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NOVEL ACTIVITIES OF 1-ADAMANTYLTHIOPYRIDINES AS ANTIBACTERIALS, ANTIMALARIALS AND ANTICANCERS

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ABSTRACT

To discover new bioactive lead compounds for medicinal purposes, herein, 2(1-adamantylthio)pyridine and derivatives (**1-10**) were prepared and tested for antibacterial (agar dilution method against 27 strains of microorganisms), antimalarial (against *Plasmodium falciparum*) and anticancer (MOLT-3, HepG2, HuCCA-1 and A549) activities. Results showed that all the tested derivatives selectively exerted antigrowth activity against *Streptococci* at 15-30 μg/mL. 3-Substituted (R) thiopyridines; **3** (R = NAc_2), **5** (R = OH) and **6** (R = Br) exhibited antibacterials, antimalarials and anticancers. Significantly, 6-(1-adamantylthio) nicotinonitrile (**10**) is a promising antibacterial which selectively displays antigrowth activity against *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Edwardsiella tarda* and β-hemolytic *Streptococcus* group A with minimum inhibitory concentration of 30 μg/mL. The findings reveal that these 1-adamantylthiopyridines represent a novel class of antibacterial, antimalarial and anticancer agents with potential medicinal values.

Keywords: 1-adamantylthiopyridines, antibacterial, antimalarial, anticancer activities

INTRODUCTION

Pyridines constituting sulfide (Ballell et al., 2005), sulfoxide (Scozzafava et al., 2001), sulfone (Centrone and Lowary, 2004) and sulfonamide (Joshi et al., 2004) have shown to be active antimicrobials and herbicides (Parrish et al., 2001; Doweyko et al., 1983). In the previous work, we have found that 3-substituted (R) pyridines having 1-adamantylthio moiety such as thiopyridines **1**-**6** (Figure 1) exert antibacterial activity (Prachayasittikul et al., 2008a). Such 3-substituents are groups or atoms with high electronegativity (R = OC_2H_5, OAc, NAc_2, Br, OH). To compare their bioactivity, it is of great interest to explore thiopyridines bearing 3-carbonyl (carboxylic acid and amide) and nitrile functions. However, antimalarial and anticancer activities of 1-adamantylthiopyridines have not yet been reported. As a continuing

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study, to search for new simple-small molecular lead compounds with medicinal values, we have focused on antibacterial, antimalarial and anticancer activities of other 3-substituted thiopyridine analogs containing 1-adamantylthio group. The interested target compounds are 1-adamantylthionicotinic acid, nicotinamide and nitrile derivatives (**7-10**) as shown in Figure 1. The compounds **7-10** were prepared using deoxydative substitution reaction of pyridine 1-oxides by thiol (Prachayasittikul and Bauer, 1985). The present study reveals antibacterial activity of the thionicotinic acid and derivatives **7-10** as well as antimalarial and anticancer activities of the thiopyridines **1-10**.

MATERIALS AND METHODS

General

Melting points were determined on an Electrothermal melting point apparatus (Electrothermal 9100) and are reported without correction. ^{1}H-NMR spectra were recorded on a Bruker AM 400 instrument with a 400/100 MHz operating frequency using deuterochloroform or DMSO-d_6 solution with tetramethylsilane as internal standard. Infrared spectra (IR) were obtained on Perkin Elmer System 2000 FTIR. 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 prior to use. Chemicals for the synthesis were of analytical grade.

Reagents for cell culture and assays were the following:

RPMI (Gibco and Hyclone laboratories, USA)

HEPES, L-glutamine, penicillin-streptomycin, sodium pyruvate and glucose (Sigma, USA)

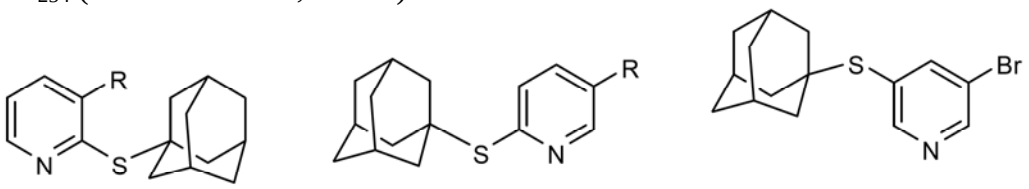
Ham's/F12, DMEM and fetal bovine serum (Hyclone laboratories, USA)

Gentamicin sulfate (Government Pharmaceutical Organization, Thailand)

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma-Aldrich, USA).

