Institute of Medical Biology Polish Academy of Sciences (IMB PAS)
Screening Laboratory for Anti-viral and Anti-bacterial Compounds

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THE PEOPLE

Zbigniew J. Lesnikowski: zlesnikowski@cbm.pan.pl

Anna Obidowska: aobidowska@cbm.pan.pl

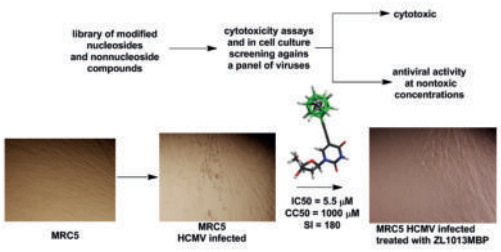
THE PROJECTS

Discovery of the innovative nucleoside modifications with antiviral activity

During the past decades, a great number of nucleoside derivatives and analogues have been synthesized and tested in the search for therapeutically useful agents. However, use of these drugs is limited by drug resistance, toxicities and other adverse effects prompting efforts to identify novel agents, new targets for chemotherapy and better treatment strategies.

One of the original avenues in search for innovative biologically active compounds are derivatives comprising in their structure a boron cluster component [Lesnikowski, Z.J., Challenges and opportunities for the application of boron clusters in drug design. J. Med. Chem., 2016, 59, 7738-7758; Leśnikowski, Z.J., Recent developments with boron as platform for novel drug design. Exp. Op. Drug Disc., 2016, 11, 569-578]. The present status of boron resembles the medicinal chemistry of fluorine four decades ago (currently, approximately 20% of pharmaceuticals on the market contain fluorine). The fact that boron compounds, especially those based on abiotic boron clusters, will be unfamiliar to life has potential advantage since organisms may be less prone to develop resistance against these molecules.

Figure 1. In cell culture screening in a search for antiviral agents.



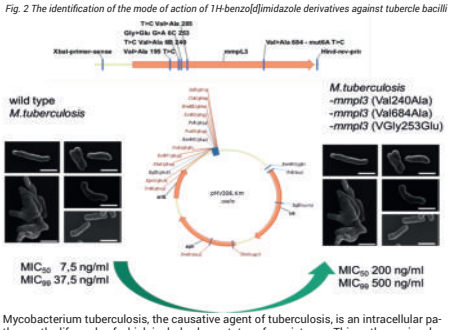
In addition, because unique properties of boron clusters, the interactions of boron cluster drugs would likely be somehow different from the purely organic systems, as a result new drug targets in a new way can be aimed.

Screening a library of as well purine as pyrimidine nucleoside boron cluster conjugates against a panel of DNA (human cytomegalovirus, herpes simplex virus type 1, HSV-1) and RNA encephalomyocarditis virus, EMCV), human parainfluenza virus type 3, HPIV-3), and vesicular stomatitis Indiana virus, VSV) viruses, allowed us to identify a lead uridine derivative highly specific towards HCMV and with high selectivity index [Białek-Pietras, M.; Olejniczak, A.B.; Paradowska, E.; Studzińska, M.; Suski, P.; Jabłońska, A.; Leśnikowski, Z.J. Synthesis and in vitro antiviral activity of lipophilic pyrimidine nucleoside/carborane conjugates, J. Organomet. Chem., 2015, 798, 99−105].

Other screening projects focused on identification of nucleoside boron cluster conjugates with antileucemic activity [Żołnierczyk, J.D., Olejniczak, A.B., Mieczkowski, A.J., Błoński, J.Z., Kiliańska, Z.M., Robak, T., Leśnikowski, Z.J., In vitro antileukemic activity of novel adenosine derivatives bearing boron cluster modification. Bioorg. Med. Chem., 2016, 24, 5076−5087] or modulatory activity towards purinergic receptors are ongoing.

