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Identification of novel protein kinase CK1 delta (CK1δ) inhibitors through structure-based virtual screening

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ABSTRACT

In eukaryotes, protein phosphorylation of serine, threonine or tyrosine residues by protein kinases plays an important role in many cellular processes. Members of the protein kinase CK1 family usually phosphorylate residues of serine that are close to other phosphoserine in a consensus motif of pS-X-X-S, and they are implicated in the regulation of a variety of physiological processes as well as in pathologies like cancer and Alzheimer's disease. Using a structure-based virtual screening (SBVS) approach we have identified two anthraquinones as novel CK1δ inhibitors. These amino-anthraquinone analogs (derivatives **1** and **2**) are among the most potent and selective CK1δ inhibitors known today (IC_{50} = 0.3 and 0.6 μM, respectively).

Protein kinase CK1 represents a unique and well-conserved group of protein kinases within the superfamily of serine/threonine kinases that is ubiquitously expressed in eukaryotic organisms. Recently, seven mammalian CK1 isoforms have been identified (α, β, γ1, γ2, γ3, δ, ε) with a molecular weight between 37 and 51 kDa. Even if all CK1 isoforms are highly conserved within their kinase domains, they show important differences in length and primary structure of the N-terminal and C-terminal domains.[1] CK1 isoforms are showed to be constitutive active with a consensus motif pS-X-X-S; this means that a prephosphorylation by other kinases (e.g., GSK3β) is required before they reach their basal activity.[2] Despite its constitutive activation, several mechanisms of CK1 activity control are known, such as the inhibitory autophosphorylation,[1] the proteolytic cleavage of the C-terminal domain,[1] and its subcellular localization and compartimentalization.[1] Members of CK1 family are involved in regulating a variety of cellular events including transduction of the Wnt signaling pathway,[3] regulation of circadian rhythms,[4,5] the DNA damage response,[6,7] and late cell cycle progression.[8,9] Consequently, the alteration of CK1 homeostasis has been possibly related to several diseases like neurodegenerative diseases, including Alzheimer's and Parkinson's disorders (CK1δ isoform),[10] the familial advanced sleep phase syndrome (CK1δ and ε isoforms),[11] hepatitis C (CK1α isoform),[12] leishmaniasis,[13] and cancer (CK1α, δ and ε isoforms).[7]

Very few potent and selective CK1 inhibitors have been described. Among these it is worthy to mention: the 4-[4-(2,3-dihydro-benzo[1,4]dioxin-6-yl)-5-pyridin-2-yl-1*H*-imidazol-2-yl] benzamide (D4476),[14] the 3-[(2,4,6-trimethoxyphenyl)methylidenyl]-indolin-2-one (IC261),[15,16] and the *N*-(2-aminoethyl)-5-chloro-isoquinoline-8-sulfonamide (CK1-7)[17] with IC_{50} of 0.3, 1.0 and 6 μM, respectively.

In recent years, we have performed an intensive screening campaign combining in silico and in vitro enzymology approaches.[18] In particular, we have focused our attention on the CK1δ isoform due to its key role in the possible pathogenesis of several neurodegenerative diseases and cancer. Following some recent successful examples of new kinase inhibitors identification through structure-based virtual screening (SBVS) approches,[18,19] we have performed an in silico study targeting the ATP-binding site of CK1 by browsing our in-house molecular database (defined as MMs-INC[20]) which contains around 4 millions of synthetic and natural compounds. Generally speaking, SBVC approach could represent a useful strategy to prioritize the synthesis and the biological screening of novel drug candidates. In our virtual screening protocol, we have used a combination of different docking protocols *in tandem* with a consensus scoring strategy, as summarized in Figure 1. In particular, due to the fact that no crystal structure is available for the human CK1δ, an homology modeling approach has been carried out to obtain a suitable three-dimensional model of the CK1δ catalytic subunit. The choice of combining different docking protocols has been dictated by the awareness that scoring is