The tested compounds 1-10

The tested compounds are 2-(1-adamantylthio)-3-ethoxypyridine (**1**), 2-(1-adamantylthio)-3-acetoxypyridine (**2**), *N*-acetyl-2-(1-adamantylthio)-3-acetamidopyridine (**3**), 2-(1-adamantylthio)-3-bromopyridine (**4**), 2-(1-adamantylthio)-5-hydroxypyridine (**5**), 3-(1-adamantylthio)-5-bromopyridine (**6**), 2-(1-adamantylthio)nicotinic acid (**7**), 2-(1-adamantylthio)nicotinamide(**8**), 2-(1-adamantylthio)nicotinonitrile (**9**) and 6-(1-adamantylthio)nicotinonitrile (**10**). The compounds **1-6** (Prachayasittikul et al., 1991) and **7-10** (Prachayasittikul and Bauer, 1985) were prepared as previously described.



1, R = OC_2H_5
2, R = OAc
3, R = NAc_2
4, R = Br
7, R = COOH
8, R = $CONH_2$
9, R = CN

5, R = OH
10, R = CN

6

Figure 1: Structure of thiopyridines **1-10**

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Cell cultures

Plasmodium falciparum chloroquine resistant (T9.94)

Human erythrocytes (type O) infected with *P. 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.

Human cholangiocarcinoma (HuCCA-1)

HuCCA-1 cells were grown in Ham's/F12 medium containing 2 mM L-glutamine supplemented with 100 U/mL penicillin-streptomycin and 10 % fetal bovine serum.

Human lung cancer (A549, non small cell)

Human lung cancer cells were grown in Ham's/F12 medium containing 2 mM L-glutamine supplemented with 100 U/mL penicillin-streptomycin and 10% fetal bovine serum.

Hepatocarcinoma (HepG2)

HepG2 cells were grown in DMEM medium supplemented with 100 U/mL penicillin-streptomycin and 10 % fetal bovine serum.

T-lymphoblast (MOLT-3, acute lymphoblastic leukemia)

MOLT-3 cells were grown in RPMI 1640 medium supplemented with 100 U/mL penicillin-streptomycin, sodium pyruvate, 4.5 g/L glucose, 2 mM L-glutamine and 10 % fetal bovine serum.

Biological activities

Antibacterial assay

Antibacterial activity of the tested compounds was performed using agar dilution method as previously described (Prachayasittikul et al., 2008b). 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 15 and 30 μg/mL. Twenty seven 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 of 3×10^9 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 testing are shown in Table 1.

Table 1: Twenty-seven strains of microorganisms for antibacterial activity testing

Bacteria	
Gram-negative bacteria	
Escherichia coli ATCC 25922	*Citrobacter freundii*
Klebsiella pneumoniae ATCC 700603	*Enterobacter aerogenes*
Serratia marcescens ATCC 8100	*Enterobacter cloacae*
Pseudomonas aeruginosa ATCC 27853	*Serratia rubidaca*
Edwardsiella tarda	*Morganella morganii*
Shigella dysenteriae	*Providencia rettgeri*
Shigella flexneri	*Providencia alcalifaciens*
Salmonella paratyphi A	*Vibrio cholera*
Salmonella typhi	*Vibrio parahaemolyticus*
Salmonella enteritidis	*Aeromonas hydrophila*
Citrobacter diversus	
Gram-positive bacteria	
Staphylococcus aureus ATCC 25923	β-hemolytic *Streptococcus* group A
Bacillus subtilis ATCC 6633	α-hemolytic *Streptococcus* spp.
Staphylococcus saprophyticus	*Streptococcus* group D enterococcus

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Antimalarial assay

Antimalarial activity of the tested compounds was evaluated against *P. falciparum* chloroquine resistant (T9.94) using the literature method (Trager and Jensen, 1976).

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 μL in each well and then add 50 μL of various tested drug concentrations. These parasite suspensions were incubated for 48 h in the atmosphere of 5 % CO_2 at 37 °C. The percents parasitemia of control and drug-treated groups were examined by microscopic technique using methanol-fixed Giemsa stained of thin smear blood preparation.

