5th International Electronic Conference on Medicinal Chemistry

1-30 November 2019

chaired by Dr. Jean Jacques Vanden Eynde

sponsored by pharmaceuticals

Acetoxystachybotrydial acetate, a natural compound isolated from *Stachybotrys chartarum* is a potent inhibitor of human protein kinase CK2

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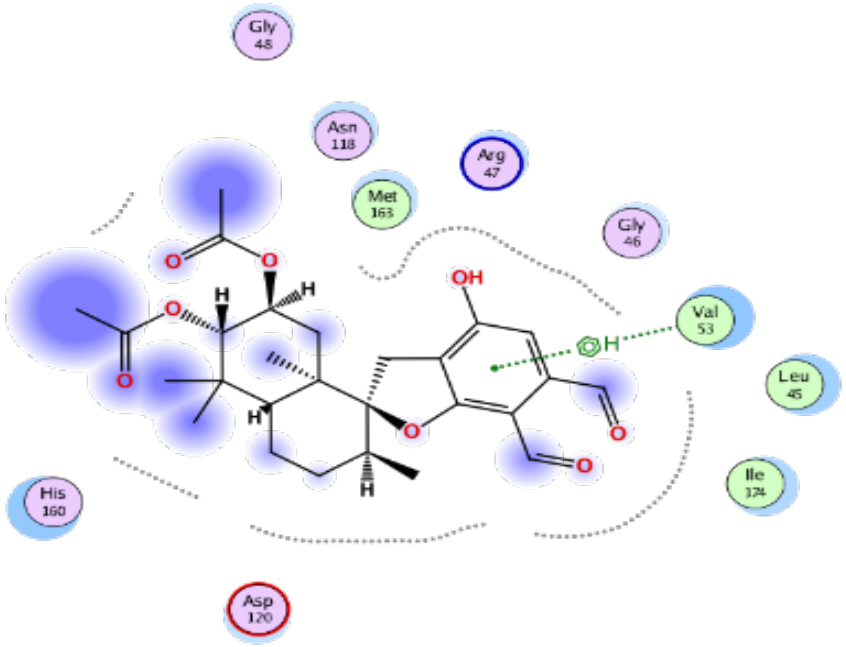
Graphical Abstract



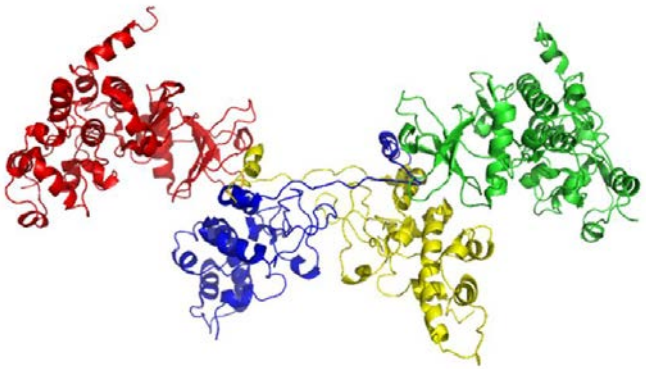
Abstract: Human protein kinase CK2 is an emerging target for drug design. Overexpression of CK2 is closely related to many types of human cancer. It was shown that elevated levels of CK2 protect tumor cells from apoptosis. Inhibition of CK2 activity can lead tumor cells into apoptosis, whereas viability of healthy cells is not affected. Up till now, one ATP competitive inhibitor of CK2, silmitasertib, is in clinical trials phase II. Here we report on the screening of natural compounds isolated from *Stachybotrys chartarum.* Twelve phenylspirodrimanes and three triphenylphenols were investigated on inhibitory activity towards CK2 using a CE-based assay. Triphenyl phenolestachybotrychromene C, phenylspirodrimanes stachybotrydial acetate and acetoxystachybotrydial acetate with IC_{50} values of 0.3 µM, 0.7 µM and 1.9 µM, respectively, were identified as potent CK2 inhibitors. Effect of these compounds on the proliferation of breast cancer cells MCF-7 was determined using an EdU assay. For comparison, viability of breast cancer cells MCF-7 as well as lung cancer cells A427 and epidermal cancer cells A431 was tested using the MTT assay. In particular, acetoxystachybotrydial acetate turned out to be the most active compound. After treatment of MCF-7 cells with 1 µM for 24 h cell proliferation was almost completely blocked (99%), whereas cell viability was decreased only by 63%.

