EUROPEAN SCIENCE FOUNDATION
SETTING SCIENCE AGENDAS FOR EUROPE

Exploratory Workshops Scheme

Standing Committee for the European Medical Research Councils (EMRC)

SCIENTIFIC REPORT

ESF Exploratory Workshop on

BioBor – Exploring New Opportunities Of Boron Chemistry Towards Medicine

Lodz, Poland, 9 - 12 May 2008

Convened by:
Zbigniew J. Lesnikowski and Agnieszka Olejniczak

Centre of Medical Biology, Polish Academy of Sciences, Lodz

EXECUTIVE SUMMARY

The aim of the workshop was to bring together scientists working on different aspects of bioorganic chemistry of boron and to discuss and define fresh potentialities offered by recent discoveries - often done in participants' laboratories. The chemistry and biology of boron carriers for Boron Neutron Capture Therapy (BNCT) was not excluded, but because the bioorganic and medicinal chemistry of boron was focused on BNCT issue for a long time, intentionally, it was not the main topic of this meeting. It was expected that such approach should give rise to new concepts and help to consolidate research programs of participants within new areas whenever possible and profitable.

Indeed, fresh ideas, new compounds and new applications were presented during the meeting. All of these form a good base for further exploration of great potential of bioorganic chemistry of boron. The general theme of cooperation and further efforts was defined as: "*Exploration of New Opportunities Towards Boron Chemistry to Improve Medicine*". Please see also chapter below "*Assessment of the results and contribution to the future direction of the field*"

Albeit, the primary mission of ESF is to provide a common platform for promotion of European research and to explore new directions for research at the European level, the needs of the European research community in a global context should not be underestimated.

The BioBor meeting in Lodz is a good example of creative opening towards such needs. In addition to colleagues from ESF Member countries leading scientists from United States, Japan and Russia have been also invited. This not only extended the scientific diversity of the presented ideas but also allowed to widen the base of the cooperation.

Many participants of the meeting are already involved in collaborative programs, however the BioBor meeting created a new frame for a broader joint initiatives which are in the process of organizing and discussion. It should be pointed out that the BioBor participants agreed that the emerging network of the laboratories

and researchers involved in studies on modern bioorganic chemistry of boron and its practical applications in molecular medicine, diagnostics and nanobiotechnology, should remain open to colleagues wishing to join it in future.

SCIENTIFIC CONTENT OF THE EVENT

All attendees of the meeting were active participants, and delivered 35 minutes presentation followed by 10 min. discussion. Since the formula of the workshop, by assumption, is less rigid than that of regular symposium, the schedule of the sessions was adjusted to the reasonable extensions of the presentations and/or discussions to provide space for additional debate.

The talks were focused not only on recent, often unpublished results (the rare and to large extend forgotten feature of scientific meetings) but shown also future directions and research planed in future in the presenting author's laboratory.

The first speaker, **prof. Vladimir I. Bregadze** (INEOS, Russia) focused his presentation on polyhedral boron compounds as molecules likely possessing a wide range of possible application in the field of medicinal chemistry. He has shown methods for synthesis of new derivatives of carboranes, polyhedral borate anions and metallacarboranes and provide preliminary data on their evaluation as potential antitumor agents. Of special interest were results on synthesis and *in vitro* antitumor activity of organotin derivatives of carboranes and synthesis of new polyhedral boron compounds as potential BNCT agents. During the discussing the necessity of careful comparison of cytotoxic activity of the potential anticancer agents in tumor and healthy cells was pointed out.

Dr Petr Cigler (VSCHT, Czech Republic), presented a series of cobalt(III) bis(dicarbollide) conjugates with different fluorophores prepared for cellular uptake studies of metallacarboranes as potential anti-HIV agents. Results of studies on cell internalization and solution properties of these novel molecules were shown. The process of molecular association was monitored using light scattering, atomic force microscopy a fluorescence spectroscopy. The possibility of aggregate formed

decomposition by action of serum albumin was demonstrated. The potential effect of fluorophore on unwanted modulation of parent compounds' cellular uptake was raised during discussion.