The identification of the mode of action of the new anti-tuberculosis compounds

Fig. 2 The identification of the mode of action of 1H-benzo[d]imidazole derivatives against tubercle bacilli



Mycobacterium tuberculosis, the causative agent of tuberculosis, is an intracellular pathogen the life cycle of which includes long states of persistence. This pathogen is relatively hard to eradicate and poses a challenge for effective chemotherapy and claims 1,5 million lives every year [WHO, 2017]. Tuberculosis treatment lasts 6 to 24 months depending on drug susceptibility of the infecting strain and requires a cocktail of at least 4 drugs to be used simultaneously in order to prevent the selection of drug-resistant Mtb mutants. Treatment of tuberculosis caused by the strains resistant to at least isoniazid and rifampicin (multi drug resistant, MDR), requires additional drugs, is often less effective, and less well tolerated. Additionally, the treatment of MDR tuberculosis is much more expensive than standard treatment, the outcomes are several times worse with a high mortality rate (50-80%) within 4 months of diagnosis [WHO, 2014], and twice the risk of relapse after the completion of treatment.

Taking the above into account the development of the alternative medical strategies based on the new generation of drugs is desperately needed to effectively cure MDR-TB, reduce the duration of current therapies, and minimize the toxicity and cost of the antituberculosis agents used [Plocinska et al, 2017]. The whole cell phenotyping (MIC) screens of chemical libraries allow to identify a number of bactericidal compounds sometimes potent against tubercle bacilli. The proper evaluation of the usefulness of these compounds requires the elucidation of their mode of action as well as the identification of the mechanisms of acquired drug resistance by tubercle bacilli.

Our collaborators reported the synthesis and tuberculostatic activity of series of pyridine [Gobis K. et al., 2012a, b], pyrazine [Gobis K. et al., 2006a, b], and other nitrogen heterocyclic compounds [Gobis K. et al., 2010, 2013]. The highest activities were observed for benzo[d]imidazoles and picolinohydrazonamides derivatives. Currently the number of derivatives will be synthesized and evaluated in respect to their bactericidal activity. The molecular mechanisms of bactericidal activity will be determined for the compounds potent against drug sensitive and MDR M. tuberculosis both in vitro and in vivo. The resistant mutants will be selected to identify the mode of action of the investigated compounds. Sequencing of genomic DNA 9NGS) of the mutants resistant for each of the selected compounds and the comparative analysis of the sequences obtained for the mutants and the parent strain will allow predicting potential molecular targets for these compounds in the cells of M. tuberculosis and suggest the mechanism of anti-tuberculosis activity. The introduction of a mutated genes controlled by a native promoter into the wild type Mtb (compound-sensitive strain) and analysis of an increasing resistance will allow to confirm the relationship between a given mutation and resistant phenotype. In order to demonstrate compounds' specificity against Mycobacterium genus they will be also tested for cytotoxicity and antimicrobial activity against other types of bacteria.