Keywords: CK2, natural compounds, cytotoxicity, antiproliferation

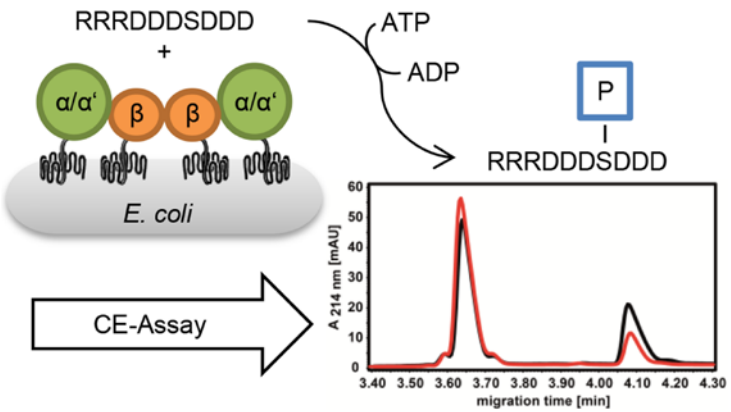
Introduction

CK2 is heterotetrameric holoenzyme and it is ubiquitously expressed in mammalian cells. Overexpressed CK2 enhances cancer phenotype by blocking apoptosis and stimulating cell growth. Thus, inhibition of this enzyme can induce the physiological process of apoptosis leading to tumor cell death. Different backbones were used as skeleton for CK2 inhibitors.

Protein kinase CK2



CK2-Aktivity test

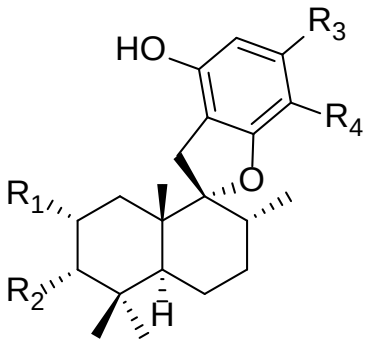


Introduction

Newly several meroterpenoids containing a chromene ring moiety as well as macrocyclic trichothecenes and phenylspirodrimanes were isolated from fungal cultures of different *Stachybotrys* strains which were grown on agar plates. Two meroterpenoid compounds exhibited moderate cytotoxic effects on HepG2 cells. The isolated compounds share structure similarity to CK2 inhibitors. In this work fourteen compounds isolated from *Stachybotrys chartarum* were tested *in silico* and *in vitro* on inhibition of CK2 holoenzyme and the most active compounds were tested on inhibition of cell viability and proliferation of different tumor cell lines.

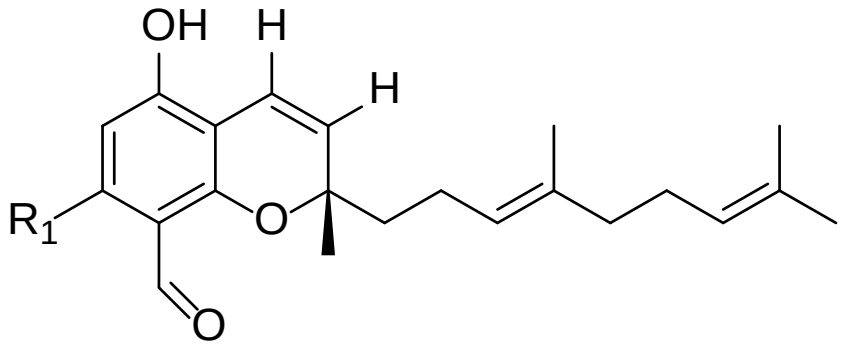
Results and discussion

The chemical structure of substituted phenylspirodrimane together with their inhibitory activity toward CK2.



