Inhibition of Protein Kinase CK2 by Condensed Polyphenolic Derivatives. An in Vitro and in Vivo Study[†]

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ABSTRACT: ATP site-directed inhibitors that can target individual kinases are powerful tools for use in signal transduction research, all the more so in the case of a pleiotropic, constitutively active protein kinase such as CK2, which is not turned on in response to specific stimuli. By screening a library of more than 200 derivatives of natural polyphenolic compounds, we have identified 16 molecules which inhibit CK2 with IC_{50} values of ≤ 1 μM. They belong to the classes of anthraquinones (six compounds), xanthenones (two compounds), fluorenones (one compound), and coumarins (seven compounds), and their inhibitory potency correlates with the presence of nitro, amino, or halogen substituents at specific positions. Three of the most potent inhibitors, MNX (1,8-dihydroxy-4-nitroxanthen-9-one), NBC (8-hydroxy-4-methyl-9-nitrobenzo[*g*]chromen-2-one), and DBC (3,8-dibromo-7-hydroxy-4-methylchromen-2-one), whose IC_{50} values range between 0.13 and 0.36 μM, are quite specific toward CK2 within a panel of 33 protein kinases tested. Treatment of Jurkat cells with these compounds promotes inhibition of endogenous CK2 and induction of apoptosis. A correlation is observed between their efficacy as CK2 inhibitors (as judged from IC_{50} values) and their capacity to induce cell death (DC_{50} values). Mutations of the unique CK2α residues Val66 and/or Ile174 to alanine have a detrimental effect on inhibition by these compounds with 16−67-fold increases in IC_{50} values. The combined usage of these reagents can be exploited to gain information about cellular functions mediated by CK2.

CK2 (an acronym derived from the misnomer "casein kinase 2") probably is the most pleiotropic protein kinase known with more than 300 protein substrates identified to date (*1*). At variance with the great majority of protein kinases which are normally inactive and are turned on only in response to specific stimuli, the catalytic subunits (α and/or α′) of CK2 are constitutively active either alone or in combination with the regulatory β subunits to give a heterotetraneric holoenzyme ubiquitously expressed in all eukaryotic cells. The striking variety of its cellular targets and its absolute requirement for viability suggest that CK2 plays some essentiale role(s) and is implicated in many cellular functions with special reference to signal transduction, gene expression, and RNA and protein synthesis. However, its constitutive activity and insensitivity to any known physiological regulator have hampered till now a detailed understanding of the role(s) of CK2 within the cell.

A powerful tool for tracking cellular functions which are affected by CK2 can be provided by the development of highly specific, cell-permeable inhibitors. In perspective, these reagents could also have pharmacological potential, considering that protein kinases currently represent the second most important target for antineoplastic drugs, and a large panel of protein kinase inhibitors are already in clinical trials or even in clinical practice to cure tumors and other diseases (*2*, *3*). Although CK2 is constitutively active under normal conditions, a number of coincidental arguments and experimental models strongly suggest that its catalytic activity under certain circumstances can be responsible for the enhancement of the tumor phenotype (*4*). CK2 is in fact invariably elevated in a wide variety of tumors (*5*), and unscheduled expression of CK2 catalytic subunits displays an oncogenic potential in a number of cellular and animal models (*6*−*8*). In perspective, therefore, CK2 inhibitors could prove useful as leads for the development of antineoplastic drugs. An added value of these reagents could also be their exploitation in infectious diseases, considering that many viruses depend on host cell CK2 for the phosphorylation of proteins which are essential to their life cycle (*9*).