Prof Stafan Eriksson (SUAS, Sweden), discussed potential of boronated nucleosides as ideal compounds for targeting tumor cells in boron neutron capture therapy (BNCT) provided that they could be metabolized and accumulate selectively in such cells at sufficient levels. His presentation summarized the current knowledge concerning borano nucleosides and DNA precursor enzymes with emphasis on their structure activity and selective expression in tumor cells compared to normal tissues. The high capacity of thymidine kinase 1 (TK1) to phosphorylate N3 substituted carboranyl nucleoside analogs with varying substitutions was reviewed as well as the recent great advances in understanding of TK1 structure and function relationships and cell cycle regulation. The successful use of one of the N3 analogs in BNCT of a brain tumor model in rats was described as well as the potential use of other DNA precursor enzymes as targets. During the discussion, several disputants pointed out that the results presented by prof. Eriksson may inspire the design of new nucleoside analogs where the bulky borano cage is tethered to be out side the active site of key metabolizing enzymes, which is of importance for practical applications of this type of nucleoside analogs as potential drugs.

Prof. Bohumir Grüner (IIC, Czech Republic) focused his talk on some aspects of the chemistry of metal bis(dicarbollide) compounds functionalized by ammonium and oxonium groups and their use in design of inhibitors of HIV-PR, the key enzyme essential for the replication of the virus. The structure of the most efficient inhibitors consists two cluster units combined together *via* an organic ammonium or amidic functions attached via several possible spacers. General aspects of use of boron cluster compounds for design of biologically active compounds were also discussed. During the discussion disputants pointed out importance of the results presented by prof. Gruner for better understanding of the mechanism of action of HIV-PR, an effect of authors' findings for design of new anti-HIV drugs as well as for development of bioorganic chemistry of metallacarboranes, in general.

Prof. M. Frederic Hawthorne (IINMM, USA), presented breakthrough results on application of boron clusters as pharmacophors in new drugs design. He focused on transthyretin, a homotetrameric transport protein found primarily in the blood and cerebrospinal fluid, is the putative causative agent in a variety of amyloid diseases. Kinetic stabilization, through small-molecule complexation, of the native tetrameric state of transthyretin may impart a protective effect through prevention of the dissociation that leads to amyloid fibril formation. However, many of the compounds known to impart this stabilization are, or are structurally similar to, non-steroidal anti-inflammatory drugs (NSAID). Consequently, such compounds exhibit deleterious concomitant inhibition of cyclooxygenase (COX) enzymes. Through judicious application of carboranes as skeletal motifs in analogs of these NSAIDs, a compound, 1-carboxylic acid-7-[3-fluorophenyl]-dicarba-*closo*-1,7-dodecaborane, was synthesized that showed effectively no COX-1 or COX-2 inhibition at a concentration more than an order of magnitude larger than the concentration at which TTR dissociation is nearly completely inhibited. Long discussion following the presentation concerned mainly the future and prospects of boron cluster application in design of various new drugs and search for suitable biological targets.

Prof. Evamarie Hey-Hawkins (Universität Leipzig, Germany), discussed new achievements in the area of carbaborane-containing bisphosphonates. High and selective accumulation in tumour cells is one important requirement for a BNCT agent. One way of increasing the tumour selectivity of BNCT agents may be the use of carbaborane-containing phosphonate groups. As some simple carbaboranylbisphosphonates already exhibit high tumour selectivity, authors are attempting to improve the BNCT potential by using glycosyl esters of phosphonic acids. Possible application of the methods developed for sugars, for the synthesis of nucleoside esters of phosphonic acids was debated in details during discussion.

Prof. Jan Konvalinka (IOCB, Czech Republic) presented some results of biological studies on metallacarboranes as strong and specific inhibitors of HIV protease. Potential of these new molecules against resistant variants of HIV was stressed. The scope of the discussion was limited due to ongoing patenting process of these new potential drugs.