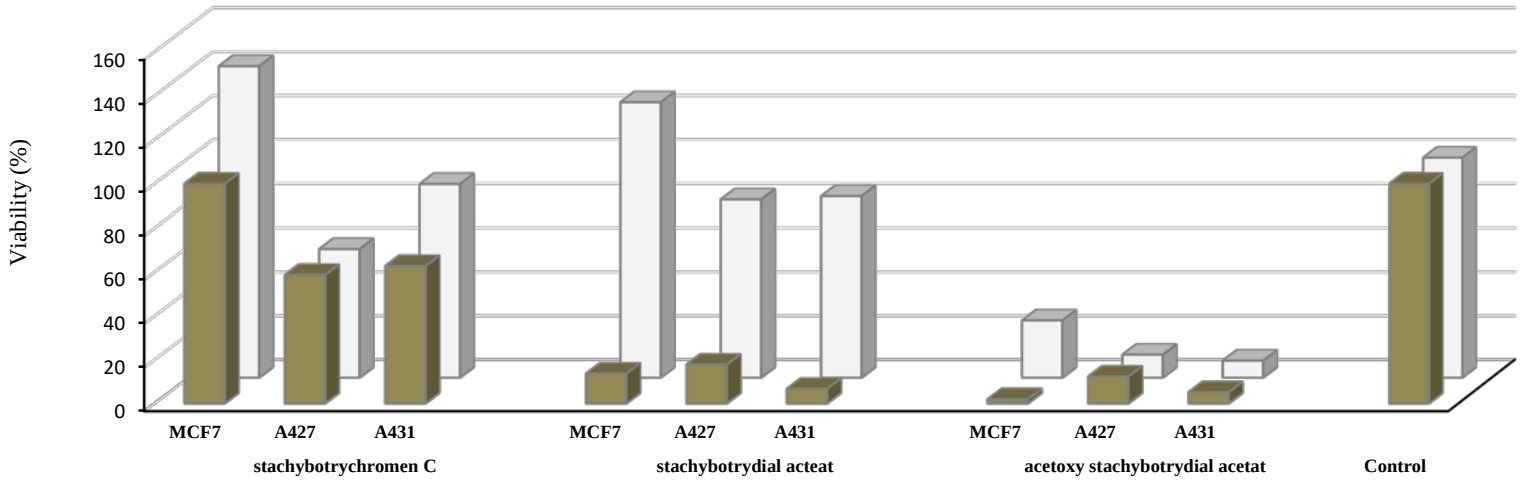
Compound	R_1	R_2	R_3	R_4	% of inhibition at 10 µM (IC_{50} µM)
Stachybotrydialacetat	H	AcO	CHO	CHO	98 **(0.70)**
Acetoxystachybotrydialacetat	AcO	AcO	CHO	CHO	87 **(1.86)**
Stachybotrydial	H	OH	CHO	CHO	72 **(4.43)**
Stachybotrysin B	H	AcO	CH_2OH	CHO	50 **(13.4)**
Stachybonoid D	AcO	AcO	CH_2OH	CHO	47
Stachybotrysin C	OH	AcO	CH_2OH	CHO	37
L-671	H	OH	CH_2OH	CHO	42
Stachybotrylactamacetate	H	AcO	CHO	CH_2NH	44

The chemical structure of three triprenylphenoles together with their inhibitory activity toward CK2



Compound	R_1	% of inhibition at 10 µM (IC50 µM)
Stachybotrychromen A	CH_3	26
Stachybotrychromen B	AcO	47
Stachybotrychromen C	CHO	95 **(0.32)**