A number of CK2 inhibitors effective in the low micromolar range have been described so far, including 5,6-dichloro-β-ribofuranosylbenzimidazole (DRB[1]), emodin, tetrabromobenzotriazole (TBB), apigenin, and [5-oxo-5,6-

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dihydroindolo(1,2-*a*)quinazolin-7-yl]acetic acid (IQA) (*10*−*14*). DRB and apigenin are marketed as "specific" CK2 inhibitors; the former however displays rather high IC_{50} values (around 20 μM) and inhibits CK1 and possibly other protein kinases with comparable efficiency (*15*). Apigenin is more potent, but as in other general flavonoids, its specificity is quite broad (*14*, *16*). Emodin, among CK2 inhibitors, is of special interest as it is the active principle of *Rheum palmatum*, an herbal medicine widely used in Asia against several diseases, including leukemia (*11*). Although emodin also inhibits receptor tyrosine kinases of the HER-2 subfamily (*17*) with IC_{50} values around 10−20 μM, its effectiveness on CK2 is significantly higher (IC_{50} around 1 μM), suggesting that its biological effects might be mediated, at least in part, by CK2 inhibition. Emodin mimicks the proapoptotic effect of another, more specific, inhibitor of CK2, TBB (*18*, *19*), and its crystal structure in complex with the CK2 catalytic subunit (*20*) and the structures of three related compounds (*21*) have been solved. This prompted us to scrutinize a library of more than 200 compounds belonging to the same class as emodin (hydroxyanthraquinones) or to related chemical classes for their ability to inhibit CK2. This led to the identification of a number of compounds which are able to inhibit CK2 with efficiency and selectivity higher than those of emodin, both in vitro and in vivo. In this paper we report on the properties of these inhibitors and the generation of CK2 mutants refractory to their action.

MATERIALS AND METHODS

Materials. The chemical synthesis of the new compounds tested in the present study as CK2 inhibitors will be detailed elsewhere (S. Moro, G. Zagotto, et al., manuscript in preparation).

Source and Purification of Protein Kinases. Native casein kinases CK1 (nCK1) and CK2 (nCK2) and Golgi casein kinase (G-CK) were purified from rat liver (*22*) and from rat lactating mammary gland (*23*), respectively. Protein tyrosine kinases Lyn (*24*), c-Fgr (*25*), and Syk (also termed TPK-IIB) were purified from rat spleen. Human recombinant α and β subunits of CK2 were expressed in *Escherichia coli*, and the holoenzyme was reconstituted and purified as previously described (*26*). The V66A and I174A CK2 mutants were obtained as described previously (*14*). Recombinant Bud32/piD261 expressed in bacteria was kindly provided by Dr. S. Facchin (Padova, Italy). V66AI174A double mutant was obtained using the QuickChange site-directed mutagenesis kit (Stratagene, La Jolla, CA), and its cloning, expression, and purification will be detailed elsewhere. The source of all the other protein kinases is either described or referenced in refs *27* and *28*.

Phosphorylation Assay. All protein kinase activities were linear with respect to time and enzyme concentration in every incubation. Phosphorylation conditions and evaluation of the phosphate incorporated were as detailed in ref *18* for CK2, CK1, G-CK, piD261, and tyrosine kinases Lyn, c-Fgr, and Syk. All the other specificity assays were carried out using an automated multichannel pipet system (Multidrop 384, Thermo Life Sciences) at room temperature in a total assay volume of 25 μL as previously detailed (*14*).

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, the 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 indicated inhibitors. Control cells were treated with equal amounts of solvent. At the end of the incubations, the cells were lysed by the addition of hypoosmotic buffer, and CK2 activity was assayed toward CK2-specific pepide substrates, as described in ref *14*. Endogenous CK2 activity was estimated by immunoprecipitation of the HS1 protein, followed by Western blot detection of the upshift band, corresponding to the HS1 protein phosphorylated by CK2 (*18*). Cell viability was assessed by means of 3-(4,5-dimethylthiazol-2-yl)-3,5-diphenyltriazolium bromide (MTT) reagent (*19*).

Determination of Kinetic Parameters. Initial velocities were determined at each of the substrate concentrations tested. Inhibition data for a range of concentrations of each competitive inhibitor at a constant concentration of the nucleotide phosphate donor were plotted as a Dixon plot ([I] vs $1/v$) to give the IC_{50} value at that [S] as the opposite value of the x intercept (*29*). 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 (K_i) were then determined by linear regression analysis of K_m/V_{max} versus inhibitor concentration plots.

