Tetraiodobenzimidazoles are potent inhibitors of protein kinase CK2

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A R T I C L E   I N F O

Article history:
Received 1 April 2009
Revised 20 July 2009
Accepted 21 August 2009
Available online 27 August 2009

Keywords:
Tetraiodobenzimidazoles
Tetrabromobenzimidazoles
CK2 inhibitors
Molecular modeling

1. Introduction

The crucial role of protein kinases in nearly all cellular processes and the growing list of kinases whose dysregulation underlies pathological conditions (www.cellsignal.com/reference/kinase_disease.html) highlight the importance of kinases as drug targets.1,2 Protein kinases have been actually implicated not only in oncology but also in a number of non-oncology indications including central nervous system disorders, autoimmune disease, post-transplant immunosuppression, osteoporosis, and several metabolic disorders like diabetes. Of special interest, among the large family of protein kinases, appears the regulation of kinases implicated in fundamental processes of cell life like proliferation, survival and apoptosis owing to the crucial commitment with the development and progression of cancer. The search of small molecules capable of down-regulating kinase activity was primarily addressed toward the nucleotide pocket of the kinase3 exploiting structural differences in this region for molecular recognition.4 However, due to the high conservation of the ATP-binding site, development of absolutely specific ATP-competitive inhibitors is hard to attain and the possibility to develop allosteric inhibitors is being explored.4

Protein kinase CK2, formerly known as casein kinase-2 or II, is a multifunctional serine/threonine protein kinase that specifically phosphorylates residues located in acidic sequences of more than 300 substrates most of which are phosphorylated also in vivo.5 It is ubiquitously distributed, constitutively active and participates in a wide variety of cellular processes including gene expression, cell differentiation and proliferation and its activity has been associated with a number of pathologies including inflammation, infection, and tumorigenesis.6–10 CK2 overexpression has been invariably observed in transformed tissues and it appears to represent an unfavorable prognostic marker in some types of cancer (e.g., prostate and lung carcinomas and in acute myeloid leukemia).11,12 The nucleotide pocket of CK2, which can utilize both ATP and GTP as phosphate donors, displays unique features for being smaller than it is in most other kinases due to the presence of some unique bulky residues hampering the entrance of large molecules like staurosporine, a very powerful, highly promiscuous inhibitor of most protein kinases but weakly effective on CK2.13,14 By contrast, smaller molecules which are not retained in the hydrophobic pocket of most kinases are firmly entrapped in that of CK2, resulting in efficient inhibition. The molecular structure of CK2 in mammals invariably consists of a heterotetrameric holoenzyme composed of two catalytic (α and/or α′) and two regulatory (β) subunits although emerging evidence would indicate specific roles also for the isolated subunits under certain circumstances.15 Searching for CK2 inhibitors, just in the late 1980s polyhalogenated benzimidazoles were found to represent a valuable scaffold to effectively compete with ATP binding16 and 4,5,6,7-tetrabromobenzimidazole (TBB) was later demonstrated to be one of the most powerful and selective cell permeable inhibitors of CK2.17,18 The presence of four bromine atoms on the benzene ring of TBB is critical to fill the hydrophilic pocket adjacent to the ATP-binding site and to assure optimal apolar interactions with...
some bulky side chains, in particular those of Val66 and Ile174.\textsuperscript{13,14} A direct correlation was also recently established for TBB derivatives between the log $K_i$ and the variation in the accessible surface area upon binding on CK2.\textsuperscript{13} From this point of view, it was known since longtime that the reduction of the number of bromine atoms or their replacement by less bulky halogens like chlorine and fluorine severely impacts the inhibitory efficiency.\textsuperscript{16,19} However, the replacement of all four bromine atoms of the benzimidazole or benzotriazole moiety by bulkier iodines was hard to attain and led to iodo-bromo or iodo-chloro benzimidazoles were unknown. Recently, structurally related tetraiodinated-isouido derivatives were synthesized and found to inhibit protein kinase CK2 in an ATP-competitive manner with IC$_{50}$ values ranging between 0.15 and 1.5 $\mu$M.\textsuperscript{20} The highest efficiency documented in the literature for a CK2 inhibitor belongs to a class of pyrazolo-triazine derivatives reported to affect CK2 with $K_i$ values in the nanomolar and subnanomolar range.\textsuperscript{21,22} Regrettably, however, the experimental conditions for these assays were not detailed; this hampers reliable comparison with other inhibitors. In this paper the synthesis of a number of tetraiodinatedbenzimidazoles is described for the first time together with a structure–activity analysis in comparison with the corresponding tetrabrominated derivatives. In most cases the replacement of bromines by iodines increases the potency of the inhibitor reaching with some derivatives IC$_{50}$ values in the low nanomolar range.