Cytotoxic assay

Cell lines were plated in 96 well microplates 100 μL/well at a density of $5x10^3$ to $2x10^4$ cells/well. Background control wells contained the same volume of complete culture medium .The microplate was incubated for 24 h at 37 °C, 5 % CO_2 - 95 % humidity, anti-cancer drugs at various concentration were added and further incubated for 48 h. Cell viability was determined (Mosman, 1983; Carmichael et al., 1987; Tominaga et al., 1999) by staining with MTT assay (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide). The reagent was dissolved in PBS at 5 mg/mL and filtered to sterilize and remove a small amount of insoluble residue present in some batches of MTT. The MTT solution (10 μL /100μL medium) was added to all wells of the assay, and plates were incubated at 37 °C, 5 % CO_2 – 95 % humidity for 2-4 h. Subsequently, 100 μL DMSO was added to dissolve the resulting formazan by sonication. The plates were read on a microplate reader (Molecular Devices, USA), using a test wavelength at 550 nm and a reference wavelength at 650 nm.

For XTT assay, the suspension cell was MOLT-3 cell line. It was seeded in flat 96-well plates at a density of 2 x 10^4 and $5x10^3$ cells/well. The incubation and exposure processes were as described in the MTT assay. Preparation of the XTT, a stock XTT solution of 5 mL (1 mg/mL) was mixed with 100 μL of PMS solution (0.383 mg/mL); then, 50 μL of this mixture was added into each well of the tested plates and incubated for 4 h. The orange-colored of formazan compound thus formed and the absorbance was measured at 492 and 690 nm, respectively.

IC_{50} values were determined as the drug and sample concentration at 50 % inhibition of the cell growth.

RESULTS AND DISCUSSION

Chemistry

1-Adamantylthio derivatives of nicotinic acid, nicotinamide and nicotinonitrile **7-10** were prepared (Prachayasittikul and Bauer, 1985) from the reaction of nicotinic acid, nicotinamide and nicotinonitrile 1-oxides with 1-adamantanethiol in refluxing acetic anhydride. Similarly, 1-adamantylthiopyridines **1-6** were obtained from the reaction of corresponding 3-substituted pyridine 1-oxides as described (Prachayasittikul et al., 1991). Structures of compounds **1-10** were confirmed by IR, ^{1}H NMR spectral data and melting points.

Biological activities

Antibacterial activity

The thionicotinic acid, nicotinamide and nitrile derivatives **7-10** were evaluated for antibacterial activity against 27 strains of microorganisms using agar dilution method as previously described (Prachayasittikul et

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al., 2008b). It was found that (Table 2) the tested thiopyridine derivatives **7-10** selectively exerted antigrowth activity against *Streptococci* at 15-30 μg/mL. Significantly, 6-(1-adamantythio) nicotinonitrile **(10)** was the most active antibacterials exhibiting complete inhibition against β-hemolytic *Streptococcus* group A with minimum inhibitory concentration (MIC) of 30 μg/mL. In addition, the compound **10** also showed complete inhibition against *V. cholerae, V. parahaemolyticus* and *E. tarda* at the same concentration. Moreover, partial inhibition of the tested compounds **7-10** was also observed. Particularly, *S. paratyphi* A was inhibited (50 %) by the tested thiopyridines **7-9** at 15 μg/mL, whereas 75 % inhibition (at 30 μg/mL) was observed for thionicotinonitrile compound **10**. The compound **10** at 30 μg/mL also showed partial antigrowth activity, 75 % inhibitions against other organisms e. g. α-hemolytic *Streptococcus* spp., *Streptococcus* group D enterococcus, *A. hydrophila* and *S. aureus* ATCC 25923. In addition, 50 % inhibitions against *S. saprophyticus, S. dsysenteriae* and *B. subtilis* ATCC 6633 at 30 μg/mL were noted for nitrile analog **10**. Recently (Prachayasittikul et al., 2008a), we have found that thiopyridine compounds **1-6** display growth inhibition (50-75 %) against *Streptococci* at 15 μg/mL. In particular the derivatives **3**, **5** and **6** exhibit complete inhibition against β-hemolytic *Streptococcus* group A with MIC of 30 μg/mL.

