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Polyhalogenobenzimidazoles: Synthesis and Their Inhibitory Activity against Casein Kinases

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Abstract—A series of novel polyhalogenated benzimidazoles have been prepared by exhaustive bromination of a variety of 2-substituted benzimidazoles. The efficacy of both new compounds and a number of their previously described cognates as inhibitors of casein kinases CK1, CK2 and G-CK was investigated. The type of N-1 alkyl substituent as well as introduction of a polyfluoroalkyl moiety at position 2 did not markedly influence the inhibitory efficacy toward CK2 of the respective 4,5,6,7-tetrabromobenzimidazole derivatives which conversely were almost ineffective toward CK1 and G-CK. However, 4,5,6,7-tetrabromobenzimidazoles substituted at position 2 with either chlorine, bromine or sulfur atom, while manifesting a still considerable inhibitory activity against CK2 (IC_{50} in the 0.49–0.93 μM range) proved to be potentially powerful inhibitors also against CK1 (IC_{50} in the 18.4–2.2 μM range).

Introduction

Halogenated benzimidazoles have raised special interest because of their diversified biological activity. For instance, 5,6-dichlorobenzimidazole ribonucleoside (DRB) has been found to inhibit casein kinases and topoisomerase, as well as cellular and viral RNA synthesis.[1,2] However, this activity has been found to be associated with relatively high cytotoxicity and therefore this compound has found no practical use as an antiviral drug.[3,4] In recent years, other nucleosides of halogeno-substituted benzimidazoles were also synthesized that showed a remarkable activity against human cytomegalovirus.[5,6] Noteworthly is also a recent finding of potent antiviral properties of another halogenosubstituted, namely the L-ribonucleoside of 5,6-dichloro-2-isopropylaminobenzimidazole (1263W94). Due to its L-configuration, this derivative shows practically no toxicity against normal human cells. For this characteristic as well as its superior pharmacokinetics properties, it has recently been chosen for clinical trials.[7,8]

Not only halogenobenzimidazole nucleosides, but also the substituted benzimidazoles themselves are biologically active compounds. Of this latter class, modified 2-trifluoromethyl derivatives are worth of special attention. Substituted 2-trifluoromethyl-benzimidazoles were found particularly promising because of their herbicidal efficacy that is effected by the inhibition of photosynthesis.[9] Most recently, potent antiprotozoal activity, antibacterial and antifungal activities of benzene ring-halogenosubstituted 2-trifluoromethyl- and 2-pentafluoroethyl-benzimidazoles has been reported.[10–13] Recently we have found that the most potent anticancer agent among the studied collection of halogenated benzimidazoles was 5,6-dichloro-2-pentafluoroethylbenzimidazole that showed considerable activity against human breast and prostate cancer cell lines.[11] However, the molecular basis of those highly interesting biological properties remain relatively unexplored.

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In contrast, the main benzotriazole analogue, 4,5,6,7-tetrabromobenzotriazole (TBB), which is probably the most studied compound with similar structure, is much better understood as far as the biochemical and structural relationship with protein kinase CK2 are concerned. TBB has been actually demonstrated to represent a quite selective and effective ATP site-directed inhibitor of protein kinase CK2 with a K_i value in the submicromolar range.[14] No inhibition, in particular, was observed toward the other members of the 'casein kinases' subfamily, CK1 and the casein kinase of Golgi (G-CK), which are structurally unrelated to each other.[15–17] Further in vivo investigation showed that, in parallel to CK2 inhibition, TBB induces time- and dose-dependent cell death which is accounted for, at least in part, by apoptosis suggesting that CK2 could indeed counteract the programmed cell death.[18] The structural basis supporting such a selectivity toward CK2 has been recently investigated on a complex between *Zea mays* CK2 α and TBB resolved at 2.2 Å.[19] TBB is filling a hydrophobic pocket that is found in all protein kinases but whose shape and size in the case of CK2 is perfectly fitting to the inhibitor molecule.