2. Results and discussion

2.1. Chemical syntheses

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

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

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

2.2. Biological activity

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

![Scheme 1. Reagents and conditions: (a) I$_2$, H$_2$IO$_6$, H$_2$SO$_4$, 3 h, 5–60 °C.](image-url)
displaying a classical double-reciprocal plot allowing to calculate, in the case of 4,5,6,7-tetraiodobenzimidazole (1a or TIBI), a $K_i$ value of 23 nM (Fig. 1A). Conversely, inhibition is non-competitive with respect to the peptide phosphoacceptor substrate (Fig. 1B) further supporting a mechanism of action implying the occupancy of the nucleotide binding pocket in competition with ATP. Moreover, the performance of compound 1a allows to definitely rank the efficiency of halogens as substituents of the benzene ring, iodine being the best, followed by bromine (0.023 vs 0.3 μM by comparing, compounds 1a and K17 (also termed TBI) of Table 1) which in turn proved to be about two orders of magnitude more effective than chlorine, 4,5,6,7-tetrachlorobenzimidazole (TCI) exhibiting a $K_i$ value of 21 μM under comparable conditions. This came not as a surprise considering the data already available for di-substituted benzimidazole derivatives in which fluorine was found to be the least effective. However, a deeper analysis of the $K_i$ values of tetraiodinated versus tetrabrominated compounds highlights that the presence of an additional substitution on the imidazole ring may affect not only the absolute potency of the inhibitor but also its relative efficacy with respect to the brominated homologue. In fact, all the four compounds bearing a substitution at position 1 of the imidazole ring (compounds 21, 23, 25 and 27 of Table 1) display inhibition constants significantly higher and close to those of the correspondent brominated derivatives.

![Scheme 2](image_url)

**Scheme 2.** Reagents and conditions: (a) 2-aminoethanol, EtOH; (b) thiourea, MeOEtOH; (c) BrCH2COOH, K2CO3, 2-butanone, reflux, 3 h; (d) acetic acid, sodium acetate, reflux, 20 h.

![Scheme 3](image_url)

**Scheme 3.** Reagents and conditions: (a) BrCH2COOEt, K2CO3, 2-butanone, reflux, 3 h; (b) NaOH, EtOH–H2O, 50 °C, 3 h; (c) ICH2CONH2, K2CO3, 2-butanone, reflux, 3 h; (d) H2NCH3, EtOH, rt, 2 d; (e) H2NNH2/H2O, EtOH or MeOEtOH.
2.3. Molecular modeling studies

Searching for a plausible structural explanation of the superiority of tetraiodinated derivatives as CK2 inhibitors, a molecular modeling approach was undertaken. Starting from the crystal structure of K17 in complex with ZmCK2α subunit (PDB code 2OXY)\(^{13}\) we determined the binding mode of compound \(1a\) by a molecular docking strategy, in comparison with that of the tetrabromo (TBI, K17) and tetrachloro (TCI) homologues, using human CK2 as target protein (PDB code: 1JWH)\(^{31}\) (Fig. 2). All three inhibitors lay inside the CK2 binding cleft exactly at the same position as K17 in ZmCK2α, revealing identical binding modes. However, compound \(1a\) is able to fill better the CK2 binding cleft due to the increased dimensions of iodine substituents with respect to bromine and chlorine. To better validate this hypothesis we calculated the percentage of human CK2 ATP-binding cleft occupancy (occupancy factor, OF) by the three analogs. The CK2 ATP-binding pocket volume was calculated as described in Section 3 by using the apo crystal structure 1JWH plus one conserved water molecule.\(^{13}\) The OF values were calculated relative to the binding cleft and the inhibitor’s volume. As also shown in Fig. 2, TCI shows a low binding cleft OF value (69%), and tetrabromine substitution is responsible of an increased occupancy up to 75% while the most potent inhibitor \(1a\) (TBI), bearing the iodine substitution, fills by 86% the CK2 ATP-binding pocket. Almost identical conclusions can be drawn by comparing the increased hydrophobicity with the increased inhibitory potency of the tetrahalogenated derivatives. Moreover, iodine atoms of derivative \(1a\) are able to perform stronger halogen bonds with the binding cleft, in particular with the backbone carbonyls of Glu114 and Val116 of the hinge region.\(^{13}\) In fact, even if all four halogen atoms are capable of acting as halogen bond donors, iodine usually forms the strongest interactions, followed by bromine and chlorine atoms, respectively.\(^{26}\) The same arguments apply also to the other tetraiodobenzimidazole derivatives considered in this paper, all displaying in general an inhibitory efficiency higher than that of their brominated counterparts. Note, however, that a double mutant of CK2 in which two bulky residues of the hydrophobic pocket have been replaced by smaller ones (V66I174AA) undergoes a 70- and 16-fold reduced inhibition by compounds \(1a\) and TBI, respectively (data not shown). This corroborates the view that, a part from halogen bonds, occupancy and hydrophobic interactions play a crucial role in optimizing the performance of tetraiododervatives.

