, *1*, 309−315.
(4) Fabbro, D.; Garcia-Echeverria C. Targeting protein kinases in cancer therapy. *Curr. Opin. Drug Discovy Dev.* **2002**, *5*, 701−12.
(5) Druker, B. J.; Talpaz, M.; Resta, D. J.; Peng, B.; Buchdunger, E.; Ford, J. M.; Lydon, N. B.; Kantarjian, H.; Capdeville, R.; Ohno-Jones, S.; Sawyers, C. L. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukaemia. *N. Engl. J. Med.* **2001**, *344*, 1031−1037.
(6) Pinna, L. A. The raison d'etre of constitutively active protein kinases: The lesson of CK2. *Acc. Chem. Res.* **2003**, *36*, 378−84.
(7) Meggio, F.; Pinna, L. A. One-thousand-and-one substrates of protein kinase CK2? *FASEB J.* **2003**, *17*, 349−368.
(8) Litchfield, D. W. Protein kinase CK2: Structure, regulation and role in cellular decisions of life and death. *Biochem. J.* **2003**, *369*, 1−15.
(9) Seldin, D. C.; Leder, P. Casein kinase IIα transgene-induced murine lymphoma: Relation to Theilleriosis in cattle. *Science* **1995**, *267*, 894−897.
(10) Kelliher, M. A.; Seldin, D. C.; Leder, P. Tal-1 induces cell acute lymphoblastic leukemia accelerated by casein kinase IIα. *EMBO J.* **1996**, *15*, 5160−5166.
(11) Landesman-Bollag, E.; Channavajhala, P. L.; Cardiff, R. D.; Seldin, D. C. p53 deficiency and misexpression of protein kinase CK2 alpha collaborate in the development of thymic lymphomas in mice. *Oncogene* **1998**, *16*, 2965−2974.
(12) Orlandini, M.; Semplici, F.; Ferruzzi, R.; Meggio, F.; Pinna, L. A.; Oliviero, S. Protein kinase CK2α' is induced by serum as a delayed early gene and cooperates with Ha-ras in fibroblast transformation. *J. Biol. Chem.* **1998**, *273*, 21291−21297.
(13) Guo, C.; Yu, S.; Wang, H.; Davis, A. T.; Green, J. E.; Ahmed, K. A potential role of nuclear matrix-associated protein kinase CK2 in protection against drug-induced apoptosis in cancer cells. *J. Biol. Chem.* **2001**, *276*, 5992−5999.
(14) Tawfic, S.; Yu, S.; Wang, H.; Faust, R.; Davis, A.; Ahmed, K. Protein kinase CK2 signal in neoplasia. *Histol. Histopathol.* **2001**, *16*, 573−582.
(15) Battistutta, R.; Sarno, S.; De Moliner, E.; Papinutto, E.; Zanotti, G.; Pinna, L. A. The replacement of ATP by the competitive inhibitor emodin induces conformational modifications in the catalytic site of protein kinase CK2. *J. Biol. Chem.* **2000**, *275*, 29618−29622.
(16) De Moliner, E.; Moro, S.; Sarno, S.; Zagotto, G.; Zanotti, G.; Pinna, L. A.; Battistutta, R. Inhibition of protein kinase CK2 by anthraquinone-related compounds. A structural insight. *J. Biol. Chem.* **2003**, *278*, 1831−1836.
(17) Battistutta, R.; De Moliner, E.; Sarno, S.; Zanotti, G.; Pinna, L. A. Structural features underlying selective inhibition of protein kinase CK2 by ATP site-directed tetrabromo-2-benzotriazole. *Protein Sci.* **2001**, *10*, 2200−2206
(18) Sarno, S.; De Moliner, E.; Ruzzene, M.; Pagano, M. A.; Battistutta, R.; Bain, J.; Fabbro, D.; Schoepfer, J.; Elliott, M.; Furet, P.; Meggio, F.; Zanotti, G.; Pinna, L. A. Biochemical and 3D-structural data on the specific inhibition of protein kinase CK2 by ((5-oxo-5,6-dihydro-indolo(1,2-a)quinazolin-7-yl)acetic acid) (IQA). *Biochem. J.* **2003**, *374*, 639−646.