Protein kinase CK2 is ubiquitously distributed among eukaryotic organisms where it most often appears to exist in tetrameric complexes composed of two catalytic (α and/or α′) and two regulatory (β) subunits. Genetic studies in yeast, worms and slime mold have demonstrated that CK2 is essential for viability (reviewed by Pinna and Meggio,[20] and Litchfield[21]) and overexpression of its catalytic subunit is correlated with lymphomas development in transgenic mice.[22] Its physiological role is still poorly understood although the preference for acidic substrates and the knowledge of its consensus sequence allowed the identification of more than 300 potential physiological targets committed with signal transduction, gene expression and development, metabolism, DNA repair and apoptosis[23] suggesting a particular role as a transducer of survival signals in the cell.[24] Much less is known from the structural point of view in the case of CK1, the other multifunctional ubiquitous casein kinase which, however, has been recently demonstrated to play fundamental roles in the transduction of the Wnt signals[25] and control of NF-AT transcription factor.[26] Even more obscure is finally the understanding of the authentic 'casein kinase', the G-CK, firstly detected in the Golgi of mammary gland but also present in the Golgi of other organs.[27]

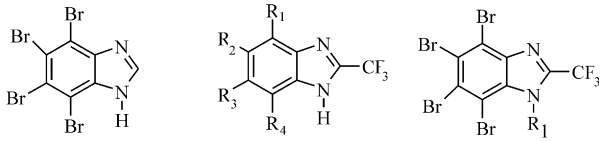
To investigate the physiological roles of these constitutively active protein kinases, the development of new specific and effective inhibitors would be of invaluable help. They could provide information about individual signal transduction pathways and, in perspective, they might have pharmacological applications in many pathologies.

In this report we present the synthesis of a number of benzimidazoles carrying two or more halogen atoms at the benzene ring of the benzimidazole core and additionally derivatized in position 2 by bromine, chlorine, sulfur or polyfluoroalkyl substituent. Additionally, their inhibitory activity against casein kinases is described and commented upon with respect to their structure.

Results and Discussion

Chemistry

To expand the group of halogenobenzimidazoles that included the previously described 4,5,6,7-tetrabromobenzimidazole[28] (**1**); 5-bromo-[29] (**2a**); 5,6-dibromo-[29] (**2b**); 4,6-dibromo-[29] (**2c**); 4,5,6-tribromo-[29] (**2d**); and 4,5,6,7-tetrabromo-[29] (**2e**) 2-trifluoromethylbenzimidazoles as well as the *N*-alkylated derivatives of **2e**, namely 1-methyl- and 1-ethyl-4,5,6,7-tetrabromobenzimidazoles[29] (**3a** and **3b**) (Scheme 1), we have synthesized several new ring-halogenated derivatives. Two important starting compounds namely 2-bromobenzimidazole (**5**) and 2,4,5,6,7-pentabromobenzimidazoles (**6**) have been mentioned in the literature.[30–33] The synthetic procedure used for synthesis of these derivatives given below have been just slightly modified compared to those previously described.[32,33] We obtained **5** in the reaction of 2-mercaptobenzimidazole (**4**) with bromine in mixture of aqueous HBr and methanol. The formed sulfinic and sulfonic intermediates are readily substituted by bromide from the reaction medium. Exhaustive bromination of **5** with bromine in water yielded **6** as the main product. Similarly to the above preparation, the bromination of 2-substituted benzimidazoles, that is 2-pentafluoroethyl-[11] (**7a**), 2-heptafluoropropyl- (**7b**), 2-nonafluorobutyl- (**7c**) and 2-chlorobenzimidazole (**7d**) provided 4,5,6,7-tetrabromocompounds **8a**,[11] **8b**, **8c** and **8d**, respectively. The mixed chloro-bromo-2-trifluoromethylbenzimidazoles **8e**, **8f** and **8g** were obtained by exhaustive bromination of 5-chloro-2-trifluoromethyl- (**7e**); 4,6-dichloro-2-trifluoromethyl- (**7f**) and 5,6-dichloro-2-trifluoromethylbenzimidazole (**7g**), respectively. The reaction of **6** with thiourea gave as expected 4,5,6,7-tetrabromo-2-thiobenzimidazole (**9**) (Scheme 2). The resulting derivatives were purified by crystallization and characterized by ^{1}H NMR, UV-, mass spectra and elemental analyses. Especially mass spectroscopy was found useful for identifying the obtained compounds, the more so because of the characteristic structure of the respective molecular peaks that was due to specific isotope composition of