RESULTS AND DISCUSSION

Identification of Novel CK2 Inhibitors. A library of 197 compounds sharing polyphenolic scaffolds have been tested for their efficacy as inhibitors of CK2 as well as of other protein kinases, either Ser/Thr- or Tyr-specific (S. Moro, G. Zagotto, et al., manuscript in preparation). Sixteen compounds exhibited a remarkable inhibitory power toward CK2, with IC_{50} values (calculated at 20 μM ATP) of ≤ 1 μM. These are on display in Figure 1. A recurrent feature of the majority of the effective inhibitors is the presence of one or two nitro groups on their condensed aromatic rings. The crucial relevance of this feature in increasing inhibitory efficiency is highlighted by the observation that its removal invariably correlates with 1−3 orders of magnitude increases in IC_{50} values (data not shown). The solution of the crystal structure of CK2α in complex with compounds A2 (also termed MNA) and X2 (also termed MNX), in conjunction with molecular modelization (*21*), has revealed that the favorable effect of the nitro groups is not due to direct interaction with structural elements of the kinase. Rather it appears to be mediated by the capability of nitro groups to increase the dissociation constant (K_a) of phenolic groups, which, in anionic form, become more prone to interact with the kinase. Our present data corroborate the general validity of this

[1] Abbreviations: DRB, 5,6-dichloro-β-ribofuranosylbenzimidazole; TBB, 4,5,6,7-tetrabromobenzotriazole; IQA, [5-oxo-5,6-dihydroindolo-(1,2-*a*)quinazolin-7-yl]acetic acid; MNA, 1,8-dihydroxy-4-nitroanthracene-9,10-dione; MNX, 1,8-dihydroxy-4-nitroxanthen-9-one; DAA, 1,4-dihydroxy-5,8-diaminoanthracene-9,10-dione; NBC, 8-hydroxy-4-methyl-9-nitrobenzo[*g*]chromen-2-one; DBC, 3,8-dibromo-7-hydroxy-4-methylchromen-2-one; HS1, hematopoietic lineage cell-specific protein 1; DC_{50}, concentration required for a 50% reduction of cell viability.

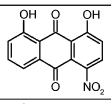
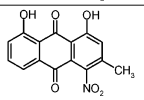
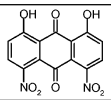
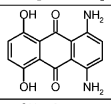
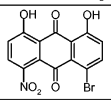
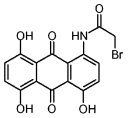
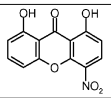
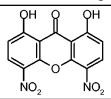
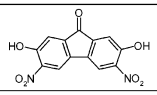
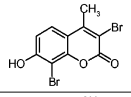
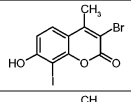
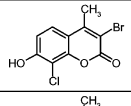
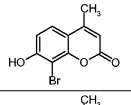
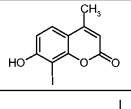
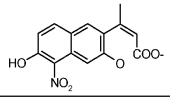
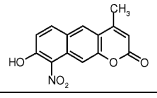
Derivative	Name	Structure	IC_{50} (μM)
A2	1,8-dihydroxy-4-nitro-anthracene-9,10-dione (MNA)		0.30 (K_i=0.78)
A7	1,8-dihydroxy-3-methyl-4-nitro-anthracene-9,10-dione		0.30 (K_i=0.95)
A70	1,8-dihydroxy-4,5-dinitro-anthracene-9,10-dione		0.30
A35	1,4-dihydroxy-5,8-diamino-anthracene-9,10-dione (DAA)		0.30 (K_i=0.42)
A54	1-bromo-4,5-dihydroxy-8-nitro-anthracene-9,10-dione		0.40
A36	1,4,5-trihydroxy-8-(2-bromoacetamido)-anthracene-9,10-dione		0.70
X2	1,8-dihydroxy-4-nitro-xanthen-9-one (MNX)		0.40 (K_i=0.80)
X3	1,8-dihydroxy-4,5-dinitro-xanthen-9-one		0.80
FL12	2,7-dihydroxy-3,6-dinitro-fluoren-9-one		1.00
C7	3,8-dibromo-7-hydroxy-4-methyl-chromen-2-one (DBC)		0.10 (K_i=0.06)
C41	3-bromo-8-iodo-7-hydroxy-4-methyl-chromen-2-one		0.28
C44	3-bromo-8-chloro-7-hydroxy-4-methyl-chromen-2-one		0.30
C24	7-hydroxy-8-bromo-4-methyl-chromen-2-one		0.70
C6	8-iodo-7-hydroxy-4-methyl-chromen-2-one		0.80
A69	3-(3,6-dihydroxy-5-nitro-naphtalen-2-yl)-but-2-enoate		1.00
C12	8-hydroxy-4-methyl-9-nitro-benzo[g]chromen-2-one (NBC)		0.30 (K_i=0.22)