Prof. Zbigniew Lesnikowski (IBM PAS, Poland), presented several general approaches for the synthesis of purine and pyrimidine nucleosides modified with carborane clusters and metallacarborane complexes. The disclosed methods included: 1) attachment of carborane modification at 2' position of nucleoside *via* formacetal linkage formation, 2) tethering of the metallacarborane group at nucleobase part of the nucleoside *via* dioxane ring opening in oxonium metallacarborane derivatives and 3) "click chemistry" approach based on Huisgen 1,3-dipolar cycloaddition. It was remarked during the discussion that the proposed methodologies extend significantly the range of nucleoside/borane cluster conjugates available for biological studies and open new areas for their applications. It was pointed out particularly that availability of the methods for synthesis of purine nucleosides - adenosine and guanosine, modified with boron cluster pharamacophore creates new perspectives in the field.

Dr. Pavel Matějíček (Charles University, Czech Republic), focused on hydrophobicity of boron clusters and on its effect on physicochemical properties of potential boron cluster containing drugs. This attribute manifests itself in a surface activity, even when they lack an amphiphilic topology. It leads to formation of small subunits consisting of several metallacarborane molecules bind together by hydrophobic interaction and hydrogen bonding in water. As a result, nanoparticles with typical radius of ca. 100 nm are formed in aqueous solutions depending on aging, concentration and ionic strength. Boron clusters can change dramatically an aggregation behavior of their conjugates with "very water soluble" species (like nucleosides or porphyrines). The light scattering measurements showed that the aggregated boron clusters can interact with conventional surfactants. Due to the formation of hydrogen and dihydrogen bonds, carboranes can interact also with polymer chains. For example, bis(dicarbollide) cobaltate anion decreases solubility of poly(ethylene oxide) and poly(2-vinylpyridine) in aqueous solutions. The phase–separation is induced by presence of inorganic cations in case of poly(ethylene oxide). The results of studies on aggregation behavior of nucleoside/boron cluster conjugates, performed in collaboration with prof. Zbigniew Lesnikowski, were also presented. During the discussion, the effect of aggregation of boron cluster containing drugs on their pharmacokinetics was pointed out.

Prof. Hiroyuki Nakamura (Gakushuin University, Japan), delivered a talk on application boronic acid as a pharmacophore in design of selected enzymes inhibitors. Aminoboronic acids as growth-factor receptor tyrosine kinase inhibitors and boronic acid containing *cis*-stilbenes as tubulin polymerization inhibitors were designed and synthesized based on the biologically active structures of Lavendustin and Combretastatin A-4, respectively. High cell growth inhibitions were observed in boronic acid containing *cis*-stilbene derivatives, in which a hydroxyl group on the aromatic ring B of the combretastatin A-4 was replaced by a boronic acid. The growth inhibition activity was tested against a Panel of 39 human cancer cell lines showing that boronic acid containing *cis*-stilbenes showed differentia growth inhibition in comparison with Combretastatin A-4 and their correlation coefficient (r) was 0.553 in the COMPARE analysis. The originality of the proposed approach - using boronic acid residue, not a boron cluster as a pharmacophore was pointed out during the discussion. The potential of this approach in drug design was also discussed.

Dr. Agnieszka Olejniczak (IBM PAS, Poland). Novel application of boron clusters and their complexes with metals - as redox labels for biomolecules was presented during this talk. A general approach to the synthesis of derivatives of four canonical nucleosides: thymidine (T), 2'-deoxycitidine (dC), 2'-deoxyguanosine (dG), 2'-deoxyadenosine (dA) and their ribocounterparts bearing metallacarborane complexes with metals such as cobalt, iron, chromium or rhenium was shown. Metallacarborane/nucleoside conjugates were prepared *via* reaction of suitable 8-dioxane-3-metal bis(dicarbollide) adduct with base-activated, protected nucleosides or by *de novo* formation of 3,3,3-tricarbonyl-3-rhenium-1,2-dicarba-*closo*-dodecaborate complex from carborane/nucleoside ligand. The conjugates display easily distinguishable, diagnostic electrochemical signals assigned to boron cluster component. The above finding lays foundation for a "multi color" electrochemical coding of DNA based on electrochemical properties of boron clusters. During the discussion the range of redox potentials required for a construction of a set of optimal electrochemical DNA labels based on boron clusters was discussed. The commercial potential of the electrochemical labels as alternative to fluorescent tags was analyzed.

