evropský sociální fond v ČR
EVROPSKÁ UNIE
MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY
OP Vzdělávání pro konkurenceschopnost

INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Propojení výzkumu a vzdělávání v oblasti medicinální chemie
reg. číslo: CZ.1.07/2.3.00/30.0060

Zpráva z mezinárodní konference/ Report from international conference

Účel cesty/Aim of travel:
1st Meeting of the International Society of Cancer Metabolism (ISCAM)
5th Meeting of the International Society for Proton Dynamics in Cancer (ISPDC)

Účastník/Participant: Mgr. Jana Štěpánková, Ph.D.

Doba trvání cesty/Duration of travel: 8. - 11. 10. 2014

Místo/Location: Smolenice, Slovensko

Zpráva/Report:

Na konferenci jsem v posterove sekci prezentovala výsledky dosažené v oblasti studie inhibitorů anhydrázy uhličitanu vápenatého 9, konkrétně karboranů se sulfonamidovým zbytkem. S účastníky konference jsem diskutovala výsledky své práce, možnosti budoucí spolupráce při řešení témat a případně možnost stáže.

Prezentované téma: Carborane-Based Carbonic Anhydrase IX Inhibitors

V Praze/Olomouci dne
In Prague/Olomouc, date: 22. 10. 2014

Jméno, podpis/
Name, signature Jana Štěpánková

Tento projekt je spolufinancován Evropským sociálním fondem a státním rozpočtem České republiky.

international society for proton
dynamics and cancer metabolism

Dear Participant,

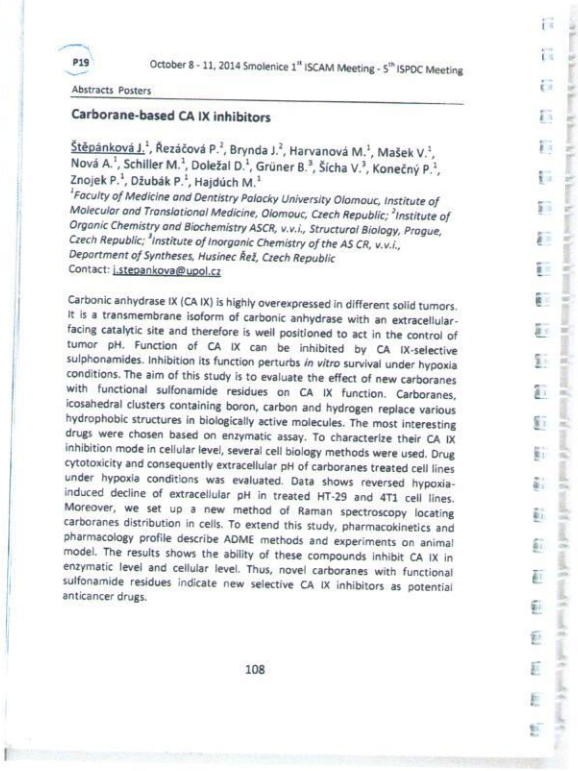
Thank you for your registration to the 1st Meeting of the International Society for Cancer Metabolism (ISCAM)/ 5th Meeting of the International Society for Proton Dynamics in Cancer (ISPDC), which will be held in Smolenice, Slovakia, from 8 – 11. October 2014.

With kind regards,

Silvia Pastorek

Prof. Silvia Pastorekova, PhD, DSc.
Department of Molecular Medicine
Institute of Virology
Slovak Academy of Sciences
Dubravska cesta 9
845 05 Bratislava
Slovak Republic

Fotodokumentace/Photos:



P19 October 8 - 11, 2014 Smolenice 1st ISCAM Meeting - 5th ISPDC Meeting

Abstracts Posters

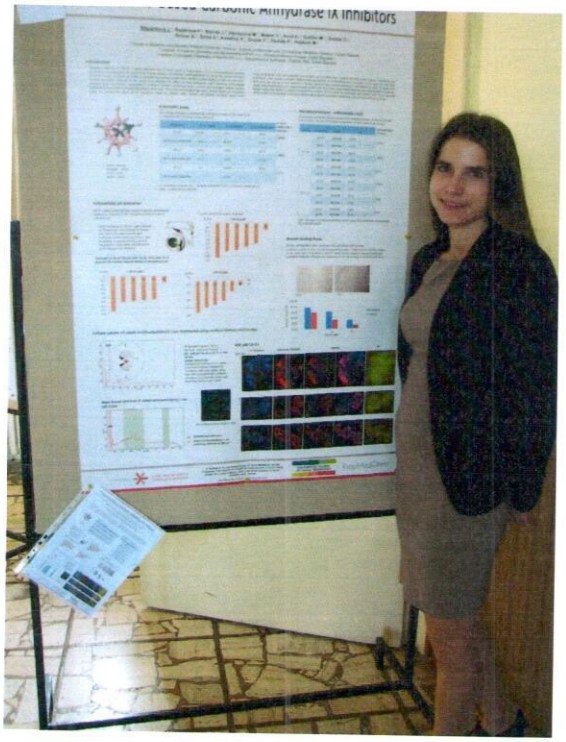
Carborane-based CA IX inhibitors

Štěpánková J.[1], Řezáčová P.[2], Brynda J.[2], Harvanová M.[1], Mašek V.[1], Nová A.[1], Schiller M.[1], Doležal D.[1], Grüner B.[3], Šícha V.[3], Konečný P.[3], Znojek P.[3], Džubák P.[1], Hajdúch M.[1]

[1]*Faculty of Medicine and Dentistry Palacky University Olomouc, Institute of Molecular and Translational Medicine, Olomouc, Czech Republic;* [2]*Institute of Organic Chemistry and Biochemistry ASCR, v.v.i., Structural Biology, Prague, Czech Republic;* [3]*Institute of Inorganic Chemistry of the AS CR, v.v.i., Department of Syntheses, Husinec Řež, Czech Republic*

Contact: j.stepankova@upol.cz

Carbonic anhydrase IX (CA IX) is highly overexpressed in different solid tumors. It is a transmembrane isoform of carbonic anhydrase with an extracellular-facing catalytic site and therefore is well positioned to act in the control of tumor pH. Function of CA IX can be inhibited by CA IX-selective sulphonamides. Inhibition its function perturbs *in vitro* survival under hypoxia conditions. The aim of this study is to evaluate the effect of new carboranes with functional sulfonamide residues on CA IX function. Carboranes, icosahedral clusters containing boron, carbon and hydrogen replace various hydrophobic structures in biologically active molecules. The most interesting drugs were chosen based on enzymatic assay. To characterize their CA IX inhibition mode in cellular level, several cell biology methods were used. Drug cytotoxicity and consequently extracellular pH of carboranes treated cell lines under hypoxia conditions was evaluated. Data shows reversed hypoxia-induced decline of extracellular pH in treated HT-29 and 4T1 cell lines. Moreover, we set up a new method of Raman spectroscopy locating carboranes distribution in cells. To extend this study, pharmacokinetics and pharmacology profile describe ADME methods and experiments on animal model. The results shows the ability of these compounds inhibit CA IX in enzymatic level and cellular level. Thus, novel carboranes with functional sulfonamide residues indicate new selective CA IX inhibitors as potential anticancer drugs.



Tento projekt je spolufinancován Evropským sociálním fondem a státním rozpočtem České republiky.

Carborane-Based Carbonic Anhydrase IX Inhibitors

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Introduction

Carbonic anhydrase IX (CA IX) is highly overexpressed in many types of solid tumors. It is a transmembrane isoform of carbonic anhydrase with an extracellular-facing catalytic site making it well positioned to act in the control of tumor pH environment. CA IX can be selective inhibited by sulphonamides what results in perturbation of cell survival under hypoxia conditions. The aim of this study is to evaluate the effect of new carboranes with functional sulfonamide residues on CA IX function. Carboranes, icosahedral clusters containing boron, carbon and hydrogen replace various hydrophobic structures in biologically active molecules. The most interesting drugs were chosen based on enzymatic assay. To characterize their CA IX inhibition mode in cellular level, several cell biology methods were used.