FIGURE 1: Effective substituents within a CK2 inhibitor molecule. The molecular structures of 16 compounds displaying $IC_{50} \leq 1.0$ μM are shown. For some representative compounds the K_i values calculated with ATP as the phosphate donor are reported in parentheses. The values of IC_{50} and K_i represent the means of at least three independent experiments with SEM never exceeding 15%.

concept since the beneficial effect of the nitro groups disappears if the hydroxyl group(s) in the *para* position in compounds A2 and A7 is methylated (data not shown).

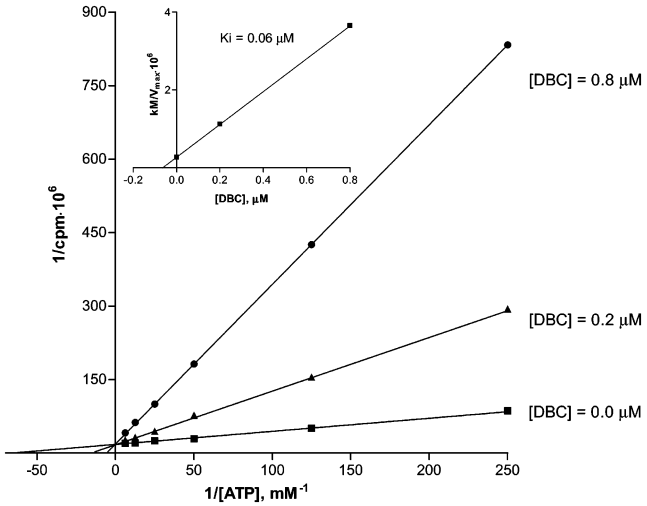


FIGURE 2: Lineweaver−Burk inhibition plots of protein kinase CK2 by DBC. The CK2 activity was determined as described in the Materials and Methods either in the absence (■) or in the presence of 0.2 (▼) and 0.8 (●) μM DBC. The data represent the means of experiments run in triplicate with SEM never exceeding 10%.

A number of compounds listed in Figure 1 display IC_{50} values of <1 μM although they are devoid of nitro groups. Of special interest are the anthraquinone derivative A35 (also termed DAA (*21*)) and the coumarin derivative C7 (thereinafter termed DBC) with IC_{50} values of 0.3 and 0.1 μM, respectively. The structural bases for inhibition by DAA have been revealed by X-ray crystallography showing that the two amino groups make polar interactions with the hinge region of CK2, thus stabilizing the complex with the inhibitor (*21*). The coumarin derivative C7 (DBC) has neither nitro nor amino groups in it; it bears instead two bromine atom substitutions at positions 3 and 8. The bromine at position 8 appears to play a crucial role as its removal causes a 40-fold increase in IC_{50} (not shown). This bromine can be effectively replaced by iodine and chlorine, giving rise to compounds with comparable inhibitory efficiencies (Figure 1, compounds C41 and C44).