3. Experimental

3.1. Chemical synthesis

3.1.1. General procedure

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

3.1.2. General procedure for iodination of benzimidazole derivatives

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

Periodic acid (2.28 g, 10 mmol) was dissolved in concd H\(2\)SO\(_4\) (50 mL). Finely powdered iodine (10.1 g, 40 mmol) was added to the solution. After 20 min of stirring the homogenous mixture was placed in ice bath. Next, the respective benzimidazole (10 mmol) was slowly added (exothermic reaction). The reaction mixture was stirred at room temperature for 1 h and then had been heated to the final (60 °C) temperature. After next 2 h the mixture was poured onto crushed ice. The resulting dark solid was filtered off, added to the solution of sodium dithionite (25 mL, 10%) and...
stirred for 10 min. The crude product was dissolved in EtOH/2 M NaOH (1:1) and filtered. The pale yellow or green filtrate was brought to pH 4–5 with acetic acid to yield respective iodinated benzimidazole as yellow or yellowish chromatographically pure precipitate. For analysis and biological investigation small amounts were crystallized from EtOH, MeOEtOH, dioxane, DMF/dioxane or purified by alkalization and precipitation with acetic acid from organic solvent–water mixtures.

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

From benzimidazole as described above: (mp 296–299 °C (decomp.) I₂ evol. >250 °C, 77%). A sample for analysis was crystallized from DMF/dioxane. UV (MeOH): 233 (28,600), 275 (9900), 295 (10,300). ¹H NMR (Me₂SO-d₆) δ (ppm): 8.21 (s, 1H, H–C), 12.85 (br s, 1H, H–N). MS m/z: 623 (9, M⁺+1), 622 (100, M⁺), 495 (16, M⁻–127), 368 (22, M⁻–254). Anal. Calcd for C₇H₂I₄N₂ (621.72): C, 13.52; H, 0.32; N, 4.51. Found: C, 13.67; H, 0.44; N, 4.40.

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

As described above starting from 5-chloro-benzimidazole. Mp 256–259 °C (with decomp.), 65%. A sample for analysis was crystallized from EtOH. UV (MeOH): 230 (23,300), 281 (14,300), 305 (sh, 5800). ¹H NMR (Me₂SO-d₆) δ (ppm): 8.32 (s, H–C), 13.05 (br s, H–N). MS m/z: 532 (33, M⁺+2), 530 (100, M⁺), 440 (19, M⁻–70), 438 (22, M⁻–22), 403 (M⁻–127). Anal. Calcd for C₇H₂ClI₃N₂ (530.27): C, 15.86; H, 0.38; N, 5.28. Found: C, 15.88; H, 0.40; N, 5.32.

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

As described above starting from 5-bromo-benzimidazole. Mp 253–255 °C (with decomp.), 65%. A sample for analysis was crystallized from methoxyethanol. UV (MeOH): 236 (19,500), 282 (12,900), 305 (sh, 5600). ¹H NMR (Me₂SO-d₆) δ (ppm): 8.28 (s, 1H, H–C), 13.0 (br s, 1H, H–N). MS m/z: 576 (M⁺+1), 574 (M⁻–1), 482 (32, M⁻–93), 449 (15, M⁻–126), 447 (M⁻–128). Anal. Calcd
for C$_7$H$_2$Br$_1$I$_3$N$_2$ (574.72): C, 14.63; H, 0.35; N, 4.87. Found: C, 14.50; H, 0.42; N, 4.76.

