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**β -(1-ADAMANTYLTHIO)PYRIDINE ANALOGS AS
ANTIMICROBIALS AND ANTIMALARIALS**

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ABSTRACT

An array of interesting activities for bioactive 3-substituted thiopyridines have previously been reported. Herein, a series of α - and β -(1-adamantylthio) analogs of 3-picoline and phenylpyridines were prepared and investigated for antimicrobial (agar dilution method against 21 strains of microorganisms) and antimalarial (against *P. falciparum*) activities. It was found that β -thiopyridines, 5-(1-adamantylthio)-3-picoline (**7**) and 3-(1-adamantylthio)-4-phenylpyridine (**8**) are novel antimicrobials and antimalarials. Significantly, analogs **7** and **8** are very potent antimicrobials with MIC range of 2-32 μ g/mL where **8** being the most potent. The β -sulfides **7** and **8** selectively inhibited the growth of tested gram-positive bacteria, but inactive against gram-negative bacilli including the members of Enterobacteriaceae. This study identified new antimicrobials that represent promising lead compounds suitable for further preclinical and clinical development.

Keywords: 1-adamantylthio, 3-picoline, phenylpyridines, antimicrobials, antimalarials

INTRODUCTION

Alkyl or arylthiopyridine analogs has been shown to exhibit a diverse array of activities (Bauer and Prachayasittikul, 1987). It has been found that 3-substituted (R) pyridines bearing 1-adamantylthio group (R = OC₂H₅, OAc, NAc₂, Br, OH) display antimicrobial activity (Prachayasittikul et al., 2008). Our findings demonstrate that these bioactive analogs possessed 1-adamantylthio moiety at either α - (2- or 6-) or β - (3- or 5-) positions on the pyridine ring.

In seeking for new lead compounds for medicinal applications, we have centered on 1-adamantylthio analogs of alkyl and arylpyridines. The present study focused on thio analogs of 3-picoline and 2-, 3-, 4-phenylpyridines as interesting target molecules as novel antimicrobials and antimalarials. The chemical structures of α - and β -(1-adamantyl thio)pyridine derivatives (**1-9**) are shown in Figure 1. The derivatives **1-9** were prepared using deoxydative substitution reaction of pyridine 1-oxides by thiol (Prachayasittikul et al., 1985, 1991).

MATERIALS AND METHODS

General

Melting points were determined on an Electrothermal melting point apparatus (Electrothermal 9100) and are uncorrected. ¹H-NMR spectra were recorded on a Bruker AM 400 instrument with a 400/100 MHz operating frequency using deuteriochloroform solution with tetramethylsilane as internal standard. Column chromatography was carried out using silica gel 60 (0.063–0.200 mm). Thin layer chromatography (TLC) was performed on silica gel 60 PF₂₅₄ (cat. No. 7747 E., Merck).

Solvents were distilled before using. Chemicals for the synthesis were of analytical grade. Reagents for cell culture were as follows:

RPMI (Gibco, USA)

HEPES (Sigma, USA)

Gentamycin sulfate (The Government Pharmaceutical Organization, Thailand).



- 1, R = 3-Me
- 2, R = 5-Me
- 3, R = 3-Ph
- 4, R = 5-Ph
- 5, R = 4-Ph
- 6, R = 6-Ph
- 10, R = 3-OEt
- 11, R = 3-OAc
- 12, R = 3-NAc₂
- 13, R = 3-Br
- 14, R = 5-OH

- 7, R = 5-Me
- 8, R = 4-Ph
- 9, R = 6-Ph
- 15, R = 5-Br

Fig. 1: α - and β -(1-adamantylthio)pyridine derivatives

Compounds 1-9

The tested thiopyridine compounds are 2-(1-adamantylthio)-3-picoline (**1**), 6-(1-adamantylthio)-3-picoline (**2**), 2-(1-adamantylthio)-3-phenylpyridine (**3**), 6-(1-adamantylthio)-3-phenylpyridine (**4**), 2-(1-adamantylthio)-4-phenylpyridine (**5**), 6-(1-adamantylthio)-2-phenylpyridine (**6**), 5-(1-adamantylthio)-3-picoline (**7**), 3-(1-adamantylthio)-4-phenylpyridine (**8**), 5-(1-adamantylthio)-2-

phenylpyridine (**9**). The compounds **1-9** were prepared as previously described (Prachayasittikul et al., 1985, 1991).

Cell culture

Plasmodium falciparum chloroquine resistant (T9/94)

Human erythrocytes (type O) infected with *Plasmodium falciparum* chloroquine resistant (T9/94) were maintained in continuous culture, according to the method described previously (Trager and Jensen, 1976). RPMI 1640 culture medium supplemented with 25 mM of HEPES, 40 mg/L gentamicin sulfate and 10 mL of human serum was used in continuous culture.

