Resorufin:
A Lead for a New Protein Kinase CK2 Inhibitor

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Abstract

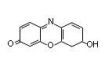
Screening of a natural compound library led to the identification of resorufin, as a highly selective and potent inhibitor for protein kinase CK2. Out of 52 kinases tested only CK2 was inhibited, in contrast to emodin, a structurally related, known CK2 inhibitor, which inhibited beside CK2, ten other kinases up to 90% .The IC50 values determined for the CK2 holoenzymes were 1.5 µM and for the free catalytic subunits ca. 4 µM. Altogether four cell lines were subjected to resorufin and emodin treatment. In the case of the three prostate carcinoma cell lines (PC-3, DU-145, LNCaP) 24h treatment with 40 µM resorufin led to 15-20% dead cells, however no caspase-mediated apoptosis was observed. In the case of the colorectal carcinoma HCT116 cell line a similar picture was obtained, yet, when resorufin was administered in cells treated with doxorubicin, apoptosis was strongly induced within 24h. Endogenous protein kinase CK2 was inhibited by resorufin by ca. 80% in the three prostate cell lines. In the case of the HCT116 cells the inhibition was only 40% supporting the notion of a cell line-specific selectivity. Moreover, did we analyse the effect of resorufin and emodin on selected signalling molecules in the cell lines under investigation.

IC50 of various CK2 inhibitors

Compound	IC50 [µM]
DRB	13.00
TBB	1.00
Emodin	0.89
Apigenin	0.80
Quercetin	0.55
FSA	0.41
MNX	0.40
IQA	0.40
Fisetin	0.35
DMAT	0.14
DBC	0.10
Ellagic acid	0.05
POM	0.01

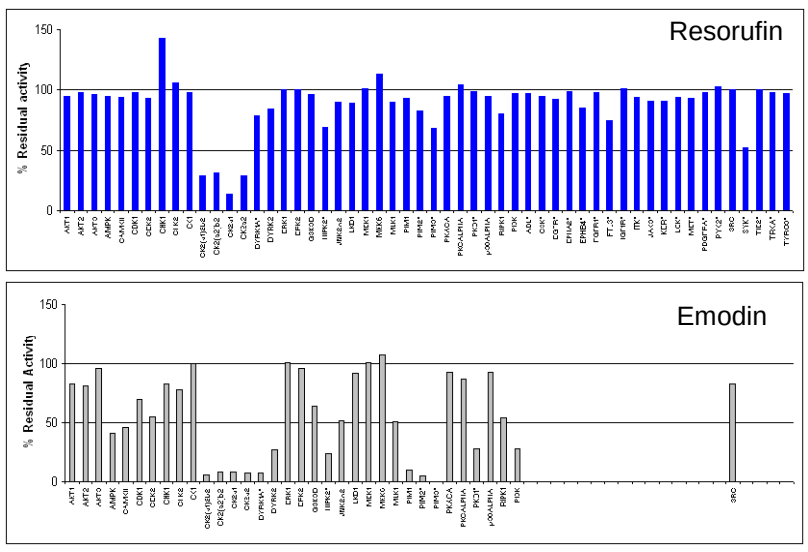
IC50 and Ki for resorufin and emodin towards protein kinase CK2.

IC50 values (in µM) were calculated for the different CK2 constructs in the presence of 125 µM ATP. The Ki values were determined at different ATP concentrations. The values are averages of at least two independent experiments.

	Resorufin		Emodin	
				
	IC_{50}	K_i	IC_{50}	K_i
$CK2\alpha^{1-335}{}_2\beta_2$	1.5	0.8	2.4	0.2
$CK2\alpha'{}_2\beta_2$	1.4	0.7	2.5	0.2
$CK2\alpha^{1-335}$	4.1	1.3	3.2	0.6
CK2α'-His	3.8	0.8	4.3	0.2

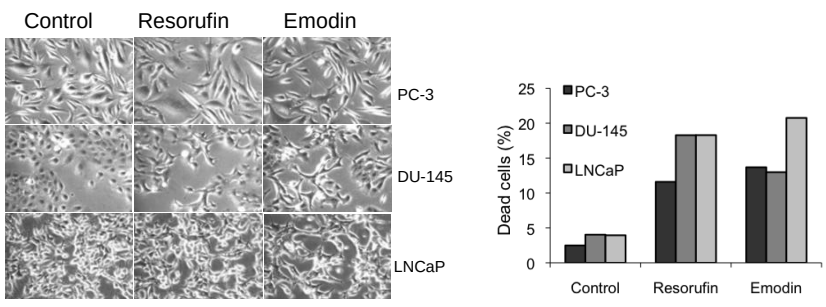
Selectivity of resorufin and emodin.