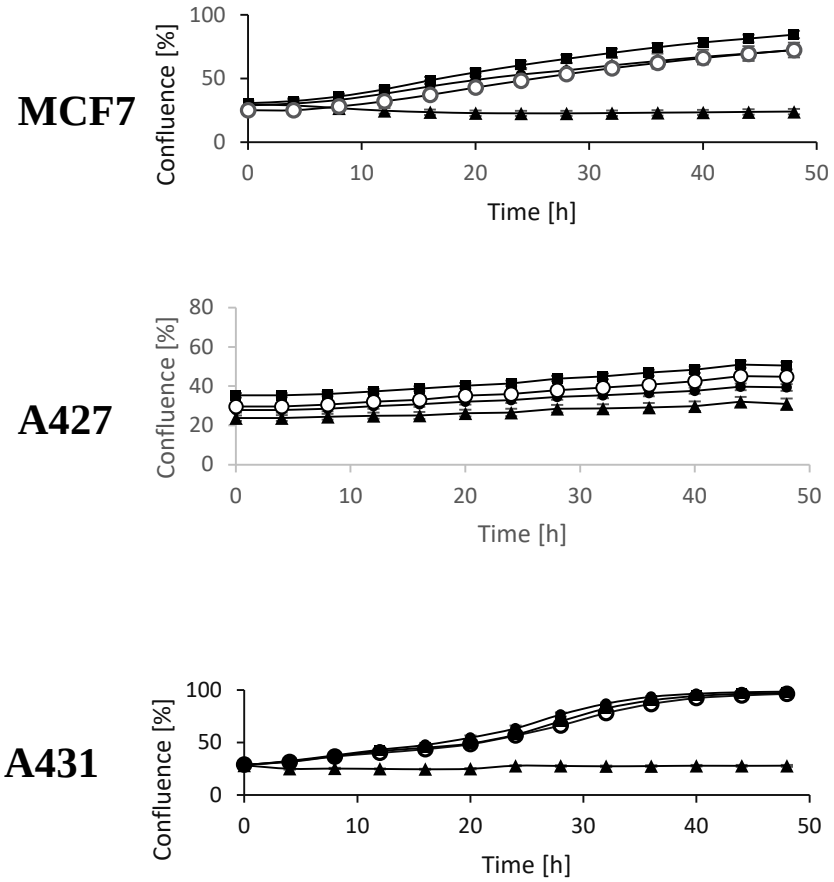
Cell viability



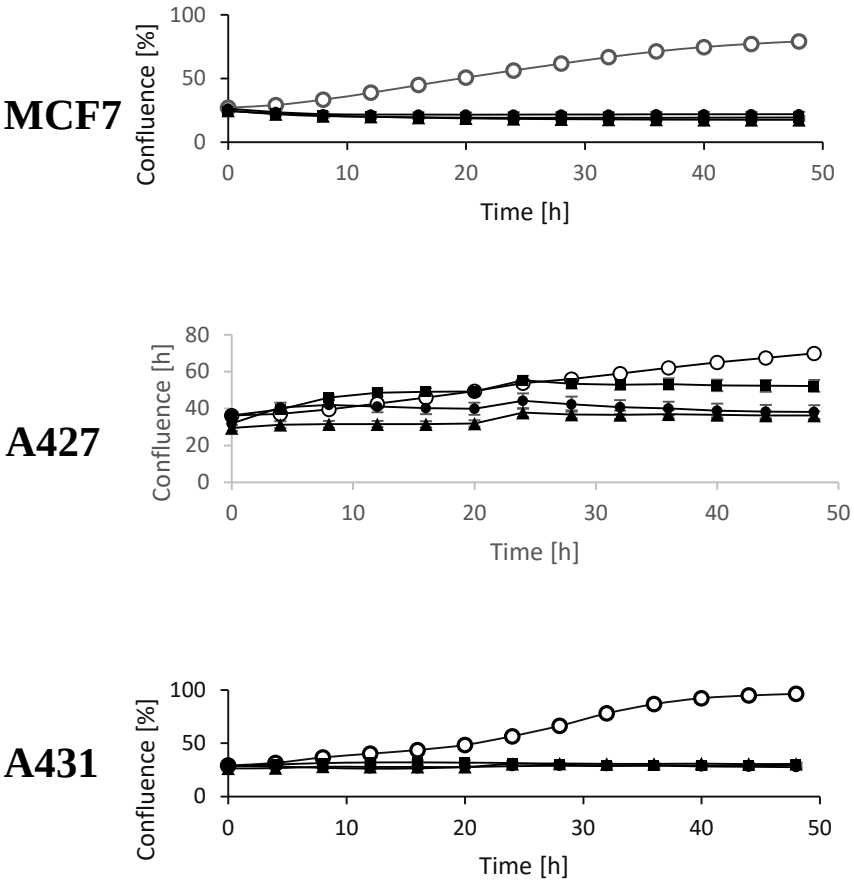
Cell viability of MCF7, A427 and A431 cells after 48 h treatment with stachybotrychromene C, stachybotrydial acetate, acetoxy stachybotrydial acetate as tested by MTT assay at concentration of 100 µM (dark) and at concentration of 10 µM (light).