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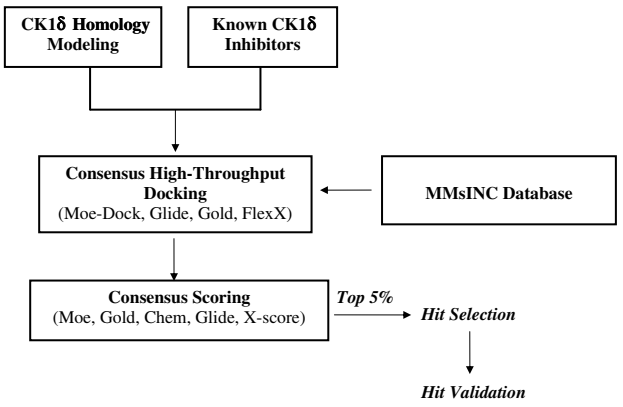


Figure 1. Flowchart of the proposed consensus high-throughput docking.

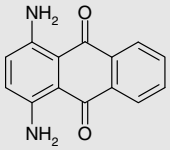
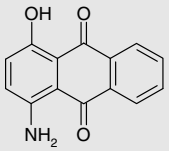
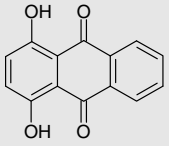
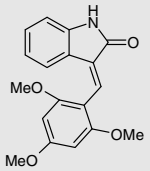
typically more important than docking in database screening, and that scoring functions performances often depend on the target active site features. However, since docking poses may significantly affect the scoring, multiple scoring functions are simultaneously used in the hit selection process, and improvements can be achieved by compensating for the deficiencies of each function. Specifically, a combination of four docking protocols (MOE-Dock,[21] Glide,[22] Gold[23] and FlexX[24]) and five scoring functions (MOE-Score,[21] GlideScore,[22] Gold-Score,[23] ChemScore[23] and Xscore[25]) has been used to appropriately dock and score all MMsINC entries with a leadlikeness profile. In particular, we have implemented a '*FiTk* consensus scoring function' to appropriately rank the possible hit compounds. This function represents the number of scoring functions for which a certain candidate docking pose is scored among the top *k*% of the database (see Supporting Information for details).

Interestingly, from our consensus SBVC protocol only few compounds (less than 150) have been scored with a full '*FiTk* consensus'. That means that these compounds appear in the top 5% of the database when ranked by every scoring function independently. After visual inspection, we have realized that two anthraquinone derivatives were among them. This finding was quite unexpected and intriguing at the same time considering that other anthraquinones were already reported to be modestly active against CK1, such as emodin[26] and 1,4-diamino-5,8-dihydroxy-anthraquinone (DAA).[27] For this reason, we decided to primarily focus our attention to these potential hit compounds. In particular, the 1,4-diamino-anthraquinone (compound **1**, in Table 1) was one of the best ranked compounds of our top 5% list. Considering the encouraging virtual screening results, we have prioritized the acquisition and the biochemical characterization of derivative **1** as new potential CK1δ inhibitor. In particular, derivative **1** is 10-fold more potent compared with the reference CK1δ inhibitor, 3-[(2,4,6-trimethoxyphenyl)methylidenyl]-indolin-2-one (IC261).[15,16]

As shown in Figure 2, inhibition of CK1δ by compound **1** is competitive with respect to the phosphodonor substrate ATP, and a 125 nM K_i value has been calculated from linear regression analysis of Lineweaver–Burk double reciprocal plots, which is the lowest K_i reported so far a CK1δ inhibitor. On the other hand, according to a preliminary selectivity study (Tables 1 and 2), derivative **1** seems to be a quite specific inhibitor of CK1δ respect the other CK1 isoforms, and also with respect to a small panel of different kinases. In particular according to our homology model, derivative **1** makes a stabilizing interaction between the amino group at the 1-position and the backbone carbonyl of Glu83 in the hinge region (Fig. 3). Moreover, one of the carbonyl groups can interact through an H-bonding with the backbone amido moiety of Leu85. These two interactions are the same generally observed in the binding of adenine moiety of ATP into the kinase active site. On the other hand, another hydrogen-bonding interaction has been detected between the amino group 4-position and the carboxylic group of Asp149. Finally, several hydrophobic interactions (Ile15, Ile23, Ala36, Leu135, Ile147) should be taken into account because they may contribute to stabilize the complex between compound **1** and CK1δ. We can also argue that the right balance of both polar and hydrophobic interactions and the appropriate shape complementarity with the ATP-binding cleft might be ultimately responsible for the appreciable selectivity presented by derivative **1** versus other kinases and in particular against the protein kinase CK2. In fact, the shape topology of the ATP-binding cleft of CK2 is clearly different respect CK1 (data not shown). Vice versa, more intriguing is the observation regarding the appreciable selectivity displayed by derivative **1** against the other CK1 isoforms. In fact, analyzing the sequence alignment of the corresponding kinase domains no crucial mutations are detectable and that would be able to justify the observed isoform selectivity spectrum of derivative **1**. Our assumption is that in this specific case the C-terminal domain of CK1 isoforms could play an important role in the control of the inhibitor's recognition as well as in the corresponding CK1 basal kinase activity. In particular, the C-terminal domain of CK1α and γ may have a deterrent effect on the interaction with derivative **1**, reducing its inhibitory effects as collected in Table 1. This experimental evidence has been already reported also for the CK1 inhibitor, IC261.[15] In fact, as suggested by a recent crystallographic information regarding the isoform CK1γ3 the C-terminal domain can closely approach the ATP-binding cleft directly interacting with the inhibitor (PDB code: 2CHL).