It is tempting to speculate that the beneficial effect of the two bromines present in DBC is due to their large size and ability to make van der Waals contacts with unique apolar residues of CK2 by analogy with the four bromines present in TBB (tetrabromobenzotriazole), a powerful and quite specific inhibitor of CK2 whose structure in complex with the enzyme has been solved, revealing an almost perfect complementarity between the brominated benzene ring of the inhibitor and the hydrophobic cavity of the kinase (*30*).

Competitive Mode of Inhibition. By analogy with emodin (*11*) the new inhibitors were expected to be competitive with respect to ATP. This point has been confirmed with a number of compounds selected among the most effective (namely, A2 (MNA), A7, A35 (DAA), X2 (MNX), C7 (DBC), and C12 (NBC)) by showing that invariably the inhibitors increase the K_m for ATP but neither the K_{cat} nor the K_m for the phosphoacceptor peptide. A typical experiment of this kind run with the inhibitor C7 (DBC) is shown in Figure 2. By this approach the inhibition constants reported in Figure 1 were calculated. Since CK2 also works with the phospho-donor substrate GTP, we wanted to see if the inhibitors were equally efficient with GTP. This turned out to be the case, although in a few cases (e.g., A35 and X2) the K_i values

Table 1: IC_{50} Values (μM) of Representative Inhibitors Determined with Wild-Type and Mutated CK2 Holoenzyme[a]

derivative	CK2 wild type	CK2 Val66Ala	CK2 Ile174Ala	CK2 Val66A, Ile174A
emodin	0.89	38.00	7.30	49.25
MNA	0.30	4.00	5.50	10.51
DAA	0.92	14.80	28.00	20.83
FL12	0.68	3.66	35.00	31.77
MNX	0.34	1.40	19.50	23.04
NBC	0.36	4.44	3.11	6.93
C6	0.77	1.18	10.33	10.65
DBC	0.13	1.07	1.79	3.34
C37	3.90	27.00	59.90	
C44	0.86	3.02	12.07	15.9
A69	2.31	6.57	13.01	8.3
C24	0.71	1.76	16.88	20.48
C41	0.68	2.70	7.11	11.02
IQA	0.39	27.00	11.00	29.00
TBB	0.50	13.00	1.74	12.51
staurosporine	14.22	28.23	7.62	1.80

[a] CK2 holoenzyme was reconstituted in vitro by mixing equimolar amounts of recombinant α and β subunits. The values are the means of the results obtained in triplicate with SEM never exceeding 15%.

calculated with ATP and GTP were significally different (data not shown).

Generation of CK2 Mutants Variably Refractory to Inhibitors. To date the crystal structure of six complexes between CK2 and ATP site-directed inhibitors have been solved, notably those of emodin (*20*), DAA, MNA, MNX (denoted in Figure 1 as compounds A35, A2, and X2, respectively) (*21*), TBB (*30*), and the indoloquinazoline derivative IQA (*14*). A common feature of all these complexes is the entrance of the inhibitors in a hydrophobic cavity which in CK2 is smaller than in the majority of the other protein kinases, due to some residues, notably Val/Ile66, Ile174, and Met163, which in most kinases are replaced by smaller side chains. The actual relevance of these bulky residues was validated by generating two mutants of human CK2α, V66A, and I174A and by showing that both mutants were less susceptible than the wild type to inhibition by emodin, TBB (*18*), and IQA (*14*). The IC_{50} values reported in Table 1 show that the same applies to the new inhibitors listed in Figure 1, consistent with the view that they occupy the same hydrophobic pocket as emodin, TBB, and IQA. Note, however, that the relative effect of the two individual mutations is quite variable depending on the nature of the inhibitor, a clear indication that the orientation and/or the interactions of each ligand inside the cavity are different. In this respect inhibitors can be roughly divided into two categories depending on whether they are more sensitive to either the V66 (e.g., emodin, TBB, IQA) or the I174 (e.g., MNX, DAA, and in general the coumarin derivatives) mutations. We have also generated the double mutant V66AI174A expected to be even more refractory to individual inhibitors. This proved true in some cases, while in others, as shown in Table 1, the double mutation is just as effective or even less effective than the single mutations individually considered. Interestingly, staurosporine, which exceptionally is a very poor inhibitor of CK2 (*31*), tends to be nearly insensitive to individual mutations, while the double mutant is actually much more sensitive to staurosporine inhibition than the wild type. Note in this respect that staurosporine, which is by far the worst inhibitor of the CK2 wild type among those listed in Table 1, becomes the most efficient one if the double mutant V66AI174A is considered. The usefulness of this mutant to probe the implication of CK2 in cells treated with either a canonical CK2 inhibitor (e.g., emodin) or staurosporine is self-evident.