1 [28]

2a-e

a R_1, R_3, R_4 = H, R_2 = Br [29]
b R_1, R_4 = H, R_2, R_3 = Br [29]
c R_2, R_4 = H, R_1, R_3 = Br [29]
d R_4 = H, R_1, R_2, R_3 = Br [29]
e R_1, R_2, R_3, R_4 = Br [29]

3a,3b

a $R_1 = CH_3$ [29]
b $R_1 = C_2H_5$ [29]

Scheme 1. Structures of previously described polyhalogenated benzimidazoles used for casein kinases tests.[28,29]

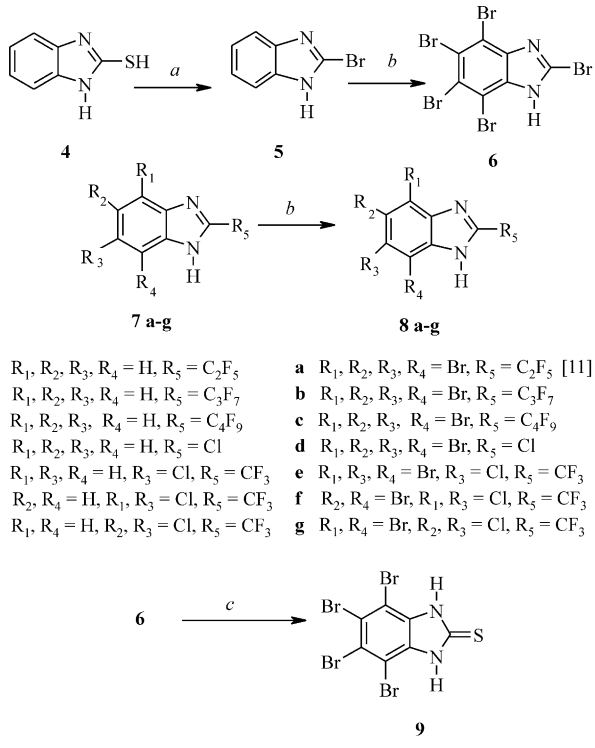
halogen substituents. In the experimental section of this report, only the structures of the molecular peaks are shown.

Inhibition of casein kinases by benzimidazoles

Halogenated benzimidazole ribonucleotides but also halogenated benzimidazoles themselves were previously found to inhibit CK1 and CK2 in a competitive manner with respect to the phosphate donors ATP and GTP.[34] In order to assess now the biochemical effect of variable substitutions introduced on the new benzimidazoles we determined the IC_{50} of various derivatives on the activity of CK1, CK2 and G-CK, each tested in in vitro phosphorylation experiments on their specific peptide substrates. The data obtained are reported in Table 1. Even a cursory examination of this data reveals that CK2 is variably affected by most of the polyhalogenated benzimidazoles as it was expected on the basis of previous work firstly performed on some ribofuranosylbenzimidazole derivatives[34] and later confirmed also on their benzotriazole analogues.[14,35] In contrast, only three compounds, namely compounds **6**, **8d** and **9** significantly inhibit CK1, while G-CK was completely unaffected by all derivatives tested. The efficient inhibition of CK2 and the rather unexpected effect on CK1, however, can be more deeply analysed leading to the following observations:

a. Most efficient inhibition of protein kinase CK2 is attained only if the benzene ring of the benzimidazoles is tetrahalogenated. This is clearly shown by comparing IC_{50} for CK2 of the five derivatives **2a–e** of Table 1, which are gradually decreasing from more than 40–0.6 μM while the number of halogens increases from 1 to 4. It is evident that bromine is preferred over chlorine at least when we replace two out of the four substituents (compare **2e** with **8f** and **8g**). This latter finding is in agreement with previous observations demonstrating that dibromo-substituted ribofuranosylbenzimidazoles were 5-fold more effective than the correspondent chlorinated derivatives.[34] In contrast, the replacement of a single bromine for chlorine at position 5 of the benzene ring (derivative **8e**) is apparently without consequences on the inhibitory activity exhibiting an IC_{50} even slightly lower than that of the control (0.39 μM vs 0.6 μM).

b. The substitution of the hydrogen atom at position 1 on the imidazole ring with a methyl (derivative **3a**) or an ethyl group (derivative **3b**) does not improve the efficiency of the trifluoromethyl substituted inhibitors giving rise to IC_{50} values for CK2 slightly but significantly higher than those observed with the control derivative **2e**. This is apparently true also in absence of the 2-trifluoromethyl substituent because the 4,5,6,7-tetrabromo-1-methylbenzimidazole showed an IC_{50} value toward CK2 only slightly higher (0.6 μM) than that obtained with control derivative **1** (data not shown).



