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BENZO[*c*]QUINOLIZINIUMS : A NEW FAMILY OF INHIBITORS FOR PROTEIN KINASE CK II

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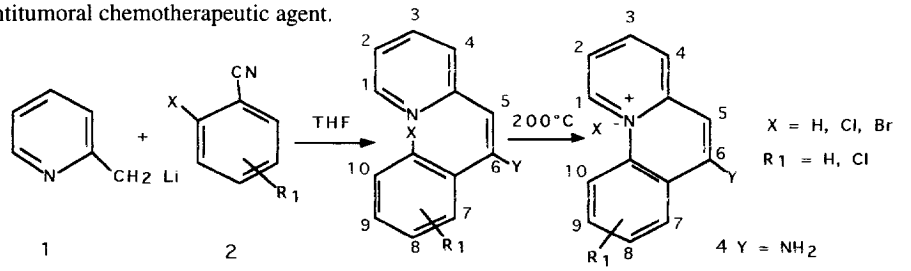
Abstract : A series of bicyclic enols **3** and tricyclic benzo[c]quinoliziniums **4** and **5** were prepared and evaluated as inhibitors of protein kinase CKII. Of the seventeen derivatives examined, 6-hydroxy-benzo[*c*]quinolizinium **5d** was the most potent inhibitor and exhibited a good selectivity for CKII in the micromolar range. © 1997 Elsevier Science Ltd.

Protein kinase CKII, formerly called casein kinase II (CKII), is a serine-threonine protein kinase found in all eukaryotic cells. CKII has a broad substrate specificity and can phosphorylate many nuclear as well as cytosolic proteins. This ubiquitous enzyme plays an important role in the regulation of metabolic pathways and the transduction of mitogenic signals [1,2]. The basal activity of CKII is elevated in normal tissue with high mitotic activity. Furthermore, some human leukemias and solid tumors exhibit high levels of CKII, suggesting a potential role in tumorigenesis [3,4]. Recently, it has been shown that the gene that encodes the catalytic subunit of CKII can serve as an oncogene, its dysregulation may give rise to transformation [5]. So the development of a specific inhibitor for the enzyme may be useful for further clarification of its physiological function and as an antitumoral chemotherapeutic agent.



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SCHEME 1

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For this aim, we wish to propose a new family of inhibitors, the benzo[*c*]quinoliziniums.

First, we synthesized bicyclic compounds **3** by condensation of 2-picolyllithium **1** and an *ortho*-halogenobenzonitrile **2**, then thermocyclisation was realized to obtain benzo[*c*]quinoliziniums **4** and **5** [6]. These different compounds were tested and a significant result was observed with benzo[*c*]quinoliziniums **4** and chiefly with derivatives **5**.

Table I : Bicyclic enols and benzo[c]quinoliziniums as protein kinase CKII inhibitors

Compound	X	Y	R_1	Rdt %	IC_{50}(mM)[a]
3a	H	OH	H	40 [b]	2.500
3b	Cl	OH	H	56 [b]	1.200
3c	Br	OH	H	40 [b]	0.820
3d	Cl	OH	10-Cl	80 [b]	0.130
3e	Cl	OH	9-Cl	45 [b]	0.900
3f	Cl	OH	7-Cl	75 [b]	0.580
4b	Cl	NH_2	H	35 [b]	0.100
4c	Br	NH_2	H	50 [b]	0.110
4d	Cl	NH_2	10-Cl	20 [b]	0.050
4e	Cl	NH_2	9-Cl	42 [b]	0.024
4f	Cl	NH_2	7-Cl	42 [b]	0.084
5b	Cl	OH	H	60 [c]	0.043
5c	Br	OH	H	50 [c]	0.050
5d	Cl	OH	10-Cl	42 [c]	0.005
5e	Cl	OH	9-Cl	72 [c]	0.039
5f	Cl	OH	7-Cl	45 [c]	0.018
5g	Cl	OH	8-Cl	68 [c]	0.210

a - human recombinant CKII holoenzyme was used to screen inhibitors, for experimental conditions see ref.7. b - from benzonitrile. c - from compound **3**.

The quinoliziniums tested, had several structural variables. Firstly, the ion X (Cl^- or Br^-), for compounds **4** or **5**, gave similar IC_{50}. Secondly, two groups of derivatives were considered : compounds **4** with Y = NH_2 and

compounds **5** with Y = OH. The latter were more efficient. Thirdly, the presence of a chlorine atom clearly increased the inhibition of CKII and especially the chlorine derivative **5d** which was 20 times more active than benzo[c]quinolizinium **4b** (Table I).

The effect of compound **5d** on CKII at different levels of peptide substrate was investigated. Kinetics analysis by double-reciprocal plots revealed a non competitive type of inhibition with respect to peptide substrate (Fig. 1A). However, the effect of compound **5d** was found to be highly dependent on the ATP concentration. It inhibited CKII in a competitive manner with respect to ATP (Fig. 1B).

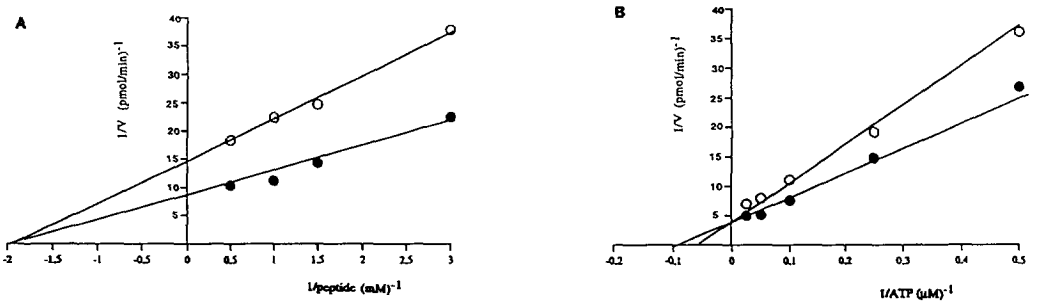

Figure 1: Double reciprocal plots for protein kinase CKII inhibition by compound **5d**. In **A**, the peptide concentration was varied while ATP was kept at 10µM. In **B**, the ATP concentration was varied and the peptide concentration was fixed at 2 mM. Filled symbols, controls; open symbols, 5µM compound **5d** present.

Table II: Effect of compound **5d** on various protein kinases

Protein kinase	IC_{50}(mM)
Protein kinase CKII	0.005
Protein kinase CKI [a]	0.033
Protein kinase C [b]	0.038
Ca^{2+}/Calmodulin kinase II [c]	0.760
cdc2 kinase [d]	e
Protein kinase A [b]	f
pp60 c-src [b]	f

For experimental conditions see ref.8 (a), ref.9 (b), ref. 10 (c) and ref.11 (d).
(e) IC_{50} > 1 mM, (f) no inhibition at 1mM inhibitor concentration.

The compound **5d** was then tested for potential inhibition of other protein kinases.

In the micromolar range, benzo[c]quinolizinium **5d** potently inhibited only CKII. At higher concentrations, it inhibited protein kinase CKI and protein kinase C. It caused only a weak inhibition of type II Ca^{2+}/calmodulin-dependent protein kinase and did not alter the activity of protein kinase A, cdc2 kinase and pp60 $^{c\text{-}src}$ (Table II).

In conclusion, benzo[c]quinolizinium **5d** exhibits a good inhibitory activity and selectivity for CKII in the micromolar range and is actually used in our laboratory as a tool to study the cellular functions of this important protein kinase.

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