(19) Sarno, S.; Reddy, H.; Meggio, F.; Ruzzene, M.; Davies, S. P.; Donella-Deana, A.; Shugar, D.; Pinna, L. A. Selectivity of 4,5,6,7-tetrabromobenzotriazole, an ATP site-directed inhibitor of protein kinase CK2 ("casein kinase-2"). *FEBS Lett.* **2001**, *496*, 44−48.
(20) Meggio, F.; Pagano, M. A.; Moro, S.; Zagotto, G.; Dal Ben, D.; Ruzzene, M.; Sarno, S.; Donella Deana, A.; Brunati, A. M.; Pinna, L. A. Unpublished results.
(21) Ruzzene, M.; Penzo, D.; Pinna, L. A. Protein kinase CK2 inhibitor 4,5,6,7-tetrabromobenzotriazole (TBB) induces apoptosis and caspase-dependent degradation of haematopoietic lineage cell-specific protein 1 (HS1) in Jurkat cells. *Biochem. J.* **2002**, *364*, 41−47.
(22) Mishra, S.; Reichert, A.; Cunnick, J.; Senadheera, D.; Hemmeryckx, B.; Heisterkamp, N.; Groffen, J. Protein kinase CKIIalpha interacts with the Bcr moiety of Bcr/Abl and mediates proliferation of Bcr/Abl-expressing cells. *Oncogene* **2003**, *22*, 8255−8262.
(23) Andrzejewska, M.; Pagano, M. A.; Meggio, F.; Brunati, M.; Kazimierczuk, Z. Polyhalogenobenzimidazoles: Synthesis and their inhibitory activity against casein kinases. *Bioorg. Med. Chem.* **2003**, *11*, 3997−4002.
(24) Pease, H. L.; Gardiner, J. A. Fluorometric and Colorimetric Procedures for Determination Residues of Benomyl. *J. Agr. Food Chem.* **1969**, *17*, 267−270.
(25) Meggio, F.; Shugar, D.; Pinna, L. A. Ribofuranosyl-benzimidazole derivatives as inhibitors of casein kinase-2 and casein kinase-1. *Eur. J. Biochem.* **1990**, *187*, 89−94.
(26) Szyszka, R.; Grankowski, N.; Felczak, K.; Shugar, D. Halogenated benzimidazoles and benzotriazoles as selective inhibitors of protein kinases CKI and CKII from *Saccharomyces cerevisiae* and other sources. *Biochem. Biophys. Res. Commun.* **1995**, *208*, 418−424.
(27) Davies, S. P.; Reddy, H.; Caivano, M.; Cohen, P. Specificity and mechanism of action of some commonly used protein kinase inhibitors. *Biochem. J.* **2000**, *351*, 95−105.
(28) Agullo, G.; Gamet-Payrastre, L.; Manenti, S.; Viala, C.; Remesy, C.; Chap, H.; Payrastre, B. Relationship between flavonoid structure and inhibition of phosphatidylinositol 3-kinase: A comparison with tyrosine kinase and protein kinase C inhibition. *Biochem. Pharmacol.* **1997**, *53*, 1649−1657.
(29) Meggio, F.; Donella Deana, A.; Pinna, L. Endogenous phosphate acceptor proteins for rat liver cytosolic casein kinases. *J. Biol. Chem.* **1981**, *256*, 11958−11961.

(30) Sarno, S.; Vaglio, P.; Meggio, F.; Issinger, O.-G.; Pinna, L. A. Protein kinase CK2 mutants defective in substrate recognition. Purification and kinetic analysis. *J. Biol. Chem.* **1996**, *271*, 10595−10601.
(31) Bain, J.; McLauchlan, H.; Elliott, M.; Cohen, P. The specificities of protein kinase inhibitors: An update. *Biochem. J.* **2003**, *371*, 199−204.
(32) Cheng, Y.-C.; Prusoff, W. H. Relationship between the inhibition constant (K1) and the concentration of inhibitor which causes 50% inhibition (I50) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099−3108.

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