However, the present study showed that among the tested thiopyridines **7-10** as compared with the reported analogs **1-6** (Prachayasittikul et al., 2008a), the thionitrile **10** (R = CN) was the most promising antibacterial. As a result, the thionicotinonitrile **10** exerted complete inhibition against many tested microorganisms e. g. β-hemolytic *Streptococcus* group A, *V. cholerae, V. parahaemolyticus* and *E. tarda* with MIC of 30 μg/mL. Apparently, 1-adamantylthiopyridine derivatives **1-10** bearing substituents with high electronegativity and or electron withdrawing effect at C-3 all selectively inhibit the growth of β-hemolytic *Streptococcus* group A, α-hemolytic *Streptococcus* spp. and *Streptococcus* group D enterococcus at concentrations range 15-30 μg/mL. Furthermore, the position of 1-adamantylthio group can be at 2-(α-) or 6-(α) and 5-(β-) positions of the pyridine ring. However, recently, β- (1-adamantylthio) analogs of 3-picoline and 4-phenylpyridine as antimicrobials and antimalarials have been reported by our group (Prachayasittikul et al., 2009).

Table 2: Antibacterial activity of 1-adamantylthiopyridines **7**-**10**

Compound	Activity	Inhibition (%)	
		15 μg/mL	30 μg/mL
7	active	50[a,b], 75[c,d,e]	50[f,g,h], 75[a,b,i,j]
8	active	50[b], 75[c,d,e]	50[h]
9	active	50[b], 75[c,d,e]	50[a]
10	active	-	50[h,k,l], 75b[d,e,f,g], 100[a,c,i,j]
Ampicillin*	active[g,l]		

Inhibition against [a]*E. tarda*, [b]*S. paratyphi* A, [c]β-hemolytic *Streptococcus* group A, [d]α-hemolytic *Streptococcus* spp., [e]*Streptococcus* group D enterococcus, [f]*A. hydrophila*, [g]*S. aureus* ATCC 25923, [h]*S. saprophyticus*, [i]*V. cholerae*, [j]*V. parahaemolyticus*, [k]*S. dysenteriae*, [l]*B. subtilis* ATCC 6633

*At 10 μg/mL showed 100 % growth inhibition against *S. aureus* ATCC 25923 and *B. subtilis* ATCC 6633

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So far, the antibacterial activity of 1-adamantylthionicotinic acid, amide and nitrile derivatives **7-10** has not been reported. Related compound e. g. 2-mercaptonicotinamide was reported to be the most active one, which inhibited the growth of *Mycobacterium kansasii*, among the tested derivatives (Klimesova and Odlerova, 1993). Recently, we have found that nicotinic acid copper complex with carboxylic acids exhibit antimicrobial activity (Suksrichavalit et al., 2008; Suksrichavalit et al., 2009). The study demonstrates that 1-adamantylthionicotinic acid, its amide and nitrile derivatives **7-10** are novel antibacterials with potential medicinal values, particularly, the thionicotinonitrile **10**.

Antimalarial activity

The thiopyridines **1-10** were assayed for antimalarial activity against *P. falciparum* chloroquine resistant (T9.94). Results (Table 3) showed that 2-(1-adamantylthio) nicotinonitriles **(9** and **10**) and 3-substituted thiopyridines **2-5** (R=OAc, NAc_2, Br, OH) and **6** exhibited fair activity with IC_{50} 10^{-6} - $<10^{-5}$ M, whereas thionicotinic acid **7** and nicotinamide **8** together with 2-(1-adamantylthio)-3-ethoxypyridine (**1)** were inactive showing $IC_{50} > 10^{-5}$ M. Interestingly, the active antimalarials (**2-6** and **9-10**) having 1-adamantylthio moiety at either α- or β-position on the pyridine ring are manifested. Such observation has been found for β-thio analogs of 3-picoline and 4-phenylpyridine (Prachayasittikul et al., 2009). Based on the literatures, antimalarial activity of the tested thiopyridines **1-10** has not been reported. Thus, thiopyridine analogs **2-6** and **9-10** are novel antimalarials.

