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Electrochemical screening of the indole/quinolone derivatives as potential protein kinase CK2 inhibitors

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ABSTRACT

An electrochemical method based on the bioorganometallic Fc-ATP cosubstrate for kinase-catalyzed phosphorylation reactions was used for monitoring casein kinase 2 (CK2) phosphorylations in the absence and presence of five indole/quinolone-based potential inhibitors. Fc-phosphorylation of immobilized peptide RRRDDDSDDD on Au surfaces resulted in a current density at approximately 460 ± 10 mV. An electrochemical redox signal was significantly decreased in the presence of inhibitors. In addition, the electrochemical signal was concentration dependent with respect to the potential inhibitors **1** to **5**, which proved to be viable CK2 drug targets with estimated IC_{50} values in the nanomolar range.

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Protein kinases are critical for cellular growth and function, and they catalyze the transfer of γ-phosphate group from ATP to the target residue of proteins [1]. The overexpression of certain protein kinases has been related to diseases such as cancer [2]. Hence, protein kinases have emerged as promising drug targets. γ-^{32}P-labeled adenosine triphosphate ([γ-^{32}P]ATP)[1] is the most commonly used approach for probing protein kinases, where the incorporation of $^{32}PO_3^-$ into the target peptide or protein is measured [3]. Due to issues related to radiation and safety protocols, alternative techniques have emerged for monitoring protein kinase activity and inhibitor screening. Currently, a number of optical, surface plasmon resonance, and electrochemical methods are being used for monitoring protein kinases and probing inhibitory activity of the potential drugs [4].

Here we focused on using the electrochemical detection method for inhibitor screening, depicted in Fig. 1 (see Results and Discussion), based on the redox signal associated with the transfer of ferrocenyl-phosphate group from adenosine 5′-[γ-ferrocene] triphosphate (Fc-ATP) to the surface-bound target peptide [5]. Casein kinase 2 (CK2) was chosen for the study because its activity promotes and regulates the cellular activity of other proteins such as Wnt, nuclear factor-κB, and phosphatidylinositol 3-kinase [6]. The CK2-driven phosphorylations also protect from caspase cleavage [7]. Because CK2 activity has been associated with tumorogenesis and regulation of other oncogenic protein kinases, the search for potential inhibitors is focused on ATP-competitive potential drugs. CK2 possesses a well-defined and small ATP binding pocket that facilitates the design of CK2 inhibitors. Based on the well-known CK2 inhibitor 5,6-dichlorobenzimidazole ribofuranoside, new halogenated benzimidazole and benzotriazole have become viable targets. For example, the inhibitor 4,5,6,7-tetrabromo-1*H*-benzotriazole (TBB) exhibits strong hydrophobic and van der Waals interactions with the CK2 binding pocket but is cross-reactive with glycogen synthase kinase and cyclin-dependent kinase 2 [8]. From TBB, two analogues that possess bulky substituents and offer improved selectivity and binding affinity toward CK2 were derived: 4,5,6,7-tetrabromo-1*H*-benzimidazole (TBBz) and 2-dimethylamino-4,5,6,7-tetrabromo-1*H*-benzimidazole (DMAT). Despite the progress made in the field of CK2 inhibition, only a limited number of inhibitors, such as CX-4945 [9], have entered clinical trials. Recently, a series of thieno[2,3-d]pyrimidine compounds have been synthesized and tested as potential CK2 inhibitors that exhibit improved IC_{50} values at approximately 100

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[1] *Abbreviations used:* [γ-^{32}P]ATP, γ-^{32}P-labeled adenosine triphosphate; Fc-ATP, adenosine 5′-[γ-ferrocene] triphosphate; CK2, casein kinase 2; TBB, 4,5,6,7-tetrabromo-1*H*-benzotriazole; TBBz, 4,5,6,7-tetrabromo-1*H*-benzimidazole; DMAT, 2-dimethylamino-4,5,6,7-tetrabromo-1*H*-benzimidazole; CV, cyclic voltammetry; SWV, square wave voltammetry.

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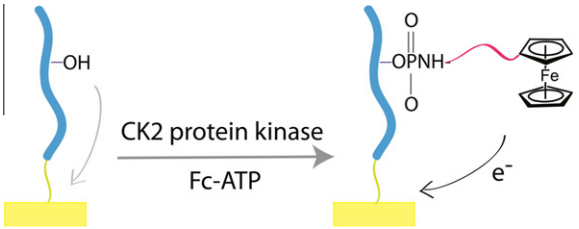
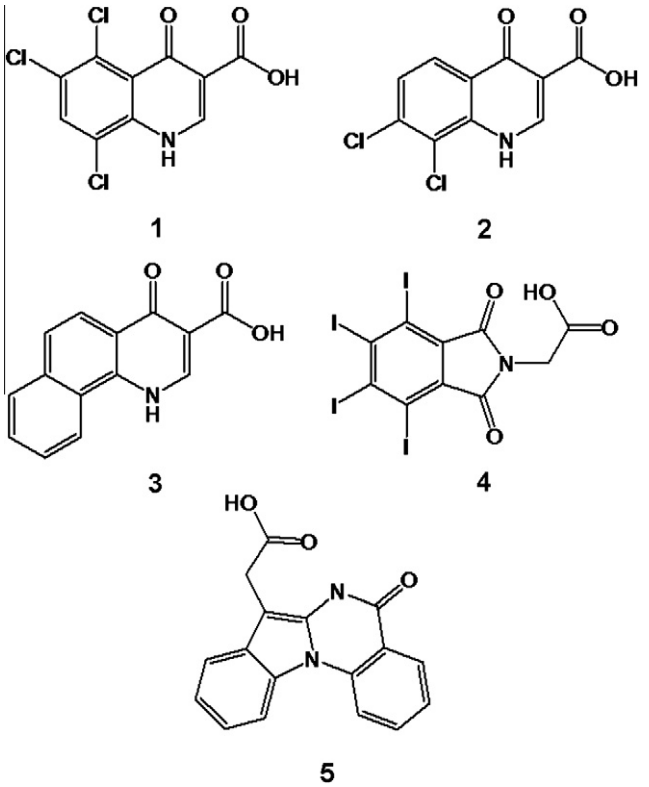


