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NEW INSIGHTS INTO ANTIBACTERIAL AND ANTITUMOR EFFECTS OF STRUCTURALLY VERSATILE AMINO ACID-BASED AND NON-PROTEINOGENIC COMPOUNDS

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Introduction

The honey bee (*Apis mellifera*) has been known to mankind since ancient times. The last few decades, honey bee populations have been declining dramatically worldwide due to various causes including pesticides, bacterial diseases and parasites [1]. That leads to a pressing problem with a huge global economic, environmental, social, and public health impact.

The American Foulbrood disease (AFB) is an infection that still belong to the most deleterious honey bee diseases (Fig. 1) [1,2]. Herein we investigate the cytotoxic potential of new small molecules against the Gram-positive bacterium *Paenibacillus larvae*, the causative agent of the American Foulbrood disease (AFB). Furthermore, we were interested to study the activity of these compounds against different cancer cell lines.

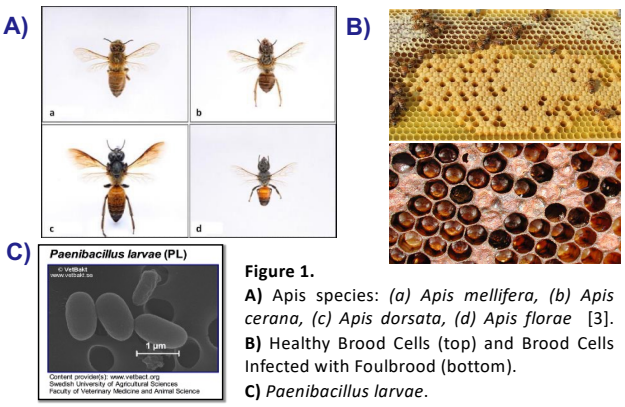

Figure 1.
A) Apis species: *(a) Apis mellifera, (b) Apis cerana, (c) Apis dorsata, (d) Apis florae* [3].
B) Healthy Brood Cells (top) and Brood Cells Infected with Foulbrood (bottom).
C) *Paenibacillus larvae*.

Anti-bacterial and antitumor efficacy

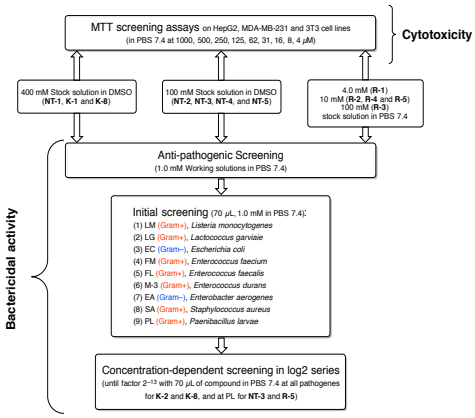

Figure 2: Work-flow of the screening procedure.

Until now, we have investigated the anti-bacterial and antitumor efficacy of a series of novel, small molecule-based compounds and compared their biological effects with those of well-known reference drugs, including cis-Pt, carbo-Pt, etoposide, and doxorubicin (Fig. 2). All compounds were further tested for their cytotoxicity on various tumor cell lines, including HepG2, MDA-MB-231, HT-29, and the non-tumor 3T3. A two-step screening procedure was applied in order to identify highly selective compounds against the target pathogen *Paenibacillus larvae* in comparison with the standard antibiotic lincomycin (Fig. 2 and 3).

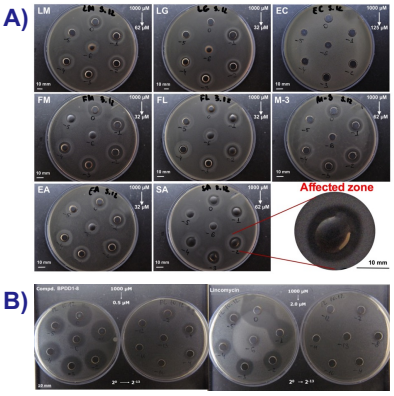

Figure 3. Anti-bacterial effects of Cmpd. **PBDD1-8** (**K-8**) on eight bacterial types (A) and against *Paenibacillus larvae* (PL) in comparison with **lincomycin** (B).

New insights into cytotoxicity

To further investigate the possible mechanism of action (MoA) of compound **BPDD1-8** (**K-8**), for example apoptotic effects, we performed initial time- and concentration-dependent fluorescence spectroscopy (Axio fluorescent microscope) using HepG2 cancer cells (Fig. 4).

After 4 h incubation time, the cells were stained with the respective fluorescence dye (DAPI, 4',6-diamidino-2-phenylindole) which showed appearance of a cytotoxic effect compared with non-treated cells. This method allowed us to obtain the appropriate effective concentrations of **BPDD1-8** in the subsequent apoptosis and cell cycle studies.

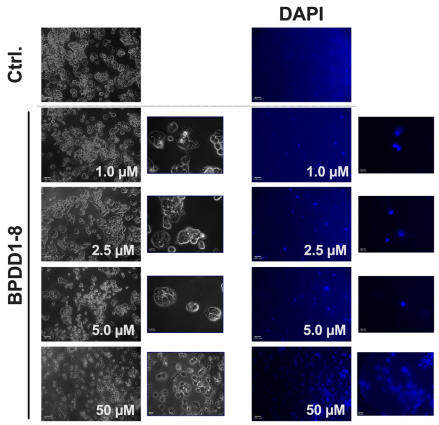

Figure 5. Representative photos of the fluorescent microscopy experiments with **BPDD1-8** on HepG2 cell line. The cells were treated for 4h at 37°C at different compound's concentrations (1.0, 2.5, 5.0, and 50 µM).

Conclusions

The biological effects of these new compounds are comparable to those of the approved antibiotic **lincomycin** and the anti-cancer drug **doxorubicin**. Based on their cytotoxic and antiproliferative activity two of the new drug candidates (designated as **BPDD1-3** / **NT-3** and **BPDD1-8** / **K-8**) were chosen for further studies identifying their mechanisms of action, e.g., apoptosis and cell cycle on cancer cells.

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