THE HARDWARE - EQUIPMENT AND BIOSAFETY LEVEL

- Biosafety level 2 (BSL-2) is suitable for work involving agents that pose moderate hazards to personnel and the environment. Although there are no specific requirements for ventilation systems, mechanical ventilation systems provide an inward flow of air without recirculation to spaces outside of the laboratory. HEPA filtered exhaust air is exhausted to the outside through a hard connection. A method for decontaminating all laboratory wastes are used, including autoclave, chemical disinfection, and incineration.
- Class II Biosafety (Biohazard) cabinets. Precision-engineered safety cabinet provides clean air solutions for a host of applications, ranging from routine work with potentially hazardous samples to handling cytotoxic compounds. It provides the level of operator, product and environmental protection. The HEPA filter traps 99.97% of particles of 0.3 μm in diameter and 99.99% of particles of greater or smaller size.
- Refrigerators, deep-freezers and liquid nitrogen containers. If very low temperatures are required, ampoules are stored in the gaseous phase above the liquid nitrogen or infectious materials are stored deep-freeze cabinets. The outer surfaces of ampoules stored are disinfected when the ampoules are removed from storage.
- The CO2 incubators are characterised by particularly safe and stable long term incubation conditions for excellent growth of cell and tissue cultures. The CO2 control system guarantees high consistency and long term stability of CO2 levels and thus of the pH value in the culture medium.
- ELISA Plate Reader detect and process biological and chemical data using absorbance, luminescence, and fluorescence detection modes. This microplate reader is used in drug discovery, research, bioassay validation, and biopharmaceutical manufacturing. Absorbance detection is used for assays such as ELISA assays, protein /nucleic acid quantification or enzyme activity assays (i.e. in the MTT assay for cell viability).
- Real-time PCR System is used to provide quantitative detection of nucleic acid sequences using real-time analysis and qualitative detection of nucleic acid sequences. It enables detection and quantification of PCR products in real-time using either SYBR green reagents or Taqman probes. It is possible to monitor PCR reactions cycle by cycle enabling quantification and rapid analysis of many different targets.
- Refrigerated, multipurpose centrifuge with the following features: variable speed (up to 20 000 g), rotors for Eppendorf and falcon tubes as well as microtiter plates (for DNA precipitation, cell harvesting and protein isolation), programmable acceleration and breaking rates, precise temperature control, automatic rotor recognition.
- Biosafety level I, II and III laboratories suitable for working with bacteria. Biosafety level I laboratory is suitable for work with well-characterized agents which do not cause disease in healthy humans, while biosafety level II laboratory is designated for work with agents that cause moderate potential hazard for humans and the environment, including genetically modified organisms. Biosafety level III laboratory enables work with bacteria causing potentially lethal diseases and genetically modified derivative strains of these bacteria. It consists of several rooms and it has been designed according to standards required by Polish law.
- Genetic modification of microorganisms- electroporators, thermoblocks, crosslinker;
- Analysis of nucleic acids: Sanger sequencer, hybridization ovens, thermocyclers, transiluminators, gel electrophoresis power packs; PCR workstations;
- Protein expression and purification: high speed centrifuge; sonicator, AKTA protein purification system, acrylamide gel electrophoresis system

Readouts/ Screening technologies

Main technologies/ competencies: Biosafety level 2 (BSL-2) and 3 (BSL-3). Cytotoxicity assays based on monitoring cell growth inhibition. Antiviral activity testing. The in vitro antiviral effects of test compounds are measured by a plaque-reduction (PR) assay and viral-induced cytopathic effects (CPE) assay. Antiviral activity assay based on qRT-PCR is also accessible. The collection of viruses available for testing includes: HCMV, HPIV-3, HSV-1, EMCV and VSV and is expanding. Antibacterial activity testing is based on laboratory and clinical tubercle bacilli including multi-drug resistance. Assessment of Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) is offered. Protein in vitro assays when the enzyme targeted by antibacterial compound is known is also available.

Compound libraries

Establishing of Polish National Library of Chemical Compounds with storage, quality control, dispensing and logistics capabilities is ongoing as one of priorities of our enterprise.

Target classes

Antiviral compounds, anticancer compounds with focus on molecules with antileucemic activity compounds with modulatory activity towards purinergic receptors, antibacterial compounds with focus on tuberculostatics.

THE OUTPUT

PATENTS:

1- Janusz Boratyński, Tomasz Goszczyński, Konrad Kowalski, Zbigniew Leśnikowski, Method for the preparation of protein-boron cluster conjugates in solution. Polish Patent Office, P. 398 380. (21.06.2016).
2- Janusz Boratyński, Tomasz Goszczyński, Konrad Kowalski, Zbigniew Leśnikowski, Method for the preparation of protein-boron cluster conjugates in the solid state, Polish Patent Office, P. 398 379 (14.12.2015)
3- Jerzy Radecki, Hanna Radecka, Iwona Grabowska, Agnieszka Sirko, Anna Góra-Sochacka, Zbigniew Leśnikowski, Agnieszka Olejniczak, Electrochemical biosensor and its application, Polish Patent Office, P. 222 314 (29.07.2016)
4- Zbigniew J. Leśnikowski and Agnieszka Olejniczak, Nucleoside Derivative, Modified Oligonucleotide, Method for Their Synthesis And Applications Thereof, U.S. Patent No. 8,026,348 (27.09.2011)