Prof. Michael D. Threadgill (University of Bath, UK), presented results of the University of Bath synthetic medicinal chemistry group attempts to address two of the outstanding issues in tumour-selective delivery of boron clusters for BNCT, tumour targetting and aqueous solubility. Syntheses of 1,2-dicarba-*closo*dodecaboranes have been linked to nitroimidazoles, nitrofurans, porphyrins and DNA-groove-binding PBDs have been achieved. Increased polarity of the targeting group carborane constructs was accomplished without charge through $(CH_2CH_2O)n$ units attached either at the periphery of the construct or as part of the linker joining the modules of the construct. A carborane–□-cyclodextrin inclusion complex with remarkable stability has been characterised; this stability may shed some light on the deleterious effect of cyclodextrins in solubilising carboranes in biological experiments. A simple cage degradation of 1,2-dicarba-*closo*-dodecaboranes to corresponding *nido*-carboranes has been developed, which may facilitate an important non-BNCT use of icosahedral carboranes as neutral scaffolds or platforms with unusual geometry for conformationally controlled display of bioligands. During the discussion a still unresolved satisfactorily issue of boron carriers selectivity toward tumor cells, in spite of progress in this area, was raised.

Prof. Werner Tjarks (Ohio State University, USA), discussed current progress and extant remaining difficulties in molecular modeling of compounds containing boron clusters. The docking of *closo*- and *nido*-carboranyl antifolates into human dihydrofolate reductase (hDHFR) investigated with Autodock 4 was shown as an example. The cage structures of these antifolates bound to a hydrophobic pocket within the active site of the hDHFR crystal structure through van der Waals interactions. The docked poses of the antifolates were similar to those of the corresponding ligand poses in the original hDHFR crystal structure. Minor variations in partial atom charges and cage geometries had no significant impact on docking accuracy. The obtained docking patterns validated methods that were previously developed for the computational design of carborane-containing compounds. Disputants stressed the importance of further improvements of computational methods for progress in design of new, boron cluster containing drugs and *in slico* evaluation of new molecules containing boron pharmacophores.

Prof. **Clara Viñas** (ICMAB, Spain), shown in her talk the great chemical and physical potential of Polypyrrole membranes doped with $[Co(C_2B_9H_{11})_2]$- in the development of novel hydrogen, potassium and sodium ion selective electrodes (IES's). To apply in biomedical measurements, these membranes have been used as the solid internal contact in potentiometric microelectrodes by using microfabrication technologies and electrochemical polymerization. It is foreseen that insoluble amine salts of $[Co(C_2B_9H_{11})_2]$- can be used in potentiometric PVC based ISE's for the detection of antibiotics. Additionally boron iodinated *closo o*-carborane derivatives have been synthesized for possible medical applications in radioimaging/radiotherapy and as X-Ray contrast agents. In addition, high boron water soluble *o*-carborane derivatives have been synthesized to be used as anti-tumor agents for BNCT. The discussion focused on versatility of boron clusters and various practical application supported by this class of compounds. Disputants agreed that it is necessary to develop not only biological aspect of boron chemistry but also take the advantage of boron clusters as useful platform in design of new materials for medical and technical applications.

Prof. Marek Zaidlewicz (Nicolaus Copernicus University, Poland), focused his presentation on application of boron compounds in organic synthesis. The asymmetric synthesis of N-substituted *N*-hydroxyureas, derived from acetophenones, 1 (benzofuran-2-yl)ethanone, 2,3-dihydrobenzofuran-3- one, 1-(benzo[*b*]thiophen-2-yl)ethanone, was shown. The enantioselective reduction of these ketones and/or their oxime ethers with borane/oxazaborolidines was used as the key step generating the stereogenic center linked to the *N*-hydroxyurea moiety. During the discussion the traditional, but still valid and important applications of boron compounds as extremely useful agents in organic synthesis was stressed.

Summary and highlights: The life is based ultimately on the organic derivatives of the element carbon. Boron compounds share some similarities with carbon but also differ in many aspects. Combination of these similarities and differences gives boron its unique potential in medicine. The fact that novel boron compounds are unfamiliar to enzymes and metabolic pathways evolved during miliards of years for carbon based compounds has potential advantages for antibiotic drugs since pathogens will be less able to develop resistance against them, and

possibly, it would take longer for this to happen in the case of boron based compounds.