Scheme 2. Reagents and conditions: (a) HBr,Br_2, 1 h, 5–10 °C; (b) Br_2, water, 24 h, reflux; (c) thiourea, EtOH, 5 h, reflux.

Table 1. Inhibition constants (μM) of benzimidazole derivatives for casein kinases CK1, CK2 and G-CK[a]

Derivative	Name	CK1	CK2	G-CK
1	4,5,6,7-Tetrabromobenzimidazole	40.0	0.50	>40.0
2a	5-Bromo-2-trifluoromethylbenzimidazole	>40.0	>40.0	>40.0
2b	5,6-Dibromo-2-trifluoromethylbenzimidazole	>40.0	28.00	>40.0
2c	4,6-Dibromo-2-trifluoromethylbenzimidazole	>40.0	>40.0	>40.0
2d	4,5,6-Tribromo-2-trifluoromethylbenzimidazole	>40.0	1.20	>40.0
2e	4,5,6,7-Tetrabromo-2-trifluoromethylbenzimidazole	>40.0	0.60	>40.0
3a	4,5,6,7-Tetrabromo-1-methyl-2-trifluoromethylbenzimidazole	>40.0	1.71	>40.0
3b	4,5,6,7-Tetrabromo-1-ethyl-2-trifluoromethylbenzimidazole	>40.0	6.12	>40.0
6	2,4,5,6,7-Pentabromobenzimidazole	18.4	0.69	>40.0
8a	4,5,6,7-Tetrabromo-2-pentafluoroethylbenzimidazole	40.0	0.40	>40.0
8b	4,5,6,7-Tetrabromo-2-heptafluoropropylbenzimidazole	>40.0	1.28	>40.0
8c	4,5,6,7-Tetrabromo-2-nonafluorobuthylbenzimidazole	>40.0	1.48	>40.0
8d	4,5,6,7-Tetrabromo-2-chlorobenzimidazole	15.4	0.49	>40.0
8e	4,6,7-Tribromo-5-chloro-2-trifluoromethylbenzimidazole	40.0	0.39	>40.0
8f	4,6-Dibromo-5,7-dichloro-2-trifluoromethylbenzimidazole	>40.0	1.25	>40.0
8g	4,7-Dibromo-5,6-dichloro-2-trifluoromethylbenzimidazole	>40.0	1.96	>40.0
9	4,5,6,7-Tetrabromo-2-mercaptobenzimidazole	2.2	0.91	>40.0

[a]The data are the mean of values obtained in triplicate with SD not exceeding 15%.

c. Position 2 on the imidazole ring, which is facing the important K68 residue of CK2 catalytic pocket according to the crystal structure of the complex CK2 α-TBB[19] is not apparently involved in the stabilization of the binding of the inhibitor. In fact, the substitution of the hydrogen atom bound to C2 in derivative **1** with other individual atoms (Br, Cl or S in derivatives **6**, **8d** and **9**, respectively) or even with larger groups (trifluoromethyl-, pentafluoroethyl-, heptafluoropropyl- and nonafluorobuthyl in derivatives **2e**, **8a**, **8b** and **8c**, respectively) only slightly reduces the inhibitory potency toward CK2. Collectively taken, these data would indicate that the main contacts between this type of inhibitors and the nucleotide cavity of CK2 are occurring only at the benzenic side of the benzimidazole molecule while the imidazolic moiety appears rather unimportant.