Selectivity of the New CK2 Inhibitors. To assess the degree of selectivity of the new inhibitors toward CK2, four of them chosen among the most effective, namely, compounds C7 (DBC), X2 (MNX), C12 (NBC), and A35 (DAA) (see Figure 1), were tested at 10 μM concentration for their ability to inhibit a panel of >30 protein kinases. The results were converted into histograms whose bars express percent inhibition, as shown in Figure 3. Collectively taken, the data indicate that while DAA is rather promiscuous the other three compounds are definitely more selective than emodin (*14*) and the flavonoids quercetin (*28*) and apigenin (*14*). DBC is less selective than MNX and NBC as, besides suppressing CK2 activity, it also drastically reduces the activities of DYRK1a, MAPKAP-K1a, MSK1, and PRAK. Interestingly, it discriminates quite well between MAPKAP-1a, which is 75% inhibited, and MAPKAP-K2, which is nearly unaffected. The other two inhibitors, MNX and, even more, NBC, are more selective: while CK2 activity is drastically reduced, all the other kinases are either unaffected or inhibited by <40%, with the only exception being DYRK1a, which is 49% inhibited by MNX. Susceptibility to CK2 inhibitors appears to be a feature of DYRK1a, considering that besides DBC also TBB inhibits DYRK1a as efficiently as CK2 (*14*), and even IQA, i.e., the most potent and specific CK2 inhibitor described so far, at 10 μM concentration inhibits by 50% DYRK1a activity (*14*). In this respect NBC is preferable over IQA itself as it displays a K_i almost identical with that of CK2 (0.22 vs 0.25 μM) while being significantly less effective on DYRK1a. The specificity of NBC and MNX is especially remarkable considering that their IC_{50} values (around 0.35 μM) are far below the inhibitor concentration used for the experiments of Figure 3. From these data it can be concluded that all the kinases of the panel display with NBC and MNX IC_{50} values which are more than 1 order of magnitude higher than the IC_{50} value of CK2.

New CK2 Inhibitors Are Cell Permeable, and Their Potency Correlates with Proapoptotic Efficacy. To check if the compounds which efficiently inhibit CK2 in vitro are also effective on it in vivo, Jurkat cells were treated with increasing concentrations of the inhibitors and the activity of endogenous CK2 was monitored by evaluating the gel upshift of the CK2 substrate HS1 protein (*18*) and by direct assay of CK2 activity in the cell lysates (*19*). By both criteria MNX, DBC, and NBC, but not related compounds devoid of inhibitory efficacy in vitro, were shown to inhibit endogenous CK2 in living cells with IC_{50} values ranging between 10 and 20 μM (data not shown). The requirement of higher concentrations as compared to those effective in vitro is a common feature of inhibitors which are competitive with respect to ATP (see, e.g., ref *19*), due, at least in part, to the high intracellular concentration of ATP (in the millimolar range) far beyond the ATP concentration used for in vitro experimentation (10−100 μM). Inhibition of endogenous CK2 by TBB and IQA is paralleled by a dose-dependent decrease in cell viability accounted for, at least in part, by apoptosis (*14*, *19*). To corroborate the concept that a cause−effect link exists between CK2 inhibition and

