



Inhibition of Bacterial Growth and Biofilm Production by Metabolites from *Hypericum* spp.

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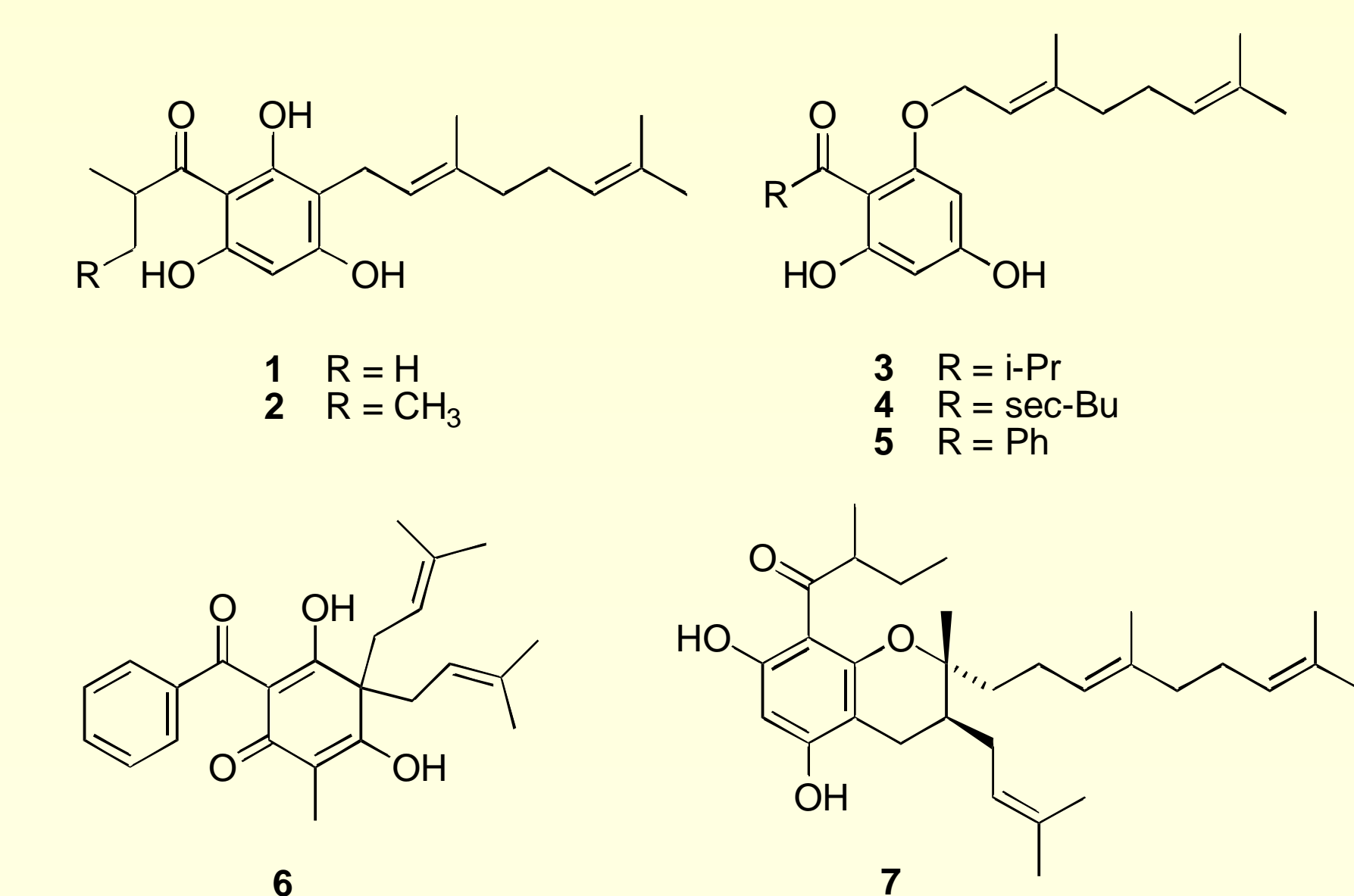
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ABSTRACT

Biofilm embedded pathogens such as *Staphylococcus* spp., *Escherichia coli*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* are difficult to eradicate and are major sources of bacterial re-infections. New drugs are needed to combat these pathogens. *Hypericum* is a plant genus that contains species known to have antimicrobial properties. However, the specific metabolites responsible for the antimicrobial properties are not entirely known, nor have these compounds been tested as inhibitors of biofilm development. This project was designed to test pure metabolites isolated from the species *H. densiflorum*, *H. ellipticum*, *H. prolificum*, and *H. punctatum* as inhibitors of bacterial growth & biofilm formation.