d. The opposite is true in the case of protein kinase CK1, whose inhibition becomes significant in the case of derivatives **6**, **8d** and **9**, in which position 2 of the imidazole has been substituted by individual atoms bromine, chlorine and sulfur, respectively. This latter displays an IC_{50} in the low micromolar range. Such a favourable effect was not observed in the case of CK2 suggesting that position 2 on the imidazole ring could represent a suitable anchor for new substituents able to generate powerful and selective inhibitors of CK1, which are not available to date. Commercially available inhibitors of CK1 in fact are poorly specific and only in a very few cases in the literature compounds have been described displaying for CK1 efficiencies below the micromolarity.[36]

Experimental

Chemical synthesis

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 in a Kontron Uvikon 940 spectrophotometer. ^{1}H NMR spectra (in ppm) were measured with a Varian Gemini 200 MHz (or a Varian UNITY plus 500 MHz) spectrometer at 298 K in DMSO-d_6 using tetramethylsilane as internal standard. Mass-spectra (70 eV) were obtained with an AMD-604 (Intectra) spectrometer. Flash chromatography was performed with Merck silica gel 60 (200–400 mesh). Analytical TLC was carried out on precoated silica gel F_{254} (Merck) plates (0.25 mm thickness). Analyses of the new compounds, indicated by the symbols of the elements, were within ±0.4% of the theoretical values. 4,5,6,7-Tetrabromo-1-methylbenzimidazole was a generous gift from Dr. M. Bretner (Institute of Biochemistry and Biophysics, Warsaw, Poland).

2-Bromobenzimidazole (5). 2-Mercaptobenzimidazole (**4**) (5 g, 0.033 mol) was dissolved in pre-cooled (5–10 °C) mixture of hydrobromic acid (48% in water, 12 mL) and methanol (30 mL). The reaction mixture was stirred and bromine (6.5 mL, 0.13 mol) was added portionswise within 45 min while temperature was maintained at 5–10 °C using ice-bath. The stirring was continued for additional 1 h and during this time a solid precipitated. This was filtered off, dissolved in MeOH–aq ammonia mixture (3:1, 40 mL) and treated with charcoal. The pale-yellow solution was brought to pH 4–5 with acetic acid to give **5** as white, chromatographically pure precipitate (3.0 g, 46%). A small amount was crystallized for analytical purposes from MeOH–water. Mp 191–193 °C (lit.[33] 190–192 °C). TLC ($CHCl_3$–MeOH, 9:1) R_f 0.63. UV (pH 2): 273 (6 200), 280 (6 700); (pH 6.5): 273 (4700), 280 (4700); (pH 12): 278 (10,300), 284 (9600). ^{1}H NMR (Me_2SO-d_6) δ (ppm): 7.22 and 7.51 (2m, H-benzimid), 13.2 (bs, H–N); MS *m*/*z*: 196 (100, M^+), 197 (9, $M^+ + 1$), 198 (98, $M^+ + 2$), 199 (9, $M^+ + 3$). Anal. calcd for $C_7H_5N_2Br$ (197.035): C, 42.67; H, 5.04; N, 14.22. Found: C, 42.55; H, 5.06; N, 14.01.

Brominated benzimidazoles. 2,4,5,6,7-Pentabromobenzimidazole (6). To a stirred and refluxed suspension of **5** (1.5 g, 7.6 mmol) in water (60 mL) bromine (8 mL, 160 mmol) was added portionwise within 6 h. The reflux was continued for 24 h. The reaction mixture was cooled and the orange precipitate was filtered off. This was dissolved in MeOH–aq ammonia (3:1, v/v), treated with charcoal and cellite. The pale-yellow solution was brought to pH 4–5 with acetic acid and the formed precipitate crystallized from MeOH–water (1:1) to give **6** (2.9 g, 69%); mp 269–270 °C. TLC ($CHCl_3$–MeOH, 9:1): R_f 0.65; UV (MeOH/H_2O, 1:1): 301 nm (7700). ^{1}H NMR (Me_2SO-d_6) δ (ppm): 14.0 (bs, H–N). MS *m*/*z*: 510 (47, $M^+ - 2$,), 511 (5, $M^+ - 1$), 512 (100, M^+), 513 (8, $M^+ + 1$), 514 (96, ($M^+ + 2$), 515 (8, $M^+ + 3$), 516 (45, $M^+ + 4$). Anal. calcd for $C_7HN_2Br_5$ (512.619): C, 16.40; H, 0.02; N, 5.46. Found: C, 16.27; H, 0.38; N, 5.28.