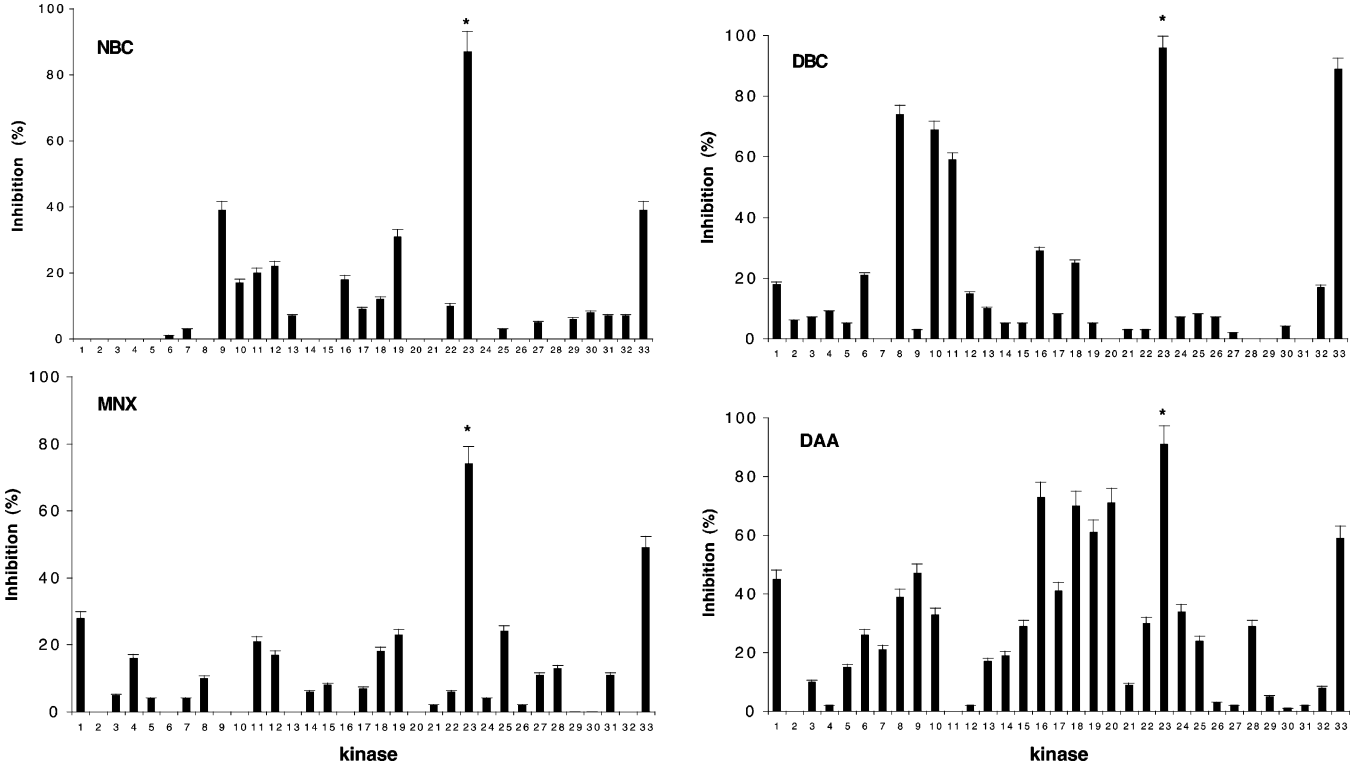


FIGURE 3: Selectivity spectrum of CK2 inhibitors NBC, MNX, DBC, and DAA. Inhibition, expressed as the percent of activity determined in the absence of inhibitor, was calculated from the residual activity measured in the presence of 10 μM inhibitor. Phosphorylation conditions are detailed in the Materials and Methods or have been described previously (*14*). All kinase assays were performed at 100 μM ATP except in the case of DAA, where each kinase was tested at an ATP concentration below K_m. The kinases examined were (1) MKK1, MAPK kinase (also called MEK), (2) MAPK2/ERK2, mitogen-activated protein kinase/extracellular signal-regulated kinase, (3) JNK/SAPK1c, c-Jun N-terminal kinase/stress-activated protein kinase, (4) SAPK2a/p38, (5) SAPK2b/p38β, (6) SAPK3/p38γ, (7) SAPK4/p38δ, (8) MAPKAP-K1a, MAPK-activated protein kinase, (9) MAPKAP-K2, (10) MSK1, mitogen and stress-activated protein kinase, (11) PRAK, p38-regulated/activated kinase, (12) PKA, cAMP-dependent protein kinase, (13) PKCα, protein kinase C, (14) PDK1, 3-phosphoinositide-dependent kinase, (15) PKBα, protein kinase B (also called Akt), (16) SGK, serum- and glucocorticoid-induced kinase, (17) p70S6K, ribosomal protein S6 kinase, (18) GSK3β, glycogen synthase kinase 3, (19) ROCKII, Rho-dependent protein kinase, (20) AMPK, AMP-activated protein kinase, (21) CHK1, checkpoint kinase, (22) PHK, phosphorylase kinase, (23) CK2, casein kinase 2, (24) CDK/cyclinA, cyclin-dependent kinase, (25) CK1, casein kinase 1, (26) G-CK, Golgi casein kinase, (27) PiD261, *Saccharomyces cerevisiae* casein kinase, (28) Lck protein tyrosine kinase, (29) Lyn protein tyrosine kinase, (30) c-Fgr protein tyrosine kinase, (31) Syk protein tyrosine kinase, (32) CSK, c-Src kinase, and (33) DYRK1a, dual-specificity tyrosine phosphorylation-regulated kinase. An asterisk denotes the position of protein kinase CK2.

Table 2: Correlation between CK2 Inhibition and Proapoptotic Efficacy of CK2 Inhibitors[a]