3.1.6. 5,6-Dichloro-4,7-diiodo-1H-benzimidazole (4a)
As described above from 5,6-dichloro-benzimidazole. Mp 271–273°C (with decomp.), 70%. A sample for analysis was crystallized from EtOH. UV (MeOH): 229 (23,000), 278 (19,000), 302 (9500). $^1$H NMR (Me$_2$SO-$d_6$) δ (ppm): 8.40 (s, 1H, H–C), 13.2 (br s, 1H, H–N).
MS m/z: 442 (10, M++4), 440 (63, M++2), 438 (100, M+), 311 (M++/C$_0$127). Anal. Calcd for C$_7$H$_2$Cl$_2$I$_2$N$_2$ (438.82): C, 19.16; H, 0.46; N, 6.38. Found: C, 19.19; H, 0.55; N, 6.23.

3.1.7. 4,6-Dichloro-5,7-diiodo-1H-benzimidazole (5a)
As described above from 4,6-dichloro-benzimidazole. Mp 254–256°C, 42%. A sample for analysis was crystallized from EtOH. UV (MeOH): 236 (22,500), 273 (8700), 302 (4400). $^1$H NMR (Me$_2$SO-$d_6$) δ (ppm): 8.39 (s, 1H, H–C), 13.08 and 13.48 (br s, 1H, H–N). MS m/z: 440 (28, M++1), 438 (100, M+/C$_0$1), 313 (12, M+/C$_0$126), 311 (M–127). Anal. Calcd for C$_7$H$_2$Cl$_2$I$_2$N$_2$ (438.82): C, 19.16; H, 0.46; N, 6.38. Found: C, 19.14; H, 0.44; N, 6.34.

3.1.8. 4,6-Dibromo-5,7-diiodo-1H-benzimidazole (6a)
As described above from 4,6-dibromo-benzimidazole. Mp 309–311°C (with decomp.), 70%. A sample for analysis was crystallized from dioxane. UV (MeOH): 236 (21,300), 277 (11,700), 303 (sh, 4200). $^1$H NMR (Me$_2$SO-$d_6$) δ (ppm): 8.34 (s, 1H, H–C), 13.09 and 13.23 (br s, 1H, H–N). MS m/z: 529 (47, M++2), 527 (100, M*), 525 (48, M*–2), 400 (M–127). Anal. Calcd for C$_7$H$_2$Br$_2$I$_2$N$_2$ (527.82): C, 15.93; H, 0.38; N, 5.31. Found: C, 15.90; H, 0.47; N, 5.22.

3.1.9. 2-Methyl-4,5,6,7-tetraiodo-1H-benzimidazole (7a)
As described above from 2-methyl-benzimidazole: Mp 289–291°C, 69%. A sample for analysis was crystallized from dioxane. UV (MeOH): 234 (29,300), 277 (10,300), 292 (10,500). $^1$H NMR (Me$_2$SO-$d_6$) δ (ppm): 2.47 (s, 3H, H$_3$C), 12.5 (br s, 1H, H–N). MS m/z: 637 (10, M++1), 636 (100, M*), 509 (17, M–127), 382 (M–254). Anal. Calcd for C$_8$H$_4$I$_4$N$_2$ (635.75): C, 15.11; H, 0.63; N, 4.41. Found: C, 15.09; H, 0.73; N, 4.27.

3.1.10. 5,6-Dichloro-4,7-diiodo-2-methyl-1H-benzimidazole (8a)
As described above from 5,6-dichloro-2-methylbenzimidazole. Mp 288–290°C (with decomp.), 52%. A sample for analysis was crystallized from EtOH. UV (MeOH): 234 (23,500), 275 (18,300), 303 (8500). $^1$H NMR (Me$_2$SO-$d_6$) δ (ppm): 2.52 (s, 3H, H$_3$C), 12.8 (br s, 1H, H–N). MS m/z: 456 (11, M++4), 454 (56, M++2), 452 (100, M*), 311 (M–128). Anal. Calcd for C$_8$H$_4$Cl$_2$I$_2$N$_2$ (452.85): C, 21.22; H, 0.89; N, 6.34. Found: C, 21.15; H, 0.95; N, 6.08.