1. *Zbigniew J. Leśnikowski. Challenges and Opportunities for the Application of Boron Clusters in Drug Design. J. Med. Chem. 2016, 59, 7738-7758.*
2. *Anna Adamska, Anna Rumijowska-Galewicz, Anna Ruszczynska, Mirosława Studzińska, Agnieszka Jabłońska, Edyta Paradowska, Ewa Bulska, Helene Munier-Lehmann, Jarosław Dziadek, Zbigniew J. Leśnikowski, Agnieszka B. Olejniczak. Anti-mycobacterial activity of thymine derivatives bearing boron clusters. Eur. J. Med. Chem., 2016, 121, 71-81.*
3. *Jagielski T, Bakuła Z, Brzostek A, Minias A, Stachowiak R, Kalita J, Napiórkowska A, Augustynowicz-Kopeć E, Żaczek A, Vasiliauskiene E, Bielecki J, Dziadek J. Characterization of mutations conferring resistance to rifampicin in Mycobacterium tuberculosis clinical strains. Antimicrob Agents Chemother. 2018 Jul 30. pii: AAC.01093-18.*
4. *Marek L. Kowalski, Aleksandra Wardzynska, Miroslawa Studzinska, Malgorzata Pawelczyk, Zbigniew Jan Lesnikowski, Edyta Paradowska, Cytomegalovirus DNA is highly prevalent in the blood of patients with asthma and is associated with age and asthma traits. Allergy, 2017, DOI: 10.1111/all.13233*
5. *Marian Vincenzi, Katarzyna Bednarska, Zbigniew J. Leśnikowski, Comparative in silico study of carborane- and phenyl-modified adenosine ligands as probes for the A2A and A3 adenosine receptors. Molecules 2018, 23, 1846, doi:10.3390/molecules23081846.*

COLLABORATIONS:

- *University of Sussex, Genome Centre, UK: Prof. Aidan Doherty – DNA repair in mycobacteria*
- *Infectious Disease Research Institute of Montpellier, French Institute of Health and Medical Research, France Dr Laurent Kremer – the putative drugs affecting cell wall metabolism in mycobacteria*
- *Faculty of Life and Environmental Sciences, Tsukuba University, Japan, Prof. Nobuhiko Nomura – Membrane vesicles of Mycobacterium tuberculosis as the regulators of the host cells.*
- *Tokyo Institute of Technology, 4259-R1-13, Nagatsuta-cho, Midori-ku, Yokohama 226-8503, Japan: Boron carriers for boron neutron capture therapy of cancers (BNCT).*
- *Royal Free and University College Medical School, Royal Free Campus, Virology Department, London, Great Brytain: Application of real time PCR in diagnostics of cytomegalovirus infections.*
- *Institute of Biochemistry and Biophysics PAS., Department of Biophysics, Warsawa, Poland: Synthesis of nucleoside analogues as potential chemotherapeutics and their biological evaluation.*
- *University of Massachusetts Medical School, Worcester, United States: Evaluating the impact of boron cluster conjugation on siRNA delivery and efficacy following local (central nervous system) or systemic (intravenous) administration.*
- *Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Wroclaw, Poland: Synthesis and evaluation of new compounds as potential anticancer agents.*

THE FUTURE

FUTURE PLANS:

Screening Laboratory for Anti-viral and Anti-bacterial Compounds is a specialist partner site designed to perform screening based on live microorganism cultures, viral and bacterial. This is in contrast to the most of HTP centers which use biochemical, in vitro assays. The major aim of our site is to expand the panel of viruses screened up to 10-15 different strains (it can be further enriched depending upon the demand) and to increase the capacity of the screening as well in virus and bacterial part of our laboratory. Close, working relationships with partners within EU-OPENSCREEN and POL-OPENSCREEN network are considered as an another, important part of our site activity.

ADDED VALUE:

Multidisciplinarity of our approache should be stressed. It takes an advantage of expertise in bioorganic chemistry (with well developed bioorganic synthesis capability), molecular virology and bacteriology available in the Laboratory of Molecular Virology and Biological Chemistry, a sit of the Screening Laboratory for Anti-viral and Anti-bacterial Compounds, together with the Laboratory of Genetic and Physiology of Mycobacterium. This allow us not only to identify biological targets and screen compounds but also perform follow up and lead optimization chemistry for selected molecules.

Institute of Medical Biology Polish Academy of Sciences (IMB PAS)
Lodowa 106, 93-232 Lodz, Poland

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