Beside derivative **1**, the 1-hydroxy-4-amino-anthraquinone (compound **2** in Table 1) also shows an appreciable inhibitory activity against CK1δ (IC_{50} = 0.6 μM). To directly verify the role of the 1,4-diaminobenzene fragment, the corresponding 1,4-dihydroxy-anthraquinone was also tested as potential CK1δ inhibitor (compound **3** in Table 1). Interestingly, compound **3** is practically inactive against the three isoforms CK1δ, γ1 and α supporting

Table 1
Inhibition of CK1 isoforms calculated as IC_{50} (μM)

Compound		CK1δ	CK1γ1	CK1α
1		0.33	34.0	4.0
2		0.66	26.2	4.0
3		>40	>40	>40
IC261		2.57	>40.0	1.24

The values of IC_{50} represent means of at least three independent experiments with SEM never exceeding 15%.

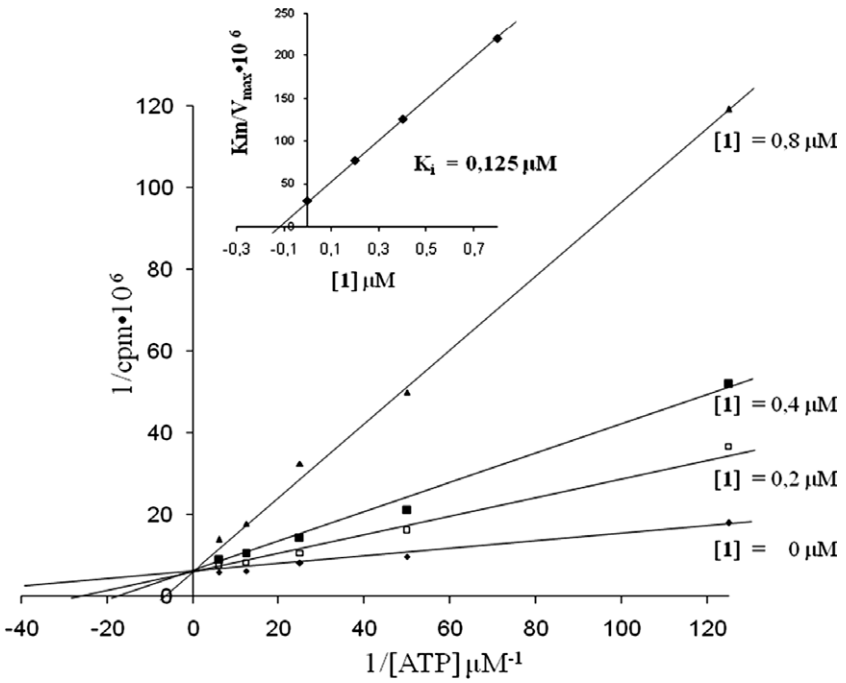


Figure 2. Kinetic analysis of compound **1**/CK1δ complexation consistent with a reversible and competitive mechanism of inhibition. CK1δ activity was determined as described in Supporting Information either in the absence or in the presence of the indicated compound **1** concentrations. The data represent means of triplicate experiments with SEM never exceeding 15%.