Biological activities

Antimicrobial assay

Antimicrobial activity of the tested compounds was performed using agar dilution method as previously described (Prachayasittikul et al., 2008). Briefly, the tested compounds dissolved in DMSO were individually mixed with 1 mL Müller Hinton (MH) broth. The solution was then transferred to the MH agar solution to yield the final concentrations of 2-256 μ g/mL. Twenty one strains of microorganisms, cultured in MH broth at 37 °C for 24 h, were diluted with 0.9 % normal saline solution to adjust the cell density to 3×10^9 cell/mL. The organisms were inoculated onto each plate and further incubated at 37 °C for 18-48 h. Compounds which possessed high efficacy to inhibit bacterial cell growth were analyzed. The microorganisms used for the activity analysis are shown in Table 1.

Antimalarial assay

Antimalarial activity of the tested compounds was evaluated against chloroquine-resistant (T9/94) *P. falciparum* using the literature method (Satayavivad et al., 2004).

Table 1: Microorganisms for antimicrobial activity testing

Gram-negative bacteria	Gram-positive bacteria	Yeast
<i>Escherichia coli</i> ATCC 25922	<i>Staphylococcus aureus</i> ATCC 25923	<i>Candida albicans</i>
<i>Pseudomonas aeruginosa</i> ATCC 27853	<i>Bacillus subtilis</i> ATCC6633	
<i>Klebsiella pneumoniae</i> ATCC700603	<i>Corynebacterium diphtheriae</i> NCTC 10356	
<i>Serratia marcescens</i> ATCC8100	<i>Staphylococcus epidermidis</i>	
<i>Shigella dysenteriae</i>	<i>Streptococcus pyogenes</i>	
<i>Salmonella typhi</i>	<i>Enterococcus</i> group D	
<i>Vibrio cholerae</i>	<i>Micrococcus flavus</i>	
<i>Aeromonas hydrophila</i>	<i>Listeria monocytogenes</i>	
<i>Plesiomonas shigelloides</i>		
<i>Acinetobacter calcoaceticus</i>		
<i>Moraxella catarrhalis</i>		
<i>Neisseria mucosa</i>		

Before performing the experiment, *P. falciparum* culture was synchronized by using sorbitol-induced hemolysis according to the method of Lambros and Vanderberg (1979) to obtain only ring-infected cells and then incubated for 48 h prior to the drug testing to avoid effect of sorbitol.

The experiments were started with synchronized suspension of 0.5 % to 1 % infected red blood cell during ring stage. Parasites were suspended with culture medium supplemented with 15% human serum to obtain 10 % cell suspension. The parasite suspension was put into 96-well microculture plate; 50 μ L in each well and then add 50 μ L of various tested drug concentrations. These parasite suspensions were incubated for 48 h in the atmosphere of 5 % CO₂ at 37 °C. The percentage of parasitemia of control and drug-treated groups were examined by microscopic technique using methanol-fixed Giemsa-stained thin smear blood preparation. The efficacy of the drugs was evaluated by determining the drug concentration that reduced parasite growth by 50 %.

RESULTS AND DISCUSSIONS

Chemistry

1-Adamantylthiopicolines (**1-2** and **7**) were prepared from the reaction of 3-

picoline 1-oxide with 1-adamantyl mercaptan in boiling acetic anhydride (Prachayasittikul et al., 1985). Similarly, 1-adamantylthio analogs of 2-, 3- and 4-phenylpyridines (**3-6**, **8-9**) were synthesized from the deoxydative substitution reaction of 2-, 3- and 4-phenylpyridine 1-oxides by 1-adamantane thiol as described previously (Prachayasittikul et al., 1991). Structure of the compounds **1-9** was confirmed by ¹H-NMR spectral data and melting points.

Antimicrobial activity

The 1-adamantylthiopyridines **1-9** were evaluated for growth inhibition against 21 strains of microorganisms using agar dilution method as described previously (Prachayasittikul et al., 2008). It was shown that all the tested compounds (α - and β -sulfides) exhibited no growth inhibition against yeast, members of the Enterobacteriaceae and other gram-negative bacilli, except for *M. catarrhalis* (Table 2). The 6-(1-adamantylthio)-3-picoline (**2**) displayed 75 % inhibition against *C. diphtheriae* NCTC 10356 and *M. catarrhalis* at a high concentration of 256 μ g/mL.

Apparently, 3-(1-adamantylthio)-4-phenylpyridine (**8**) is the most potent antimicrobial as it exerted complete inhibition against *C. diphtheriae* NCTC 10356

