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Original article:

ACTIVITIES OF THIOTETRAHYDROPYRIDINES AS ANTIOXIDANT AND ANTIMICROBIAL AGENTS

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ABSTRACT

Tetrahydropyridines have been reported previously as important medicinal agents. The present study, thiotetrahydropyridines were prepared and tested for antioxidants (DPPH and SOD assays) and antimicrobials (agar dilution method). The results show that 1-acetyl-1,2,3,4- and 1,2,3,6-thiotetrahydropyridines **15a-b**, **16**, **17** and **18a** are new antioxidants that scavenge superoxide and free radicals. Whereas the analogs 1**5a** and **16** are novel antimicrobials. Significantly, 1-acetyl-2-(1-adamantylthio)-3,4-diacetoxy-1,2,3,4-tetrahydropyridine (**15a**) is the most potent compound that inhibits the growth of *Streptococcus pyogenes* and *Moraxella catarrhalis* with MIC of 32 μg/mL, of *Corynebacterium diphtheriae* NCTC 10356 and of *Vibrio cholerae* (MIC of 64 μg/mL). Remarkably, the analog **15a** is the most potent antioxidant and antimicrobial agent. This finding reveals a new and unique group of 1-acetyl-1,2,3,4-thiotetrahydropyridines as interesting lead compound with potential to be further developed for medicinal applications.

Keywords: thiotetrahydropyridine, 3-picoline, phenylpyridines, antioxidants, antimicrobials

INTRODUCTION

Tetrahydropyridine is a moiety constituting in many bioactive compounds. It can present as three possible isomeric forms; 1,2,3,6-, 1,2,3,4- and 2,3,4,5-tetrahydropyridines. 1-Methyl-4-phenyl–1,2,3,6–tetrahydropyridines (**1**) is a potent neurotoxin in dopaminergic system. Tetrahydropyridine **1** was a byproduct in the synthesis of meperidine analog. However **1** itself is not toxic, but requires metabolic activation *via* monoamine oxidase B to form 1-methyl-4-phenylpyridinium ion accounting for its uptake into dopaminergic neurons producing Parkinson like syndrome. Since the discovery of the tetrahydropyridine **1**, synthesis and bioactivities of analog **1** were extensively studied (Mateeva et al., 2005). The syntheses of tetrahydropyridine analogs were reported such as 4-phenyl-1,2,3,6-tetrahydropyridine analogs **2** and **3** (Figure 1) from the reaction of α-and β-prodinol in 36 % HCl (Fries et al., 1986). *N*-(Carbonylimino) pyridinium ylide **4** underwent facile sodium borohydride reduction to furnish alkyl- or aryl-1,2,3,6-tetrahydropyridine **5** as shown in Figure 2 (Knaus and Redda, 1976; Redda et al., 1990). Furthermore, a series of *N*-acetyl-

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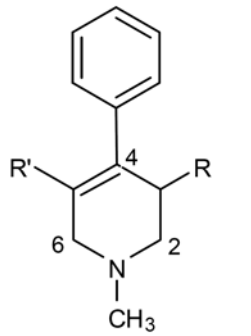
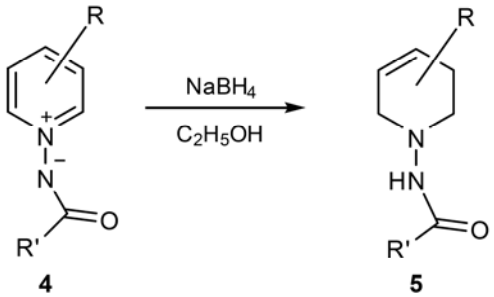
hydroxyl (or acetoxy) alkylthio substituted 1,2,3,4- and 1,2,3,6-tetrahydropyridines had been reported (Hershenson and Bauer, 1969; Egan et al., 1969; Kokosa et al., 1975; Prachayasittikul et al., 1985, 1991). The synthesis involved the reaction of pyridine 1-oxides with thiols in boiling acetic anhydride with or without inclusion of triethylamine. 1,2,3,4-Tetrahydropyridines **6**-**8** (Figure 3) were achieved from the reaction of picoline or phenylpyridine 1-oxides with *t*–butyl or 1-adamantyl (1-Adm) mercaptan in refluxing acetic anhydride (Prachayasittikul et al., 1985, 1991). From such reactions, 1,2,3,6-tetrahydropyridines **9** and **10** (Figure 4) were also found. These tetrahydropyridines are 1-adamantylthio and *t*-butylthio analogs bearing bis-oxy functions.



1, R, R' = H
2, R = CH_3, R'= H
3, R = H, R' = CH_3

Figure 1: 1,2,3,6-Tetrahydropyridines **1**-**3**.



R = H, alkyl, aryl
R' = phenyl, pyridyl

Figure 2: Tetrahydropyridine **5** from reduction of pyridinium ylide **4**.