Hypericum metabolites included in this study:



ACKNOWLEDGEMENT

This study was supported by RI-INBRE Grant # P20 RR016457 from the NCRR, NIH. Printing service provided by the RI-INBRE Centralized Research Core Facility supported by Grant # P20RR16457-10 from NCRR, NIH.

BACKGROUND



Hypericum prolificum

Certain members of the *Hypericum* genus, including St. John's Wort (*Hypericum perforatum* L.), produce metabolites possessing antimicrobial properties. These molecules may play defensive roles in protecting the plants from assaults by environmental pathogens.



Hypericum punctatum

OBJECTIVES

Evaluate novel acylphloroglucinols as promising new antimicrobial agents against significant bacterial diseases.

Measure the *in vitro* growth and biofilm inhibitory properties of *Hypericum* spp. metabolites against the bacterial pathogens *A. baumannii*, *P. aeruginosa*, *E. coli*, *S. aureus* and *S. epidermidis*.

METHODS

Susceptibility testing. The minimum inhibitory concentrations (MIC) & minimum bactericidal concentrations (MBC) of seven *Hypericum* spp. metabolites were measured against *A. baumannii*, *P. aeruginosa*, *E. coli*, and clinical *Staphylococcus* spp. using Clinical Laboratory Standards Institute (CLSI) methods. MIC is the lowest concentration of an antimicrobial agent that results in no visible bacterial growth. MBC is the minimum concentration required to kill 99.9% of a bacteria inoculum.

Prevention of staphylococcal biofilm formation by metabolites from *Hypericum* spp. Pure metabolites from *Hypericum* spp. that demonstrated growth inhibitory properties against staphylococci were tested for their ability to prevent biofilm formation by planktonic *S. aureus* and *S. epidermidis*. Biofilm formation was quantified using a microtiter plate assay.

RESULTS

Table 1. Antimicrobial activities of *Hypericum* metabolites. All values are reported in µg/mL.

Compounds	Biofilm Producing <i>S. epidermidis</i> (RP62A; ATCC 35984)			Non-Biofilm Producing <i>S. epidermidis</i> (M7)			Biofilm Producing <i>S. aureus</i> (ATCC 35556)			Clinical MRSA (L32)		
	MIC	MBC	MBIC	MIC	MBC	MBIC	MIC	MBC	MBIC	MIC	MBC	MBIC
1	7.81	7.81	1.95	7.81	7.81	3.91	7.81	7.81	3.91	3.91	3.91	3.91
2	3.91	3.91	1.95	3.91	3.91	1.95	7.81	7.81	1.95	3.91	3.91	7.81
3	7.81	7.81	1.95	7.81	7.81	3.91	3.91	3.91	3.91	7.81	7.81	3.91
4	3.91	7.81	3.91	3.91	7.81	3.91	3.91	3.91	7.81	7.81	7.81	7.81
5	3.91	7.81	3.91	3.91	3.91	3.91	1.95	3.91	3.91	3.91	3.91	3.91
6	>125	>125	125	>125	>125	125	>125	>125	125	>125	>125	125
7	>125	>125	>125	>125	>125	>125	>125	>125	>125	>125	>125	>125

CONCLUSION

Of the seven *Hypericum* metabolites tested, five demonstrated potent antimicrobial activity against the Gram-positive pathogens. None of the metabolites demonstrated activity against the Gram-negative pathogens. Interestingly, four of the metabolites also inhibited biofilm formation at sub-MIC concentrations. These results suggest that these molecules deserve further preclinical evaluation as agents to treat and prevent staphylococcal infections.

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