The presentations addressed the following, important aspects of medicinal chemistry of boron containing compounds: 1) new methods of synthesis and new biologically active compounds, 2) biological and physicochemical evaluation of boron containing molecules, 3) computational design of boron containing drugs, 4) new applications of boron containing compounds as therapeutics and diagnostics, 5) new materials based on bioorganic/inorganic, boron containing conjugates. The proposed follow-up activities and actions are shown below.

The papers presented during the meeting, in the form of 5-14 pages chapters, were published in the “BioBor” proceedings.

ASSESSMENT OF THE RESULTS, CONTRIBUTION TO THE FUTURE DIRECTION OF THE FIELD, OUTCOME.

In conclusion of the workshop, as result of the after-presentation discussions and exchange of ideas during the meeting, and final brainstorming session the following was agreed:

Proposed follow-up activities:

1. Research Networking and organizing of the Consortium (in preparation for ESF Research Networking Programmes - 2008 Call for Proposals)
2. Preparation and submitting of Expression of Interest
3. Preparation and submitting of joint project proposal (EUROCORE)

Proposed theme:

“Exploration of New Opportunities Towards Boron Chemistry to Improve Medicine”

Topics and directions:

1. Initiating the development of new, economically attractive methods for synthesis of boron precursors.
2. Building of data base of boron containing bioorganic compounds.
3. Study and development of new drugs and diagnostics containing essential boron component.
    a. Screening and physicochemical and biochemical evaluation of libraries of compounds for specific activities:
    Antiviral
    Anticancer
    Boron carriers for BNCT
    Other
    b. Developing of new diagnostic methods;
    Imaging
    Biosensing
    c. Developing of new materials based on bioorganic/inorganic, boron containing conjugates

FINAL PROGRAMME

Friday 9 May 2008

Afternoon/Evening	*Arrival and registration at "Qubus" hotel*

Saturday 10 May 2008

Session 1

09:00-09:30	**Meeting introduction by the convenors**
	Alexandra Polakova (Standing Committee for the European Medical Research Councils) **Presentation of the European Science Foundation (ESF)**
09:30-10:15	**Vladimir I. Bregadze** (INEOS) Synthesis of new polyhedral boron compounds as potential antitumour agents
10:15-11:00	**Petr Cigler** (VSCHT) Fluorescently labeled metallacarboranes: solution behavior and interaction with serum proteins
11:00-11:15	*Coffee break*
11:15-12:00	**Staffan Eriksson** (SUAS) DNA precursor enzymes and carborano nucleosides

12:00-12:45	**Bohumir Grüner** (IIC) Metallacarborane building blocks and their use in design of inhibitors of HIV protease
12:45-13:30	**M. Frederic Hawthorne** (IINMM) Roles for polyhedral boranes and carboranes in nano and molecular medicine
13:30-14:45	*Lunch*

Session 2

14:45-15:30	**Evamarie Hey-Hawkins** (Universität Leipzig) Imitation and Modification of Biologically Relevant or Active Molecules via Integration of Carbaborane Clusters
15:30-15:45	*Coffee break*
15:45-16:30	**Jan Konvalinka** (IOCB) Metallacarboranes as potent and specific inhibitors of HIV protease and its resistant variants: more inhibitors, more enzymes, more structures
16:30-17:15	**Zbigniew Lesnikowski** (IBM PAS) Beyond pyrimidine nucleosides and carboranes - New nucleoside/boron cluster conjugates and their applications
18:00-21:00	*Workshop Dinner* and Bar Discussion