compound	name	IC_{50} (μM)	DC_{50} (μM)
TBB	4,5,6,7-tetrabromobenzotriazole	0.50	17
MNX	1,8-dihydroxy-4-nitroxanthen-9-one	0.40	17
X1	1,8-dihydroxyxanthen-9-one	>40	>100
MNA	1,8-dihydroxy-4-nitroanthraquinone	0.30	16
A1	1,8-dihydroxyanthraquinone	>40	>100
NBC	8-hydroxy-4-methyl-9-nitrobenzo[*g*]chromen-2-one	0.30	18
C11	8-hydroxy-4-methylbenzo[*g*]chromen-2-one	40	>100
IQA	(5-oxo-5,6-dihydroindolo(1,2-*a*)quinazolin-7-yl)acetic acid	0.30	16

[a] DC_{50} values (concentrations required for a 50% reduction of cell viability) were calculated from cell viability assays (see the Materials and Methods) run with increasing concentrations of CK2 inhibitors. Reported values represent the means ± SEM from three separate experiments. Cell death was shown to be due at least in part to apoptosis as judged from the generation of the PARP fragment and DNA laddering.

induction of apoptosis, advantage has been taken of the new CK2 inhibitors by assessing their ability to induce dose-dependent cell death. As shown in Table 2 a fairly good correlation was found between IC_{50} and DC_{50} values, consistent with the concept that inhibition of endogenous CK2 is instrumental to the observed cell death. This in all cases was shown to be accounted for, at least in part, by apoptosis, as judged from the generation of the PARP fragment and DNA fragmentation (not shown). In summary, these data provide further support to the emerging idea, originally grounded on experimentation with prostate cancer cell lines where the CK2 catalytic subunits were either down-regulated or overexpressed (*32*), that CK2 is an antiapoptotic agent whose catalytic activity is essential to cell survival under certain circumstances. A practical consequence would be that in perspective CK2 inhibitors may develop into antineoplastic agents to treat tumors whose aberrant phenotype is mainly due to defective apoptosis.

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