Anticancer activity

Anticancer activity of the 1-adamantylthiopyridines **1-10** was performed against four cell lines; MOLT-3, HepG2, HuCCA-1 and A549 using etoposide and/or doxorubicin as positive controls. It was observed (Table 4) that 1-adamantylthionicotinic acid, its amide and nitrile derivatives (**7-10**) were inactive ($IC_{50} > 50$ μg/mL) to all the tested cell lines, except for thionicotinonitrile compound **9** which begin to show some activity with IC_{50} of 50 μg/mL against HepG2 cell line. However, nicotinonitrile without thio functionality analog of 3,4-disubstituted pyrazoles was reported to be anti-tumor cyclin-dependent inhibitor (Lin et al., 2007). The 3-substituted thiopyridines **5** (R = OH) and **6** were active against all the tested cell lines with IC_{50} range 17.90-45.00 μg/mL, while *N*-acetyl-2-(1-adamantylthio)-3-acetamidopyridine (**3**) selectively exerted anticancer activity against only MOLT-3 cell line with IC_{50} of 16.10 μg/mL. Particularly, MOLT-3 cell line was inhibited not only by the compound **3**, but also by 2-(1-adamantylthio)-5-hydroxypyridine (**5**) and 3-(1-adamantylthio)-5-bromopyridine (**6**). Such thiopyridines (**3**, **5** and **6**) showed comparable anticancer activity, in which the thioacetamidopyridine **3** was the most active with IC_{50} of 16.10 μg/mL. Interestingly, thionicotinic acid (**7**), amide **(8)** and nitrile **(10)** derivatives are inactive agents. However, anticancer activity of these compounds has not been reported. So far, *N*-acetyl-3-acetamidopyridine and its 2-isomer were shown to significantly inhibit the replication of influenza A virus (Pushkarskaya et al., 1971). Now, it comes to the point that the active 1-adamantylthiopyridines **3**, **5** and **6** are new types of analogs with anticancer activity.

Table 3: Antimalarial activity of 1-adamantylthiopyridines **1-10**

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

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Table 4: Anticancer activity of 1-adamantylthiopyridines **1**-**10**

Compound[c]	IC_{50} (μg/mL)[a,b]			
	MOLT-3	**HepG2**	**HuCCA-1**	**A549**
3	16.10±1.11	inactive	inactive	inactive
5	17.90±0.18	35.00±0.00	44.00±2.83	45.00±0.00
6	18.34±0.84	41.00±1.41	37.00±4.24	45.00±0.71
7	inactive	inactive	inactive	inactive
8	inactive	inactive	inactive	inactive
9	inactive	50.00±0.00	inactive	inactive
10	inactive	inactive	inactive	inactive
etoposide	0.02±0.00	12.00±0.00	-	-
doxorubicin	-	0.23±0.00	0.50±0.00	0.45±0.07

a : When IC_{50} > 50 μg/mL denote inactive for anticancer activity.
b : The tests were performed in triplicate.
c : Results of compounds **1**, **2** and **4** were not obtained, due to their insolubility.

Based on the outcome of activity testings, it is noteworthy that 1-adamantylthiopyridines **3**, **5** and **6** all exert antibacterial (against β-hemolytic *Streptococcus* group A), antimalarial and anticancer (against MOLT-3) activities. In addition, the compounds **5** and **6** also showed anticancer activity against all other tested cell lines (HepG2, HuCCA-1 and A549). Furthermore, the 6-(1-adamantylthio) nicotinonitrile (**10**) selectively showed complete inhibition against β-hemolytic *Streptococcus* group A, *V. cholerae, V. parahaemolyticus* and *E.tarda*. Antimalarial activity was also observed for the nicotinonitrile **10** as well as its 2-isomer; 2-(1-adamantylthio) nicotinonitrile **9**. In relating their structures and activities, it can be concluded that 1-adamantylthio analogs of 3-substituted thiopyridines require substituents with high electronegativity atoms (O, Br) and/or electron withdrawing groups (NAc_2, CN). Moreover, such active 1-adamantylthiopyridines are simple-small molecules which are readily achieved by one pot reaction. Significantly, the thionicotinonitrile (**10**) is a promising antibacterial which selectively exhibits antigrowth activity against β-hemolytic *Streptococcus* group A, *V. cholerae*, *V. parahaemolyticus* and *E. tarda* with MIC of 30 μg/mL.

CONCLUSION

The findings demonstrate that 1-adamantylthiopyridines represent a novel class of bioactive compounds, particularly, **3**, **5** and **6** are analogs of interesting antibacterials, antimalarials and anticancers. However, all the tested derivatives selectively exhibit growth inhibition against *Streptococci* at 15-30 μg/mL. Significantly, 6-(1-adamantylthio) nicotinonitrile (**10**) is a promising antibacterial agent with potential for further development as pharmaceutical uses and applications.

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