Sunday 11 May, 2008

Session 3

09:30-10:15	**Pavel Matějíček** (Charles University) Behavior of metallacarboranes in aqueous solutions and their interaction with polymers
10:15-11:00	**Hiroyuki Nakamura** (Gakushuin University) Boronic acid as an alternative functional group for drug design
11:00-11:15	*Coffee break*
11:15-12:00	**Agnieszka Olejniczak** (IBM PAS) Boron clusters as electrochemical labels for biomolecules
12:00-12:45	**Michael D. Threadgill** (University of Bath) 1,2-Dicarbadodecaboranes: chemical strategies fordelivery and opportunities as ligand platforms
12:45-13:30	**Werner Tjarks** (Ohio State University) The carborane cluster in computational drug design
13:30-15:00	*Lunch*

Session 4

15:00-15:45	**Clara Viñas** (ICMAB) Carboranes and metallacarboranes as building blocks for biomaterials
15:45-16:30	**Marek Zaidlewicz** (Nicolaus Copernicus University) Asymmetric synthesis of 5-lipoxygenase Inhibitors

16:30-16:45 *Coffee break*

16:45-18:15 **Brainstorming and plans for action**

18:15-20:30 *Dinner* and Bar Discussion

Monday 12 May 2008

08:00 *Breakfast and departure*

FINAL LIST OF PARTICIPANTS

1. Vladimir I. Bregadze
A.N.Nesmeyanov Institute of Organoelement Compounds RAS
Vavilov Str. 28, Moscow, 119991
Russia

2. Petr Cigler
VSCHT (Institute of Chemical Technology)
Technicka 5
Prague 6 – Dejvice 166 28
Czech Republic

3. Staffan Eriksson
Swedish University of Agricultural Sciences
Biomedical Center
Box 575
S-75123 Uppsala
Sweden

4. Bohumir Grüner
Institute of Inorganic Chemistry
Academy of Sciences of the Czech Republic
250 68 Rez near Prague
Czech Republic

5. M. Frederic Hawthorne
International Institute of Nano & Molecular Medicine
University of Missouri-Columbia
202 Schlundt Hall
Columbia, MO 65211
USA

6. Evamarie Hey-Hawkins
Institut für Anorganische Chemie
Universität Leipzig
Johannisallee 29
04103 Leipzig
Germany

7. Jan Konvalinka
Institute of Organic Chemistry and Biochemistry ASCzR
Flemingovo nam. 2
166 10, Prague 6
Czech Republic

8. Zbigniew J. Lesnikowski
Polish Academy of Sciences
Institute of Medical Biology
106 Lodowa St.
93232 Lodz Poland

9. Pavel Matějíček
Charles University in Prague
Albertov 2030
128 40 Prague 2
Czech Republic

10. Hiroyuki Nakamura
Faculty of Science
Gakushuin University Mejiro
Toshima-ku, Tokyo 171-8588
Japan

11. Agnieszka Olejniczak
Polish Academy of Sciences
Institute of Medical Biology
106 Lodowa St.
93232 Lodz Poland

12. Clara Viñas
Institut de Ciència de
Materials de Barcelona
Campus U.A.B.
08193 Bellaterra
Barcelona
Spain

13. Michael D. Threadgill
Department of Pharmacy
and Pharmacology
University of Bath
(5-West -3.9) Claverton Down
Bath, BA2 7AY
United Kingdom

14. Werner Tjarks
The Ohio State University
College of Pharmacy
Div. of Medicinal Chemistry
& Pharmacognosy
Parks Hall Room 421
500 West 12th Avenue
Columbus, OH 43210
USA

15. Marek Zaidlewicz
Nicolaus Copernicus
University
Faculty of Chemistry
7 Gagarina St.
87-100 Torun
Poland

ESF Representative

16. Prof. Katarina Polakova
Slovak Academy of
Sciences,
Bratislava
Slovakia

STATISTICAL INFORMATION

Total number of participants:	15
Number of participants from ESF Member countries:	11 (75%)
Number of non ESF Member countries participants:	4 (25%)
Breakdown by individual countries:	
Czech Republic	4 (25%)
Germany	1 (7%)
Japan	1 (7%)
Poland	3 (20%)
Russia	1 (7%)
Sweden	1 (7%)
Spain	1 (7%)
United Kingdom	1 (7%)
United States of America	2 (13%)
Number of females:	3 (20%)
Number of males:	12 (80%)
Number of participants below 35 years old	3 (20%)
Number of participants above 35 years old	12 (80%)