Drug cytotoxicity and consequently extracellular pH of carboranes treated cell lines under hypoxia conditions was evaluated. Data shows reversed hypoxia-induced decline of extracellular pH in treated HT-29 and 4T1 cell lines. Moreover, we set up a new method of Raman spectroscopy locating carboranes distribution in cells. To extend this study, pharmacokinetics and pharmacology profile was described using ADME methods and experiments on animals. Results show the ability of tested compounds to inhibit CA IX activity on enzymatic and cellular level. Thus, selective CA IX inhibitors containing functional sulfonamide residues becomes a promising class of novel anticancer drugs.

Colour scheme:
hydrogen – white,
boron – pink,
carbon – black.

Enzymatic assay

Decreasing carborane-sulfonamides inhibition activity of CA II and CA IX in presence 0.05 % human serum albumin.

Compound	K_i CA II [μM]		K_i CA IX [μM]		Ki CA II/CA IX ratio	K_i CA IX 0.05% HSA/ HSA free
CB-30	0.287	± 0.043	0.01	± 0.004	28.70	
CB-30 with 0.05% HSA	128.3	± 48.135	10.332	± 1.721	12.42	1033x
CB-31	0.905	± 0.16	0.0016	± 0.0007	565.6	
CB-31 with 0.05% HSA	70.11	± 8.927	0.627	± 0.182	111.82	391x
CB-33	2.3	± 0.35	0.0642	± 0.0618	35.83	
CB-33 with 0.05% HSA			0.079	± 0.0096		1.2x

Ki, inhibition constant; CA II, carbonic anhydrase II; CA IX, carbonic anhydrase IX
HSA - human serum albumin

Metallocarborane - sulfonamides IC50

Decreasing carborans IC50 for human colon adenocarcinoma cell line HT-29 and colon cancer cell line HCT116 after treatment of cells in serum free media.

cell line	compound	media	IC50		IC50 10% FCS/ FCS free
HT-29	CB-30	10 % FCS	78.90	± 8.54	
	CB-30	FCS free	4.13	± 1.49	19.1x
	CB-31	10 % FCS	165.47	± 0.46	
	CB-31	FCS free	13.58	± 4.22	12.2x
	CB-33	10 % FCS	52.61	± 0.73	
	CB-33	FCS free	43.16	± 10.38	1.2x
HCT116	CB-30	10 % FCS	50.45	± 0.83	
	CB-30	FCS free	2.17	± 0.07	23.2x
	CB-31	10 % FCS	79.14	± 1.15	
	CB-31	FCS free	15.82	± 7.43	5.0x
	CB-33	10 % FCS	56.81	± 1.64	
	CB-33	FCS free	30.14	± 6.17	1.9x

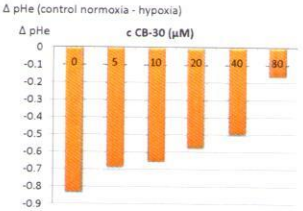
IC50 - the value of drug concentration which cause 50 % inhibition of cell growth
FCS, fetal calf serum

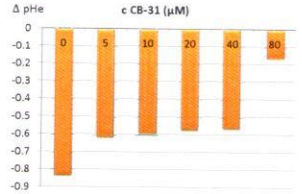
Extracellular pH alteration

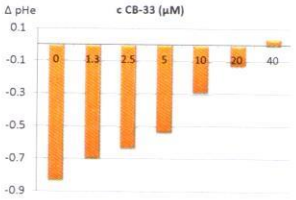
CA IX controls tumor pH and hypoxia-induced, extracellular acidosis is a measure of the biological activity of CAIX in cultured cells.

CAIX expressing HT-29 cells were seeded on 96 well plate and allowed to recover overnight. Cells were treated with carborane-sulfonamides and after 3 days incubation in hypoxia (1% O_2), pH was measured in single wells with the aid of pH-4 microsensors (PreSens).

Treatment of the HT-29 cells with CB-30, CB-31 and CB-33 reversed the hypoxia-induced decline of extracellular pH.

