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## Original article:

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

Supaluk Prachayasittikul[1*], Phattrapoom Limnusont[1], Ratchanok Pingaew[1], Somsak Ruchirawat[3], Virapong Prachayasittikul[2*]

[1] Department of Chemistry, Faculty of Science, Srinakharinwirot University, Bangkok 10110, Thailand
[2] Department of Clinical Microbiology, Faculty of Medical Technology, Mahidol University, Bangkok 10700, Thailand
[3] Laboratory of Medicinal Chemistry, Chulabhorn Research Institute, Bangkok 10210, Thailand

*Corresponding authors:
[1] E-mail: supaluk@swu.ac.th; Telephone: 662-664-1000 ext 8209, Fax: 662-259-2097
[2] E-mail: mtvpr@mahidol.ac.th; Telephone: 662-418-0227, Fax: 662-412-4110

## 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 μg/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 = $OC_2H_5$, OAc, $NAc_2$, 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).

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## MATERIALS AND METHODS

### *General*

Melting points were determined on an Electrothermal melting point apparatus (Electrothermal 9100) and are uncorrected. $^1$H-NMR spectra were recorded on a Bruker AM 400 instrument with a 400/100 MHz operating frequency using deutero-chloroform 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 $PF_{254}$ (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-$NAc_2$
**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:** $\alpha$- and $\beta$-(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-adaman tylthio)-3-phenylpyridine (**3**), 6-(1-adaman tylthio)-3-phenylpyridine (**4**), 2-(1-adaman tylthio)-4-phenylpyridine (**5**), 6-(1-adaman tylthio)-2-phenylpyridine (**6**), 5-(1-adaman tylthio)-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 $\mu$g/mL. Twenty one strains of microorganisms, cultured in MH broth at 37 $^o$C for 24 h, were diluted with 0.9 % normal saline solution to adjust the cell density to $3\times10^9$ cell/mL. The organisms were inoculated onto each plate and further incubated at 37 $^o$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).

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**Table 1:** Microorganisms for antimicrobial activity testing

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

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 $\mu$L in each well and then add 50 $\mu$L of various tested drug concentrations. These parasite suspensions were incubated for 48 h in the atmosphere of 5 % $CO_2$ at 37 $^{o}$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 $^{1}$H-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 ($\alpha$- and $\beta$-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 $\mu$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 $\mu$g/mL, against *S. pyogenes* and *S. aureus* ATCC 25923 with MIC of 4 and 32 $\mu$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 $\mu$g/mL. In addition, $\beta$-sulfides (**7** and **8**) exhibited complete inhibition against *L. monocytogenes* with MIC of 32 $\mu$g/mL. The $\beta$-sulfide **8** showed activity against *B. subtilis* ATCC 6633 and *M. catarrhalis* with MIC of 4 $\mu$g/mL, while the compound **7** inhibited the growth of *B. subtilis* ATCC 6633 with MIC of 32 $\mu$g/mL. It was observed that a presumably drug resistant strain of *Enterococcus* group D was inhibited by $\beta$-sulfides **7** (75 %) and **8** (50 %) at 256 $\mu$g/mL. Significantly, $\beta$-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 $\alpha$- and $\beta$-(1-adamantylthio) analogs of picolines and phenyl pyridines have not been reported. Our previous study reported antimicrobial activity of $\alpha$- and $\beta$-(1-adamantylthio)-3-substituted (R) pyridines e. g. $\alpha$-thiopyridines **10**-**14** (R = 3-OEt, 3-OAc, 3-$NAc_2$, 3-Br, 5-OH) and $\beta$-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; $\beta$-(1-adamantylthio) pyridines **7** and **8** for pharmaceutical applications.

### *Antimalarial activity*

The activity of $\alpha$- and $\beta$- 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 $IC_{50}$ $10^{-6}$ to < $10^{-5}$ M. However, $\alpha$- and $\beta$- sulfides **2** and **4**-**6** were inactive antimalarials showing $IC_{50}$ >$10^{-5}$ M. It is interested to note that such active antimalarials constitute 1-adamantylthio group at $\alpha$-(2-) and $\beta$-(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 $\alpha$- and $\beta$-(1-adamantylthio)picolines and (1-adamantylthio)phenylpyridines (**1**-**9**) provides significant results that only $\beta$-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 $\beta$-thio analogs of 3-picoline and 4-phenylpyridine, namely 5-(1-adamantyl thio)-3-picoline (**7**) and 3-(1-adamantyl thio)-4-phenlypyridine (**8**), as antimicrobials and antimalarials. Significantly, both analogs **7** and **8** are very potent antimicrobials in which **8** being the most potent one. The $\beta$-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* μg/mL |
|---|---|---|---|
| **1** | inactive | - | - |
| **2** | active[a] | - | - |
| **3** | inactive | - | - |
| **4** | inactive | - | - |
| **5** | inactive | - | - |
| **6** | inactive | - | - |
| **7** | active[b] | *M. flavas, M. catarrharis* | 4 |
| | | *S. pyogenes, N. mucosa, C. diphtheriae* NCTC 10356 | 8 |
| | | *B. subtilis* ATCC 6633, *L. monocytogenes* | 32 |
| **8** | active[c] | *M. flavas, C. diptheriae* NCTC 10356 | ≤ 2 |
| | | *S. pyogenes, B. subtilis* ATCC 6633, *M. catarrhalis* | 4 |
| | | *S. aureus* ATCC 25923, *S. epidermidis, L. monocytogenes* | 32 |
| **9** | Inactive | - | - |

*MIC: Minimum inhibitory concentration was the lowest concentration that inhibited the growth of microorganisms. At 256 μg/mL showed inhibition against [a]*M. catarrhalis* and *C. diphtheriae* NCTC 10356 (75%), against *Enterococcus* group D [b]75 %, [c]50 %

**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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