and *M. flavas* both with MIC value of 2 $\mu\text{g/mL}$, against *S. pyogenes* and *S. aureus* ATCC 25923 with MIC of 4 and 32 $\mu\text{g/mL}$, respectively. Moreover, *S. pyogenes* and *C. diphtheriae* NCTC 10356 were also completely inhibited by 5-(1-adamantylthio)-3-picoline (**7**) with MIC of 8 $\mu\text{g/mL}$. In addition, β -sulfides (**7** and **8**) exhibited complete inhibition against *L. monocytogenes* with MIC of 32 $\mu\text{g/mL}$. The β -sulfide **8** showed activity against *B. subtilis* ATCC 6633 and *M. catarrhalis* with MIC of 4 $\mu\text{g/mL}$, while the compound **7** inhibited the growth of *B. subtilis* ATCC 6633 with MIC of 32 $\mu\text{g/mL}$. It was observed that a presumably drug resistant strain of *Enterococcus* group D was inhibited by β -sulfides **7** (75 %) and **8** (50 %) at 256 $\mu\text{g/mL}$. Significantly, β -sulfides **7** and **8** are very potent antimicrobials. Interestingly, such compounds **7** and **8** provide antimicrobial activity selectively against growth of gram-positive bacteria, but not against gram-negative bacilli. So far, bioactivities of the α - and β -(1-adamantylthio) analogs of picolines and phenyl pyridines have not been reported. Our previous study reported antimicrobial activity of α - and β -(1-adamantylthio)-3-substituted (R) pyridines e. g. α -thiopyridines **10-14** (R = 3-OEt, 3-OAc, 3-NAc₂, 3-Br, 5-OH) and β -sulfide **15** (R = 5-Br) as shown in Figure 1. However, 3-phenylpyridine was reported to be a component in antimicrobial cosmetics for skin such as vanishing cream (Kosuge et al., 1975). This suggests a potential development of novel antimicrobials; β -(1-adamantylthio) pyridines **7** and **8** for pharmaceutical applications.

Antimalarial activity

The activity of α - and β - sulfides (**1-9**) were tested against *P. falciparum* (T9/94). Results (Table 3) showed that 2-(1-adamantylthio)-3-picoline (**1**), 5-(1-adamantylthio)-3-picoline (**7**) and 3-(1-adamantylthio)-4-phenylpyridine (**8**) displayed fair antimalarial activity with IC₅₀ 10⁻⁶ to < 10⁻⁵ M. However, α - and β - sulfides **2** and **4-6** were inactive antimalarials showing IC₅₀ >10⁻⁵

M. It is interesting to note that such active antimalarials constitute 1-adamantylthio group at α -(2-) and β -(3- or 5-) positions on the pyridine rings of 3-picoline and 4-phenylpyridine. The antimalarial activity of such compounds (**1**, **7** and **8**) has not been reported. Thus, these represent a new group of antimalarials.

As a result, the investigation on α - and β -(1-adamantylthio)picolines and (1-adamantylthio)phenylpyridines (**1-9**) provides significant results that only β -sulfides of 4-phenylpyridine (**8**) and 3-picoline (**7**) are very potent antimicrobials and antimalarials. However, the sulfides of 2- and 3-phenylpyridines are inactive antimicrobials and antimalarials.

CONCLUSION

The study shows a novel group of β -thio analogs of 3-picoline and 4-phenylpyridine, namely 5-(1-adamantylthio)-3-picoline (**7**) and 3-(1-adamantylthio)-4-phenylpyridine (**8**), as antimicrobials and antimalarials. Significantly, both analogs **7** and **8** are very potent antimicrobials in which **8** being the most potent one. The β -sulfides **7** and **8** selectively inhibited the growth of gram-positive bacteria, but inactive against gram-negative bacilli. This study identified new antimicrobials that represent promising lead compounds suitable for further preclinical and clinical development.

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Table 2: Antimicrobial activity of α - and β -thiopyridines 1-9

Compound	Activity	Microorganism	MIC* $\mu\text{g/mL}$
1	inactive	-	-
2	active ^a	-	-
3	inactive	-	-
4	inactive	-	-
5	inactive	-	-
6	inactive	-	-
7	active ^b	<i>M. flavas</i> , <i>M. catarrhalis</i> <i>S. pyogenes</i> , <i>N. mucosa</i> , <i>C. diphtheriae</i> NCTC 10356 <i>B. subtilis</i> ATCC 6633, <i>L. monocytogenes</i>	4 8 32
8	active ^c	<i>M. flavas</i> , <i>C. diphtheriae</i> NCTC 10356 <i>S. pyogenes</i> , <i>B. subtilis</i> ATCC 6633, <i>M. catarrhalis</i> <i>S. aureus</i> ATCC 25923, <i>S. epidermidis</i> , <i>L. monocytogenes</i>	≤ 2 4 32
9	Inactive	-	-

*MIC: Minimum inhibitory concentration was the lowest concentration that inhibited the growth of microorganisms. At 256 $\mu\text{g/mL}$ showed inhibition against ^a*M. catarrhalis* and *C. diphtheriae* NCTC 10356 (75%), against *Enterococcus* group D ^b75 %, ^c50 %

Table 3: Antimalarial activity of α - and β -thiopyridines 1-9

Compound	Activity	IC ₅₀ (M)
1	fair	10^{-6} - $<10^{-5}$
2	inactive	$>10^{-5}$
3	NA	-
4	inactive	$>10^{-5}$
5	inactive	$>10^{-5}$
6	inactive	$>10^{-5}$
7	fair	10^{-6} - $<10^{-5}$
8	fair	10^{-6} - $<10^{-5}$
9	NA	-

NA : not tested

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