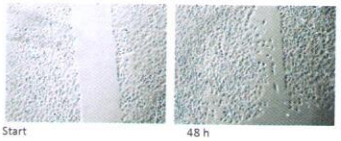


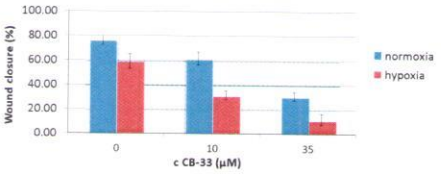




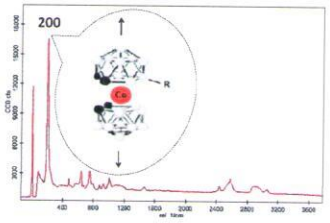
Wound Healing Assay

Change cell migration after treatment with carborane-sulfonamides.
Confluent culture of HeLa cells were starved overnight in DMEM with 0.5% FCS. Images of calls were taken immediately and 48 h after wound initiation. Wound healing was quantified using Zen software and expressed as the percentage of closed wound area.



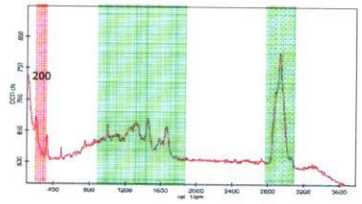


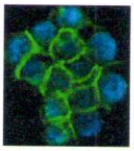
Cellular uptake of cobalt bis(dicarbollide)(1-) ion monitored using confocal Raman microscopy



- **HT-29 cells,** hypoxia (1% O_2) 48 hours, methanol fixation
- **50 – 400 μM CB-30 or CB-31 in PBS 30 min**
- **Raman microscopy**
- immunofluorescence (CA IX rabbit poloclonal antibody followed by incubation with anti–rabbit Alexa fluor 488-conjugated IgG antibody, cells were mounted onto slides with Mounting Medium with DAPI)

Mean Raman spectrum of cobalt bis(dicarbollide)(1-) ion cell cluster

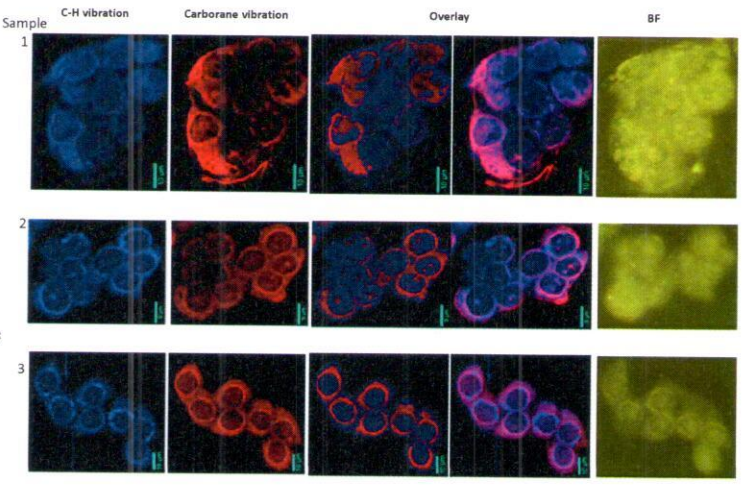




imunofluorescence, Zeiss m., 40x

biomolecular vibrations
cobalt bis(dicarbollide)(1-) ion stretching vibration at 200 cm^{-1}

400 μM CB-31



1st Meeting of the International Society of Cancer Metabolism (ISCAM)
5th Meeting of the International Society for Proton Dynamics in Cancer (ISPDC)
October 08-11, 2014, Congress center of the Slovak Academy of Sciences, Smolenice castle, Slovakia