Cell proliferation

stachybotrydial acetate

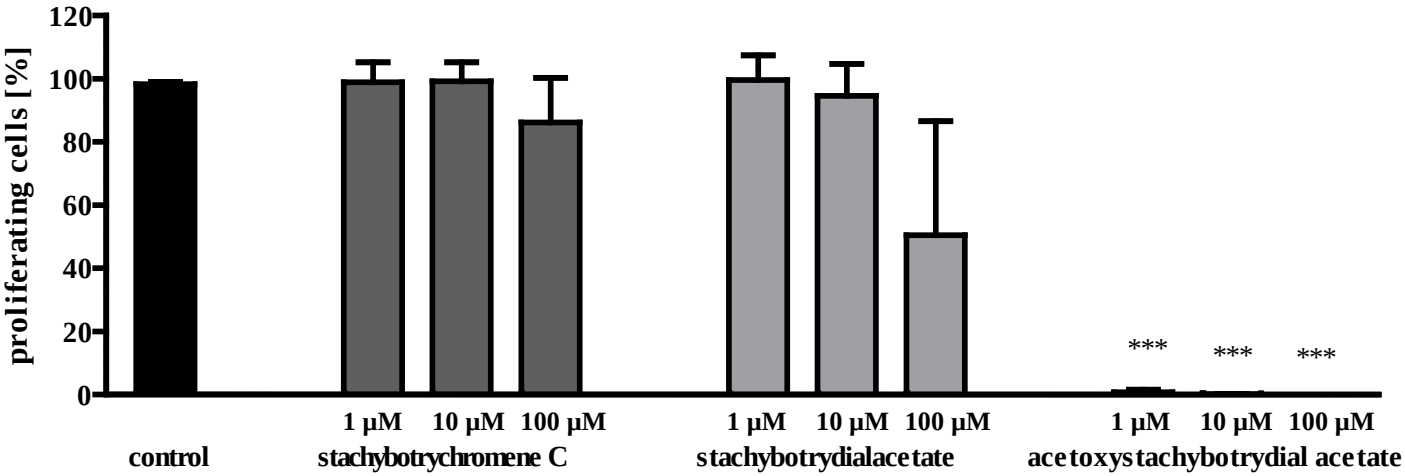


acetoxy stachybotrydial acetate

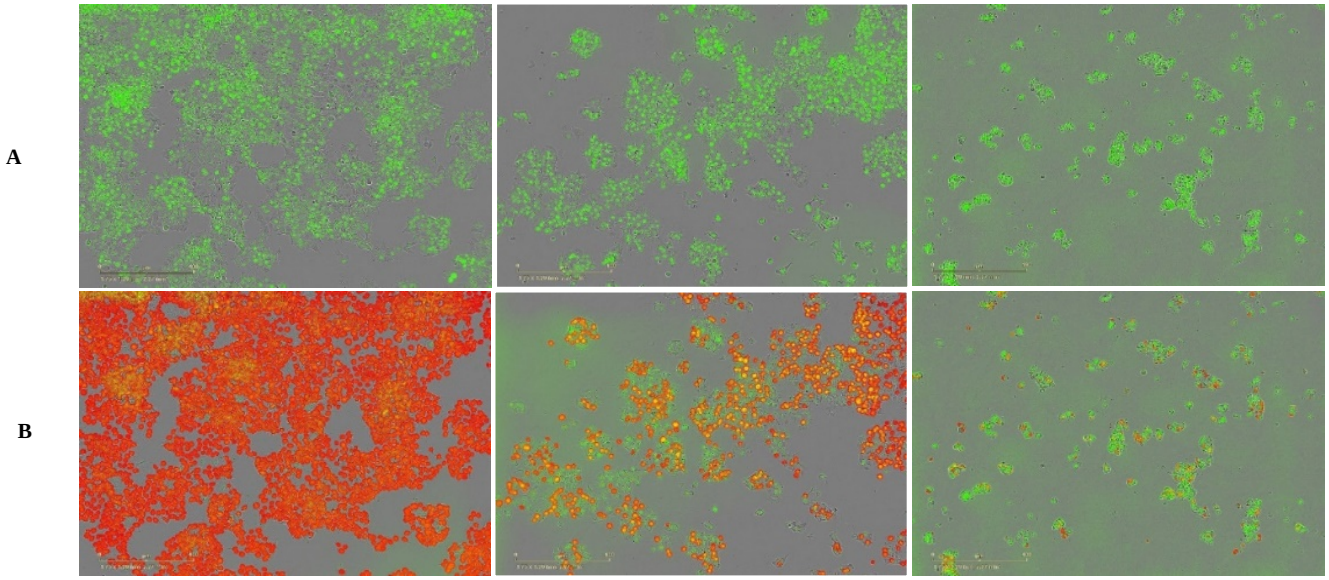


IncuCyte® cell proliferation assay with different concentration of stachybotrychromen C (A-C), stachybotrydialacetat (D-F), and acetoxystachybotrydialacetat (G-I), used for treating (A, D, G) MCF7 cells (B, E, H) A427 cells (C, F, I) A431 cells. 1 µM (■), 10 µM (•) and 100 µM (▲). As a control 1 % DMSO was used (o).