2-Heptafluoropropyl-4,5,6,7-tetrabromobenzimidazole (8b). Similar to **6**, from **7a**: (mp 248–250 °C, from EtOH/H_2O, 55%). TLC ($CHCl_3$–MeOH, 9:1): R_f 0.76. UV (MeOH/H_2O, 1:1): 292 (10,900), 304 (8900). ^{1}H NMR (Me_2SO-d_6) δ (ppm): 14.4 (bs, H–N). MS *m*/*z*: 599 (66, $M^+ - 2$), 600 (8, $M^+ - 1$), 601 (100, M^+), 602 (12, $M^+ + 1$), 603 (63, $M^+ + 2$), 604 (7, $M^+ + 3$), 605 (15, $M^+ + 4$). Anal. calcd for $C_{10}HN_2Br_4F_7$ (601.278): C, 19.98; H, 0.17; N, 4.66. Found: C, 19.77; H, 0.31; N, 4.50.

2-Nonafluorobutyl-4,5,6,7-tetrabromobenzimidazole (8c). Similar to **6** starting from **7c**: (mp 221–223 °C, from EtOH/H_2O, 37%). TLC ($CHCl_3$–MeOH, 9:1): R_f 0.80. UV (MeOH/H_2O, 1:1): 294 (11 400), 304 (10 100). ^{1}H NMR (Me_2SO-d_6) δ (ppm): 14.5 (bs, H–N). MS *m*/*z*: 649 (69, $M^+ - 2$), 650 (9, $M^+ - 1$), 651 (100, M^+), 652 (12, $M^+ + 1$), 653 (66, $M^+ + 2$), 654 (8, $M^+ + 3$), 655 (16, $M^+ + 5$). Anal. calcd for $C_{11}HN_2Br_4F_9$ (651.741): C, 20.27; H, 0.15; N, 4.30. Found: C, 20.04; H, 0.33; N, 4.14.

2-Chloro-4,5,6,7-tetrabromobenzimidazole (8d). Similar to **6** starting from **7d**: (mp 298–300 °C, from EtOH/

H_2O, 55%). TLC ($CHCl_3$–MeOH, 9:1): R_f 0.56. UV (MeOH/H_2O, 1:9): (pH 2): 267 (8500), 302 (5300), (pH 6): 291 (6000), 302 (4800), (pH 12): 272 (10,400) 302 (6500). ^{1}H NMR (Me_2SO-d_6) δ (ppm): 14.2 (bs, H–N). MS *m*/*z*: 466 (62, M^+-2), 467 (5, M^+-1), 468 (100, M^+), 469 (9, M^++1), 470 (83, M^++2), 471 (7, M^++3), 472 (31, M^++4). Anal. calcd for $C_7HN_2Br_4Cl$ (468.168): C, 17.96; H, 0.22; N, 5.98. Found: C, 17.80; H, 0.34; N, 5.77.

4,6,7-Tribromo-5-chloro-2-trifluoromethylbenzimidazole (8e). Similar to **6** starting from **7e**: (mp 261–263 °C, from EtOH/H_2O, 82%). TLC ($CHCl_3$–MeOH, 9:1): R_f 0.80. UV (MeOH/H_2O, 1:9): (pH 2): 279 (9100), 303 (5200), (pH 6): 280 (8800), 301 (6400), (pH 12): 301 (10,600). ^{1}H NMR (Me_2SO-d_6) δ (ppm): 13.6 (bs, H–N). MS *m*/*z*: 456 (82, M^+-2), 457 (8, M^+-1), 458 (100, M^+), 459 (9, M^++1), 460 (47, M^++2), 461 (37, M^++3), 462 (8, M^++4). Anal. calcd for $C_8HN_2Br_3ClF_3$ (457.269): C, 21.01; H, 0.22; N, 6.13. Found; C, 20.88; H, 0.34; N, 5.93.