Table 2
Inhibition of a preliminary protein kinases panel by compound **1** calculated as IC_{50} (μM)

CK2	HIPK2	PIM1	DYRK1a	PKA	CSK	Lyn	Syk	Fgr	GST-ALK
18.0	3.3	24.7	3.6	>40.0	>40.0	>40.0	>40.0	24.0	>40.0

The activity of each protein kinase was determined as described in Supporting Information. The values of IC_{50} represent means of at least three independent experiments with SEM never exceeding 15%.

the critical role mediated by the aminobenzene fragment into CK1δ recognition. Indeed, a robust and quantitative structure–activity relationship is underway in our laboratories to explore the possibility of decorating or modifying the 1,4-diamino-anthraquinone moiety increasing the pharmacodynamics of the second generation of these CK1δ inhibitors and, at the same time, improving their pharmacokinetics profiles.

Finally, considering the encouraging inhibitory and selectivity properties of compound **1** and **2** against isolated CK1δ, we have also acquired a very preliminary cytotoxicity profile on human ovarian carcinoma cell line (2008) and on its cisplatin-resistant clone (C13). Results showed that after 48 h of exposure to compound **1** IC_{50} values were 14.4 and 87.9 μM in 2008 and C13 cells, respectively (±95% confidence interval from three different experiments). Interestingly, compound **2** was slightly more potent on the cisplatin resistant cell line (IC_{50} 8.0 μM) than in cisplatin sensitive cancer cells (IC_{50} 122.4 μM) (see also Supporting Information). Considering the wealth of kinase and non-kinase mediate biological activities of anthraquinones, further investigations are in progress to clarify in detail the cytotoxicity pathway(s) activated and regulated in different human tumor cell lines.

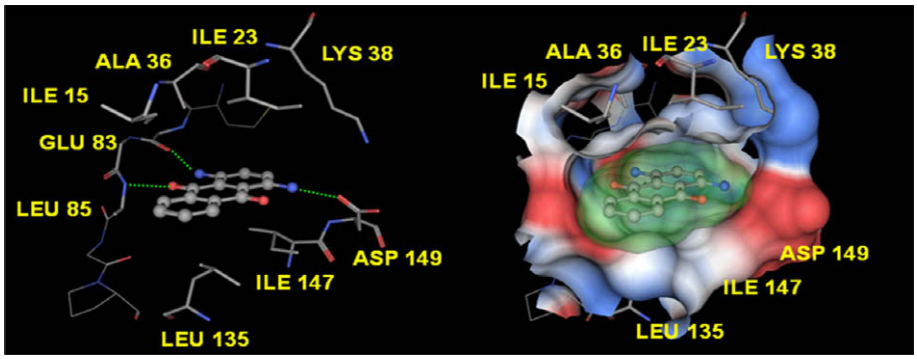


Figure 3. Molecular docking of compound **1** bound to the active site of the CK1δ catalytic subunit. On the left, analysis of the binding mode of derivative **1** whose interactions with the most crucial amino acids are highlighted. On the right, Connolly's electrostatic charge distribution surface of ATP-binding cleft of CK1δ (blue indicates positive surface charge and red indicates negative surface charge).

Concluding, we have demonstrated the usefulness of our structure-based virtual screening (SBVS) approach to identify novel CK1δ inhibitors. In particular, two amino-anthraquinone analogs (derivatives **1** and **2**) have demonstrated being among the most potent and selective CK1δ inhibitors known today (IC_{50} = 0.3 and 0.6 μM, respectively). Indeed, we have conformed that anthraquinone scaffold is a versatile scaffold to design specific protein kinase inhibitors, as already reported for other classes of kinases such as for the protein kinase CK2,[28] for the Janus-activated kinase 2 (Jak2),[29] for the dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A (DYRK1A)[26,27] and the serum and glucocorticoid-inducible kinase (SGK).[26,27] An ongoing project is now running in our laboratories to clearly understand the mechanism of action of this new class of promising CK1δ inhibitors with the aim to design and synthesize a second generation of more potent and selective anthraquinone-driven CK1δ inhibitors.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.08.072.

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