Residual kinase activities were determined in the presence of 10 µM resorufin or emodin and 125 µM ATP and are expressed as percentage of the control. PI3K was tested in the presence of 1 µM ATP. Kinases marked with * were tested by Carna Biosciences at the Km of ATP.



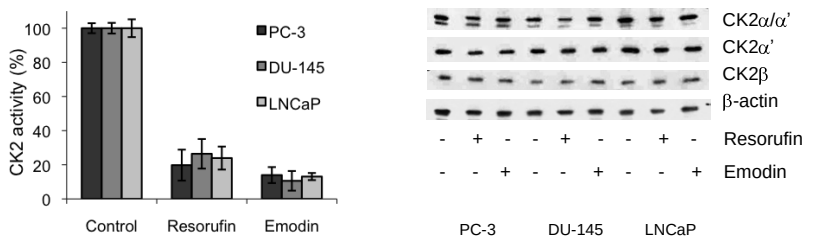
Effect of resorufin and emodin on cell morphology and cell death.

Prostate cancer cell lines (PC-3, DU-145, LNCaP) were treated for 24h with 40 µM resorufin or emodin. Cell death was measured as the percentage of trypan blue dye positive cells.

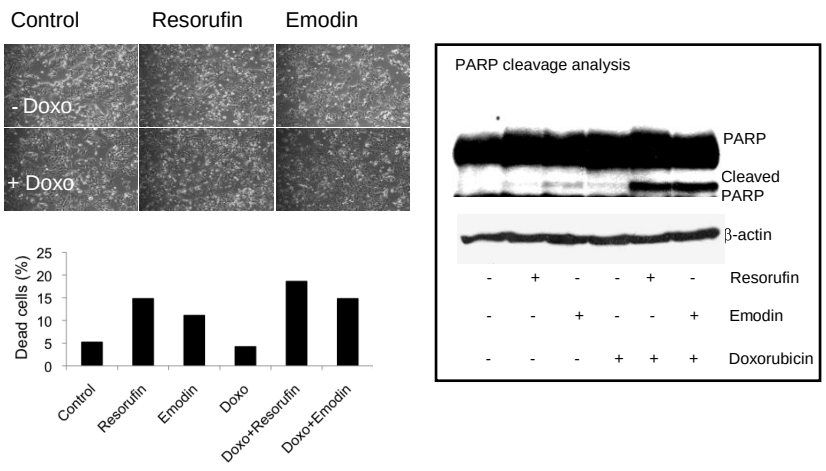


Inhibition of endogenous CK2 by resorufin or emodin.

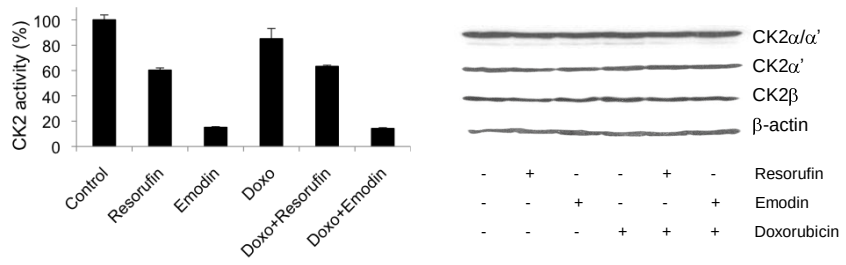
Prostate cancer cell lines (PC-3, DU-145 and LNCaP) were treated for 24h with 40 µM resorufin or emodin. CK2 kinase activity in cell lysates was determined against the synthetic CK2 peptide RRRADDSDDDDD in the presence of 125 µM ATP. The activities represent the means ± standard deviation from three independent experiments.



The human colorectal cell line, HCT116 was treated for 24h with 40 µM resorufin, 40 µM emodin and/or 0.5 µM doxorubicin (Doxo). Cell death was measured as the percentage of trypan blue dye positive cells.



The human colorectal cell line, HCT116 was treated for 24h with 40 µM resorufin, 40 µM emodin and/or 0.5 µM doxorubicin. CK2 kinase activity in cell lysates was determined. The activities represent the means ± standard deviation from triple determinations.



Conclusion

Resorufin was identified as a highly selective and potent inhibitor for protein kinase CK2.

In different cancer cell lines treatment with resorufin led to cell death and endogenous CK2 was inhibited.

Reference

Sandholt et al., Anticancer Drugs, 2009 Jan 27 [Epub ahead of print]

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