4,6-Dibromo-5,7-dichloro-2-trifluoromethylbenzimidazole (8f). Similar to **6** starting from **7f**: (mp 264–266 °C, from EtOH/H_2O, 87%). TLC ($CHCl_3$–MeOH, 9:1): R_f 0.63. UV (MeOH/H_2O, 1:9): (pH 2): 276 (7200), 302 (3200), (pH 6): 291 (6000), 302 (4800), (pH 12): 300 (10,000). ^{1}H NMR (Me_2SO-d_6) δ (ppm): 14.0 (bs, H–N). MS *m*/*z*: 410 (39, M^+-2), 411 (4, M^+-1), 412 (100, M^+), 413 (10, M^++1), 414 (92, M^++2), 415 (9, M^++3), 416 (32, M^++4). Anal. calcd for $C_8HN_2Br_2Cl_2F_3$ (415.818): C, 23.11; H, 0.24; N, 6.74. Found: C, 22.91; H, 0.40; N, 6.55.

4,7-Dibromo-5,6-dichloro-2-trifluoromethylbenzimidazole (8g). Similar to **6** starting from **7g**: (mp 252–254 °C, from EtOH/H_2O, 87%). TLC ($CHCl_3$–MeOH, 9:1): R_f 0.62. UV (MeOH/H_2O, 1:9): (pH 2): 279 (10,200), (pH 6): 291 (8400), 300 (8100), (pH 12): 300 (12,200). ^{1}H NMR (Me_2SO-d_6) δ (ppm): 14.0 (bs, H–N). MS *m*/*z*: 410 (38, M^+-2), 411 (5, M^+-1), 412 (100, M^+), 413 (9, M^++1), 414 (88, M^++2), 415 (9, M^++3), 416 (29, M^++4). Anal. calcd for $C_8HN_2Br_2Cl_2F_3$ (415.818): C, 23.11; H, 0.24; N, 6.74. Found: C, 22.93; H, 0.41; N, 6.58.

4,5,6,7-Tetrabromo-2-mercaptobenzimidazole (9). To the stirred solution of **6** (510 m, 1 mmol) in EtOH (25 mL) thiourea (83 mg, 1.1 mmol) was added. The reaction mixture was refluxed for 5 h. A precipitate formed was filtered, dissolved in EtOH/aq ammonia (1:1) and precipitated by addition of acetic acid to pH 4–5. The purification was repeated to give chromatographically pure **9** (200 mg, 43%); mp >320 °C (decomp.) (for analysis cryst. from EtOH). TLC ($CHCl_3$–MeOH, 9:1): 0.81. UV (MeOH/H_2O, 1:9): (pH 2): 256 (16,800, 293 (4600), 327 (12,900), (pH 6): 255 (17,600), 292 (4600), 327 (14,900), (pH 12): 293 (9100). ^{1}H NMR (Me_2SO-d_6) δ (ppm): 13.3 (bs, H–N). MS *m*/*z*: 464 (70, M^+-2), 465 (7, M^+-1), 466 (100, M^+), 467 (10, M^++1), 468 (67, M^++2), 469 (6, M^++3), 470 (19, M^++4). Anal. calcd for $C_7H_2N_2Br_4S$ (465.783): C, 18.05; H, 0.43; N, 6.01. Found: C, 18.23; H, 0.55; N, 5.85.

Biological evaluation

Materials. CK1 and CK2 were partially purified from rat liver cytosol as previously described.[37] G-CK was purified from Golgi apparatus of rat lactating mammary gland as described by Lasa et al.[27] Synthetic peptide substrates were kindly provided by Dr. O. Marin (Padova, Italy).

Phosphorylation. Phosphorylation assays were performed by incubating the three protein kinases CK1, CK2 and G-CK in the absence and in the presence of various inhibitors under their optimal conditions. In detail, the activities of protein kinases CK1 and CK2 were tested in 50 mM Tris–HCl pH 7.5 containing 10 mM $MgCl_2$, 100 mM NaCl, 20 μM γ^{33}P-ATP (specific activity of about 1000 cpm/pmol) and the specific peptide substrate (100 μ M RRRADDSDDDDD and 200 μ M RRKHAAIGDDDDAYSITA for CK2 and CK1, respectively). The activity of G-CK was assayed on the specific peptide substrate KIEKFQSEEQQQ (0.5 mM) under identical conditions except that $MnCl_2$ was replacing $MgCl_2$ and NaCl was omitted.

Acknowledgements

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