Antiproliferative effect



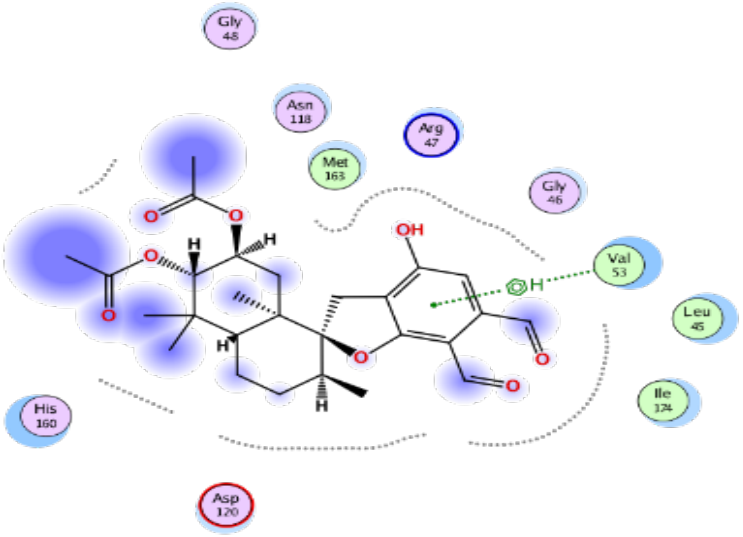
Quantification of the antiproliferative effect of stachybotrychromene C, stachybotrydial acetate, acetoxy stachybotrydial acetate on MCF7 NucLight Green cells after 24 h of incubation. Results are shown as a percent of proliferating cells relative to control cells (with 1% DMSO) and represent the mean (± SD) of three independent experiments. *** $p < 0.001$.