Tetrahydropyridine analogs are compounds of diverse biological activities, e. g. dopaminergic, nicotinic and muscarinic receptor agonist/antagonist actions including analgesics, antiinflammatory and chemotherapeutic agents as well as nerve gas antidotes. Examples of 1,2,3,6-tetrahydropyridines (Figure 5) are arecoline (**11**) as the muscarinic agonist (Dunbar et al., 1994), 1-aminoaryl analog **12** exhibiting antiinflammatory activity comparable to that of indomethacin (Yeung et al., 1982), tetrahydropyridinium analog **13** as nerve agent poisoning antidotes (Gray et al., 1988) and 1-carbonyloxy tetrahydropyridine **14** containing 4-substituted aryl moiety as antibacterial (Barbachyn et al., 2003).

However, biological activities of thiotetrahydropyridines have not been reported. Our previous studies showed that alkylthiopyridines; 1-adamantylthio analog of 3-picoline, phenylpyridine, ethoxy-, acetoxy-, bromo- and *N,N*-diacetylamino pyridines exhibited antimicrobial actions (Prachayasittikul et al., 2008, 2009a). To search for new bioactive thiotetrahydropyridines, thus, it is of interest to investigate 1-adamantylthio analogs of tetrahydropyridine as antioxidants and antimicrobials. The study is directed towards the activities of 1,2,3,4- and 1,2,3,6-tetrahydropyridines. Therefore, 1-adamantylthiotetrahydropyridines **15**-**18**; analogs of 3-picoline and 3-, 4-phenylpyridines were prepared (Prachayasittikul et al., 1985, 1991) and evaluated for antioxidant and antimicrobial properties. The structure of target lead compounds is shown in Figure 6.

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 deuterochloroform solution with tetramethylsilane

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as internal standard. Infrared spectra (IR) were obtained on Perkin Elmer System 2000 FTIR. Ultraviolet spectra (UV) were measured with Milton Roy Spectronic 3000 Array. Elemental analysis was carried out using a Perkin Elmer Elemental Analyzer 2400 CHN. 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 and assays were of analytical reagent grade.

Compounds 15-18

The tested compounds were prepared by the reaction of 3-picoline or 3- and 4-phenylpyridine 1-oxides with 1-adamantanethiol (1-AdmSH) in refluxing acetic anhydride as described (Prachayasittikul et al., 1985, 1991). The compounds are 1-acetyl-2-(1-adamantylthio)-3,4-diacetoxy-5-methyl (or phenyl)-1,2,3,4-tetrahydropyridines (**15a**-**b**), 1-acetyl-2-(1-adamantylthio)-3-hydroxy-3-methyl-4-acetoxy-1,2,3,4-tetrahydropyridine (**16**), 1-acetyl-2-(1-adamantylthio)-3-acetoxy-4-phenyl-6-hydroxy-1,2,3,6-tetrahydropyridine (**17**) and 1-acetyl-2,6-dihydroxy-3-(1-adamantylthio)-4-phenyl-1,2,3,6-tetrahydropyridine (**18a**). Tetrahydropyridine **18a** was isolated (1.15g, 4.05 %); m.p. 158-159°C; IR(KBr)υ_{max}: 3473 (OH), 1650 (CO) cm^{-1}; UV(95 % ethanol)λ_{max} nm (logε): 202 (4.33), 243 (4.07); ^{1}H-NMR (300 MHz, $CDCl_3$): δ6.12 (d, J = 4.1 Hz, 1H, H-5), 5.95 (d, J = 4.1 Hz, 1H, H-6), 5.64 (d, J = 1.2 Hz, 1H, H-2), 3.95 (d, J = 1.2 Hz, 1H, H-3), 1.85 (s, 3H, $NCOCH_3$), 2.33 (s, 3H, OCOCH3), 1.59-1.90 (m, 1-Adm); ^{13}C-NMR (75 MHz, $CDCl_3$): δ142.5 (C-4), 126.4 (C-5), 84.2 (C-2), 77.1 (C-6), 44.7 (C-3). EIMS m/z (% relative intensity): 381(M^+-18, 3), 320 (23), 135 (100), 93 (21), 79 (28), 43 (24), 18 (62). *Anal*. Calcd. for $C_{23}H_{29}NO_3S$: C, 69.14; H, 7.32; N, 3.50. Found: C, 70.28, H, 7.13, N, 3.47.