3.1.11. 2-Ethyl-4,5,6,7-tetraiodo-1H-benzimidazole (9a)
As described above from 2-ethyl-benzimidazole. Mp 261–262°C (from EtOH), 68%. UV (MeOH): 234 (28,500), 277 (9700), 293 (11,100). $^1$H NMR (Me$_2$SO-$d_6$) δ (ppm): 1.28 (t, 3H, H$_3$C, J = 7.6 Hz), 2.82 (q, 2H, H$_2$C, J = 7.6 Hz), 13.28 (br s, 1H, H–N). MS m/z: 637 (10, M++1), 636 (100, M*), 509 (17, M–127), 382 (M–254). Anal. Calcd for C$_9$H$_6$I$_4$N$_2$ (649.76): C, 16.64; H, 0.93; N, 4.31. Found: C, 16.59; H, 0.99; N, 4.20.

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

Figure 2. Molecular docking of the tetraiodinated (A), tetrabrominated (B), and tetrachlorinated (C) benzimidazole derivatives bound to the active site of protein kinase CK2. Computational analysis was performed as described in Section 3. Connolly's distribution surfaces of the three inhibitors inside the nucleotide pocket are represented in light green. The occupancy factor (OF) values, discussed in the text, are also indicated.
3.1.13. 2-Pentfluoroethyl-4,5,6,7-tetraiodo-1H-benzimidazole (11a)

As described above from 2-pentfluoroethylbromobenzimidazole. Mp 247–249 °C (with decomp.), 48% (from EtOH). UV (MeOH): 237 (36,100), 284 (11,000), 304 (11,200). 1H NMR (MeSO-d4) δ (ppm): 14.3 (br s, 1H, H–N). MS m/z: 741 (10, M+1*), 740 (100, M*). 1H NMR (MeSO-d6) δ (ppm): 13.7 (br s, 1H, H–N). MS m/z: 658 (42, M+2*), 656 (100, M*), 529 (24, M+–127*), 402 (35, M–254*). Anal. Calcld for C14HClI4N2S: C, 15.91; H, 0.15; N, 3.79. Found: C, 15.95; H, 0.31; N, 3.69.

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

As described above from 2-chlorobenzimidazole. Mp 303–306 °C (with decomp., >250 °C i2 evol.), 37%. A sample for analysis was crystallized from DMF/dioxane. UV (MeOH): 239 (35,200), 273 (12,100), 302 (12,900), 315 (sh). 1H NMR (MeSO-d6) δ (ppm): 13.6 (br s, 1H, H–N). MS m/z: 701 (86, M*), 699 (M–2*), 574 (19, M+–127*), 572 (17, M–129*). Anal. Calcld for C14HClI4N2S (656.17): C, 12.81; H, 0.15; N, 4.00. Found: C, 12.75; H, 0.20; N, 3.92.

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

As described above from 2-bromobenzimidazole. Mp 296–299 °C (i2 evol. >230 °C), 88%. A sample for analysis was crystallized from DMF/dioxane. UV (MeOH): 239 (38,100), 273 (12,100), 300 (12,700). 1H NMR (MeSO-d6) δ (ppm): 13.6 (br s, 1H, H–N). MS m/z: 701 (86, M*), 699 (M–2*), 574 (19, M+–127*), 572 (17, M–129*). Anal. Calcld for C14HBrI4N2S (700 mg, 1.15 mmol), K2CO3 (1 g), bromoacetic acid (400 mg, 2.87 mmol) in butanone (45 mL) was refluxed for 6 h. The insolubilities were filtered off and washed with 50 mL of hot butanone. The filtrate was evaporated to dryness. The residue was crystallized from dioxane; mp 274–276 °C (decomp. >240); yield 70% (4.98 g). UV (MeOH): 254 (13,100), 309 (12,300). 1H NMR (MeSO-d6) δ (ppm): 4.16 (s, 2H, H2C–N). TOF MS ESI*: 712.6257 (M+H*), 712.6251. Anal. Calcld for CsH9Br2N2O2S (711.83): C, 15.19; H, 0.57; N, 4.50. Found: C, 15.20; H, 0.51; N, 4.43.

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

To a mixture of 13a (600 mg, 0.85 mmol) in MeOEtOH (15 mL), ethanolaamine (2 g, 3 ml, 55 mmol) was added. The reaction mixture was stirred and heated at 110–115 °C (bath temp) for 10 h. The reaction mixture was diluted with water (20 mL) and brought to pH 4–5. The precipitate was filtered and deposited on silica gel column (2 × 12 cm). Chromatography was performed with CHCl3 (200 ml) and CHCl3–MeOH (95:5, v/v). The product containing fractions were evaporated to dryness and the residue crystallized from dioxane–water to give pale yellow powder (320 mg, 55%). UV (MeOH): 236 (39,600), 306 (6400). 1H NMR (MeSO-d6) δ (ppm): 11.04 (s, H–N-imid.). MS m/z: 741 (10, M+H*), 740 (100, M*), 512 (25, M+–128*), 384 (27, M–256*). Anal. Calcld for CsH9Br2N2O2S (700.62): C, 12.00; H, 0.14; N, 4.00. Found: C, 12.00; H, 0.19; N, 3.84.

