

# PHARMACEUTICAL APPLICATIONS OF BIRCH BARK EXTRACTIVE BETULIN

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## INTRODUCTION

- **Betulin 1** (Fig. 1) is an abundant naturally occurring triterpene
- Principal extractive (up to 35% of dry weight) of the bark of birch trees
- No economical use for this easily isolable compound at present
- Pulp mill plant can produce up to 1000 t/a of 95% pure betulin
- Possible source for polymers and a precursor of biologically active compounds
- Betulin and its derivatives have many interesting pharmacological properties:<sup>1</sup>
  - Cytotoxic activity against many tumour cell lines
  - Anti-HIV activity with new mechanisms of action
- **Leishmaniasis** is a disease caused by protozoan parasites
- Affects millions of people in developing countries
- Transmitted by sand flies
- Can be fatal if not treated
- Currently, no effective treatment
- **Alphaviruses** cause polyarthritis in avian and mammalian hosts
- Consists of enveloped viruses having a single-stranded RNA genome
- Distributed by *Aedes sp.* mosquitoes
- Currently, available treatment is limited to relatively inefficient ribavirin
- **Bacterial** species cause illnesses from minor skin infections to life-threatening diseases such as pneumonia and endocarditis
- Antibacterial resistance to current drugs have become increasing problem
- Occurrence of multidrug-resistant *Staphylococcus aureus* (MRSA) is increasing
- In this study betulin derivatives were screened against:
  - *Leishmania donovani* axenic amastigotes<sup>2</sup>
  - (+)-Stranded RNA virus *Semliki Forest virus* (SFV)<sup>3</sup>
  - Bacterial species (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis* and *Micrococcus luteus*)<sup>4</sup>

## EXPERIMENTAL

### Chemistry

- Simple betulin derivatives were synthesized by modifying OH groups at C-3 and C-28, and the double bond at C-20–C-29
- More complex heterocyclic derivatives **2** of betulin were also synthesized

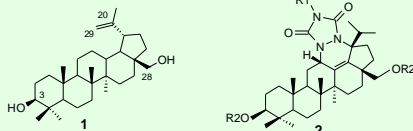


Fig. 1. Structure of betulin 1 and heterocyclic betulin derivatives 2.

### Antileishmanial assay

- Screened using a fluorescent viability microplate assay at 50  $\mu\text{M}$  of betulin derivative<sup>5,6</sup>
- The  $\text{GI}_{50}$  (concentration for 50% growth inhibition) of the most promising compounds was also determined

### Antiviral assay

- Screened using luminescence reporter-gene assay at 50  $\mu\text{M}$  of betulin derivative<sup>7</sup>
- The  $\text{IC}_{50}$  (50% inhibition concentration) values of the most potent betulin derivatives was also determined
- Cytotoxicity was screened using intracellular ATP counter-screen with hepatocellular Huh-7 cells<sup>8</sup>

### Antibacterial assay

- Screened using microdilution assay at 50  $\mu\text{M}$  of betulin derivative<sup>9</sup>

## RESULTS AND DISCUSSION

### Antileishmanial activities

- The most effective antileishmanial betulin derivatives are presented in Figure 2
- The hetero cycloadduct **2b** and betulonic acid **3** were the most potent derivatives
- In heterocyclic betulin derivatives less bulky R1 and R2 groups were more potent
- In simple betulin derivatives oxidation of betulin had beneficial impact on activity

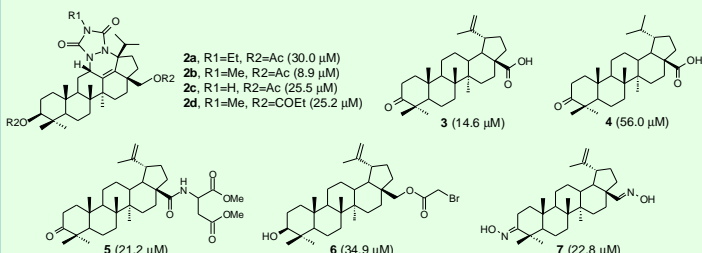


Figure 2. Chemical structures and  $\text{GI}_{50}$  values of the most potential betulin derivatives against *L. donovani* axenic amastigotes. Positive control Amphotericin B 95% inhibition at 1  $\mu\text{M}$ .

### Antiviral activities

- The most effective antiviral betulin derivatives are presented in Figure 3
- Betulin **1** was moderately active against SFV ( $\text{IC}_{50}$  45  $\mu\text{M}$ )
- Potency was enhanced by oxidation of C-28 to betulonic acid **8** and by certain cyclic ether or ester substituents, e.g. 28-O-tetrahydropyranylbetulin **9** and heterocycle **10**
- Betulin derivatives generally show no or only low cytotoxicity

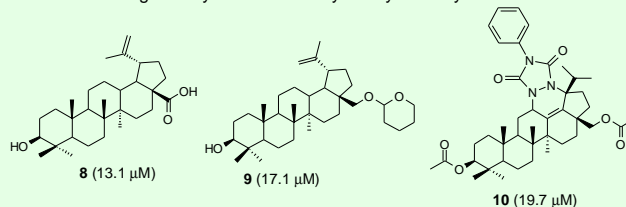


Figure 3. Chemical structures and  $\text{IC}_{50}$  values of the most potent betulin derivatives against SFV. Positive control: 3'-amino-3'-deoxyadenosine (20  $\mu\text{M}$ ) yielding surviving fractions 12% - 25% in the assay.

### Antibacterial activities

- The most potent antibacterial betulin derivatives are presented in Figure 4
- Inhibition of betulin derivatives at 50  $\mu\text{M}$  was dependent on the bacterial strain:
  - Betulonic acid **3** (inhibition between 50% and 100%)
  - Betulonic acid **8** (from 10% to 40%)
  - 28-O-(*N*-acetylthraniloyl)betulin **11** (from 60% to 100%)
- Most of the betulin derivatives inactive
- MIC value (minimum inhibitory concentration) for **11** against *S. aureus* was 0.4  $\mu\text{M}$  (For comparison: MIC for known antibiotic erythromycin was 0.7  $\mu\text{M}$ )

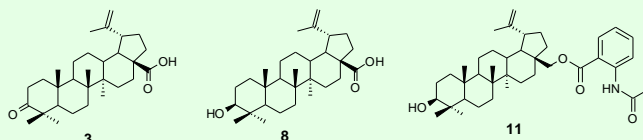


Figure 4. Chemical structures of the most active betulin derivatives against *S. aureus*, *S. epidermidis*, *B. subtilis* and *M. luteus*.

## CONCLUSIONS AND FUTURE PERSPECTIVES

- Betulin scaffold has potential for new antimicrobial agents
- Betulonic acid seems to be effective against several microorganisms
- Future perspectives:
  - Comprehensive evaluation of the antimicrobial potential of betulin derivatives by:
    - Synthesis of more potent derivatives
    - Screening with larger collection of derivatives
    - In-depth analyses of the active compounds through selectivity profiling and functional characterisation

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