Fig.1. Illustration of the electrochemical biosensor for monitoring CK2 protein kinase and inhibitor screening.

nM [10]. Advances in the design and synthesis of novel protein kinase inhibitors is ongoing, and the need for facile, rapid, and affordable analytical methods for the screening of protein kinases, peptide targets, and inhibitors is immediate.

The electrochemical approach presented here focuses on screening of the potential CK2 inhibitors based on indole/quinolone motifs. The inhibitors under study are shown in Scheme 1 and consist of 5,6,8-trichloro-4-oxo-1,4-dihydroxo-quinoline-3-carboxylic acid (**1**), 7,8-dichloro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (**2**), 4-oxo-1,4-dihydrobenzo[h]quinoline-3-carboxylic acid (**3**), (4,5,6,7-tetraiodo-1,3-dioxo-1,3-dihydro-isoindol-2-yl)-acetic acid (**4**), and 5,6-dihydro-5-oxo-indolo[1,2-a]quinazoline-7-acetic acid (**5**) [11]. 4(1*H*)-Quinolones exhibit a variety of biological activities that include antibacterial, immunomodulating, and antineoplastic in addition to EGFR (epidermal growth factor receptor) inhibition [12]. The inhibition of CK2 protein kinase by several selected indole/quinolones has been investigated with respect to the RRREEETEEE peptide substrate in a radiolabeled approach [11]. However, these inhibitors have not been studied with respect to RRRDDDSDDD target peptide by other analytical methods.



Scheme 1. Structures of inhibitors **1** to **5** under electrochemical study for CK2 protein kinase.

Here we present an electrochemical assay based on the Fc-ATP for detection of Fc-phosphorylation of RRRDDDSDDD peptides on Au surfaces in the presence of potential inhibitors **1** to **5**. In this biosensor format, the electrochemical signal-on indicated efficient Fc-phosphorylation, and a decrease in the current response was associated with inhibition. The IC_{50} values for this series of compounds were estimated by electrochemical means and were in the nanomolar range.

Materials and methods

General consideration

The substrate peptide RRRDDDSDDD and CK2 protein kinase were donated by the Litchfield laboratory (D. W. Litchfield, Department of Biochemistry, University of Western Ontario, Canada). Human osteosarcoma U2-OS cells with tetracycline-regulated expression of CK2 were used to isolate the wild-type CK2α form. Inhibitors **1** to **5** were received from Otava (Kyiv, Ukraine). Ethanol was freshly distilled, and the experiments in aqueous solutions were prepared using ultrapure water (18.3 MΩ·cm) from a Millipore Milli-Q system. Fc-ATP was synthesized according to a procedure published elsewhere [5]. All electrochemical experiments, including cyclic voltammetry (CV) and square wave voltammetry (SWV), were carried out using a CH Instruments potentiostat model 660B (Austin, TX, USA). Gold disk electrodes (99.99% purity) with a surface area of 0.02 cm^2 were obtained from CH Instruments. All electrochemical experiments were carried out in 0.1 M sodium phosphate buffer (pH 7.5). For CV experiments, a scan rate at 0.1 V s^{-1} and a potential range of 0.2 to 0.7 V were used unless otherwise specified. For SWV measurements, the potential was scanned from 0.2 to 0.6 V with a step potential of 4 mV, frequency at 15 Hz, quiet time at 2 s, and pulse amplitude of 25 mV in the same buffer.

Fabrication of peptide-based biosensor

The cleaning procedure was carried out using the following steps. The gold disk electrodes were cleaned by immersing in Piranha solution (3:1 H_2SO_4/H_2O_2; caution: handle with extreme care) for 3 min and polished with slurry of 0.05 μm Al_2O_3 until a mirror finish was obtained. After 5 min of sonication in Milli-Q water, the gold electrodes were rinsed with water and ethanol. The electrodes were then cleaned electrochemically by CV in 0.5 M H_2SO_4 in a range from 0 to 1.2 V and then by cycling in a negative potential range from –2.3 to –0.6 V. Next, the gold electrodes were incubated with 2 mM lipoic *N*-hydroxysuccinimide active ester ethanolic solution for 2 days at 273 K. Then, the electrodes were washed with fresh ethanol and incubated with 100 μM substrate peptide solution RRRDDDSDDD in Milli-Q water for 20 h at 273 K. Following the peptide incubation, the electrodes were rinsed with Milli-Q water and blocked with ethanolic 100 mM ethanolamine solution (1 h) followed by ethanolic 10 mM hexanethiol solution (20 min) for the backfilling of the remaining uncovered empty spots on the Au surface.

Electrochemical kinase assays

In a typical electrochemical experiment, the peptide-modified Au electrode served as the working electrode, Ag/AgCl as the reference electrode, and Pt wire as the auxiliary electrode. The CK2 kinase assay buffer was based on 50 mM Tris (pH 7.5), 150 mM NaCl, and 10 mM $MgCl_2$. The phosphorylation reaction was performed by incubating the working electrode in 20 μl of kinase reaction buffer that contained CK2 (10 ng ml^{-1}) and Fc-ATP (200 μM) unless stated

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