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

To the stirred solution of 13a (700 mg, 1 mmol) in MeOEtOH (25 mL) thiourea (156 mg, 2 mmol) was added. The reaction mixture was stirred and heated at 105–110 °C (bath temp) for 14 h. A precipitate formed was filtered, dissolved in EtOH–water 0.2 M aq. NaOH (1:1, v/v) and precipitated by addition of acid to pH 4–5 to give as pale yellow chromatographically pure precipitate (520 mg, 80%); mp (decomp. >290 °C with i2 evol.) for analysis crystallized from MeOEtOH–water. UV (MeOH): 222 (18,600), 264 (35,450), 336 (20,500). 1H NMR (MeSO-d6) δ (ppm): 12.8 (br s, 1H, H–N–CH2, J = 6.6 Hz). MS m/z: 681 (46, M+), 680 (100, M*), 662 (19, M–18*), 637 (100, M–43*), 510 (60, M–170*). Anal. Calcld for CsH11I4N2S (680.79): C, 15.88; H, 1.04; N, 6.17. Found: C, 15.91; H, 1.15; N, 6.06.

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

The mixture of 2-mercapto-4,5,6,7-tetraiodo-1H-benzimidazole (751 mg, 1.15 mmol), K2CO3 (1 g), bromoacetic acid (400 mg, 2.0 mmol) in butanone (35 mL) was refluxed for 6 h. The insolubilities were filtered off and washed with 50 mL of hot butanone. The filtrate was evaporated to dryness. The residue was crystallized from dioxane; mp 274–276 °C (decomp. >240); yield 70% (4.98 g). UV (MeOH): 254 (13,100), 309 (12,300). 1H NMR (MeSO-d6) δ (ppm): 4.16 (s, 2H, H2C–N). TOF MS ESI*: 712.6257 (M+H*), 712.6251. Anal. Calcld for CsH9Br2N2O2S (711.83): C, 15.19; H, 0.57; N, 4.50. Found: C, 15.20; H, 0.51; N, 4.43.
The white chromatographically pure precipitate was obtained for 2 h. The mixture was chilled and left for the crystallization. The precipitate was filtered off and crystallized from DMF/dioxane gave white powder (195 mg, 80%). Mp >325 °C.

Method B: To the stirred and refluxed mixture of TBI (260 mg, 0.5 mmol), anhyd K2CO3 (600 mg) in dioxane iodoacetamide (130 mg, 0.7 mmol) was added. The stirring and reflux was continued for 2 h. The mixture was chilled and water (20 mL) was added. The precipitate was filtered off and crystallized from DMF/dioxane gave white powder (180 mg, 73%). Mp >325 °C. UV (MeOH): 230 (30,100), 267 (10,800), 273 (10,400), 302 (3500). 1H NMR (Me2SO-d6) δ (ppm): (s, H2C), 7.37 and 7.72 (2s, H2N), 8.43 (s, H, HC). MS m/z: 495 (6), 493 (22, M+4), 491 (36, M+2), 489 (26, M−2), 487 (7, M−4), 447 (65, M−44), 410 (100, M−81). Anal. Calcd for C9H4Br4N4O (678.78): C, 21.44; H, 1.30; N, 8.08. Found: C, 15.55; H, 0.89; 8.05.

4. Biological evaluation

4.1. Materials

4.1.1. Phosphorylation

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

This work was supported by grants to L.A. from AIRC, European Commission (PRO-KINASERESEARCH 503467), and by the Italian Cystic Fibrosis Research Foundation (Grant FFC#4/2007) with the contribution of ‘Banca Popolare di Verona e Novara’ and ‘Fondazione Giorgio Zanotto’. The Molecular Modelling Section (MMS) coordinated by Professor S. Moro (Padova, Italy) is gratefully acknowledged. The authors (A.O. and Z.K.) are grateful to Foundation for the Development Diagnostics and Therapy for partial financial support.

References and notes