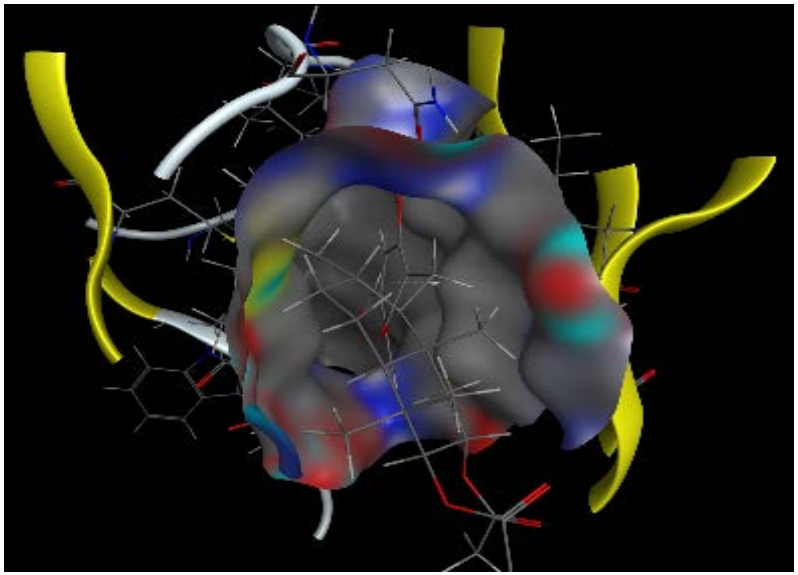


Fluorescence images of MCF7 NucLight green cells treated with 1% DMSO, 100 µM stachybotrydial acetate or 1 µM acetoxy stachybotrydial acetate for 24 h. Detection of cells achieved using green fluorescent nuclei (A). Proliferating cells were detected by an additional staining of cell nuclei by EdU-assay using 5-TAMRA-PEG3-azide as a coupled fluorophore (B). Proliferating cells were monitored by red fluorescence. The pictures in lane B are overlay of the fluorescence images of MCF7 NucLight green cells (green fluorescence) and TAMRA-labeled proliferating cells. The cells that are emitting only green fluorescence are not proliferating, in contrast to those emitting an additional red fluorescence. Live Cell Imager “IncuCyte” was used to obtain the pictures using 10 folds lens.

Docking

Acetoxystachybotrydial acetate was docked in the ATP binding site of the CK2 crystal structure (PDB ID: 3C13, resolution 1.95 Å) using MOE software
The docked compound fits well in the ATP binding site of the enzyme





2-D interaction and snapshot representing the docking complex of Acetoxystachybotrydial acetate with the ATP binding site of CK2 (MOE).

Conclusions

In this work, we demonstrated that three natural compounds from our in house database namely stachybotrychromene C, stachybotrydial acetate and acetoxystachybotrydial acetate were active CK2 inhibitor with IC_{50} values in the low micro molar range. The acetoxystachybotrydial acetate was very effective in different cancer cell lines. In concentration of 1 µM acetoxystachybotrydialacetat was able to inhibit the growth of the MCF-7 cells by 99% and the cell viability was reduced to 63% as well. This compound can be considered as an important hit for further investigations.

THE END