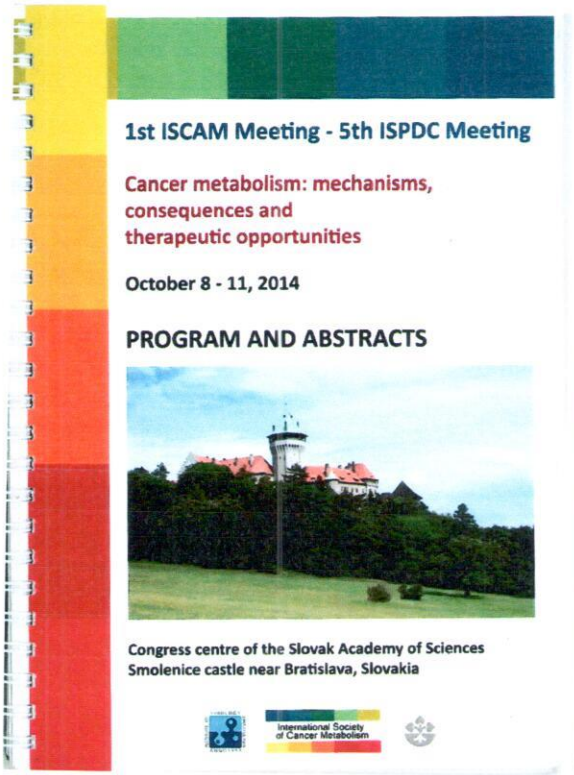
Jana Štěpánková, Contact: j.stepankova@upol.cz

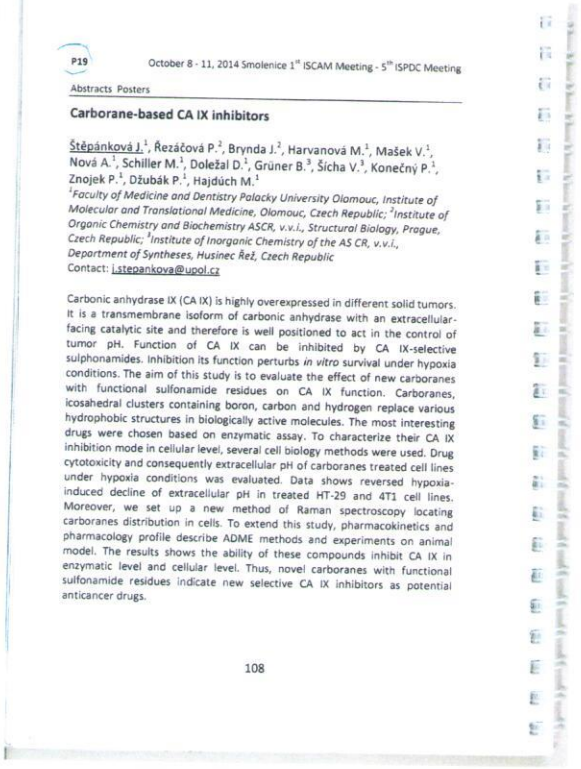
BIOMEDREG ÚSTAV MOLEKULÁRNÍ A TRANSLAČNÍ MEDICÍNY

International Society of Cancer Metabolism

PropMedChem

Fotodokumentace/Photos:





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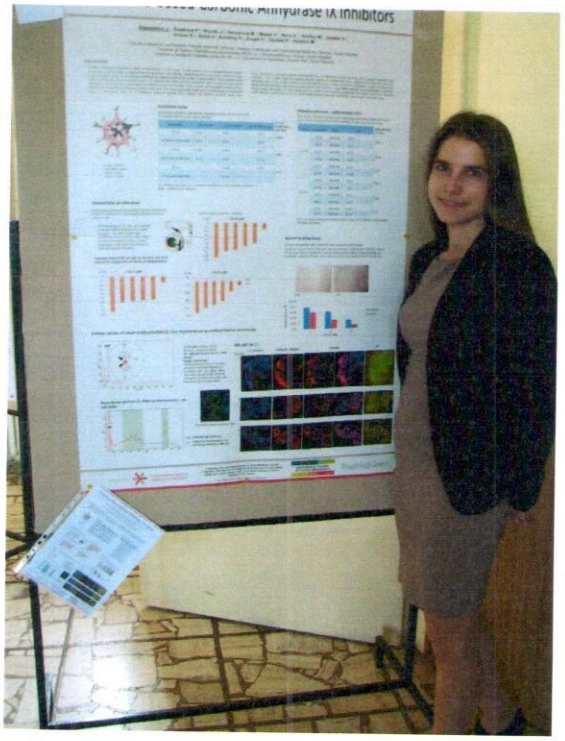

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