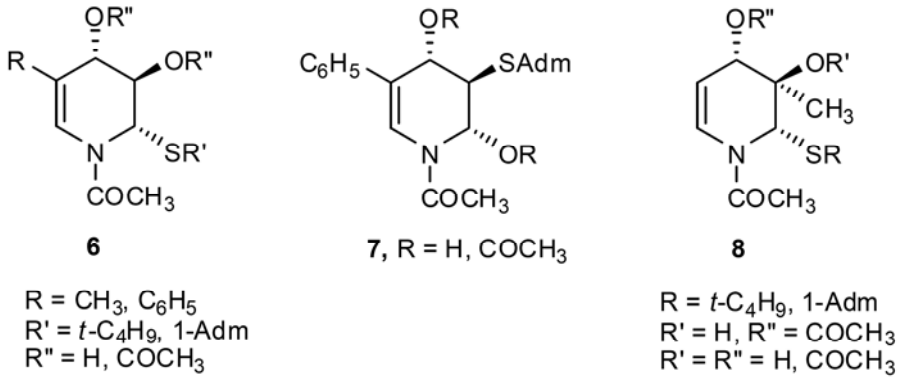

Figure 3: 1,2,3,4-Tetrahydropyridines bearing alkylthio bis-oxy **6**-**8**.

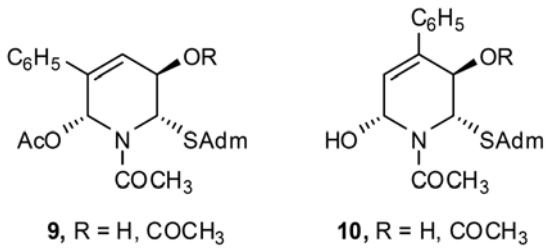

Figure 4: 1,2,3,6-Tetrahydropyridines **9**-**10**.

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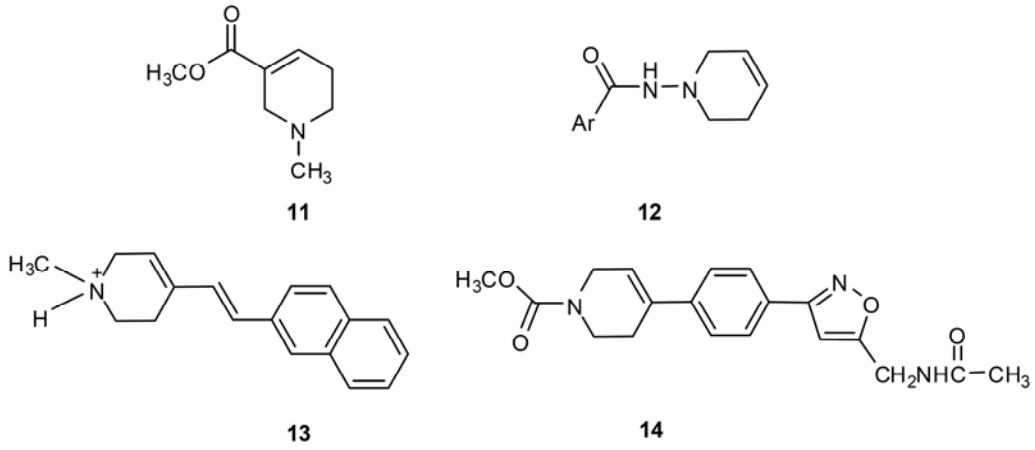

Figure 5: Bioactive compounds of 1,2,3,6-tetrahydropyridines.

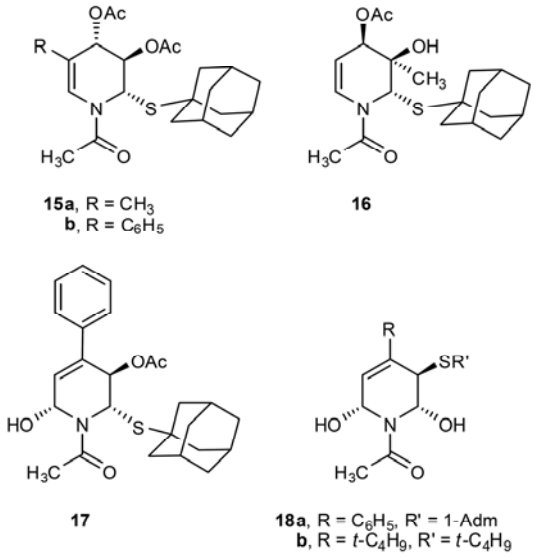

Figure 6: Structures of target thiotetrahydropyridines **15**-**18**.

Antioxidative activity

Antioxidative activity of the compounds was determined by DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging (Prachayasittikul et al., 2009b) and superoxide dismutase (SOD) (Piacham et al., 2006) assays. The DPPH (a stable purple color) reacts with an antioxidant compound; it is reduced to yield a light-yellow color of diphenylpicrylhydrazine. Changes of the color can be spectrophotometrically measured. In this study, experiment was initiated by preparing 0.2 mM DPPH in methanol. One millilitre of this solution was added into 0.5 mL of sample solution (1 mg/mL dissolved in methanol). The reaction was mix vigorously. Absorbance was measured at 517 nm after 30 min incubation at room temperature in the dark. The percentage of radical scavenging activity was calculated from the following equation:

$$\% \text{ Radical Scavenging} = (1\text{-}Abs._{sample}/Abs._{control}) \times 100$$

where $Abs._{control}$ is the absorbance of the control reaction and $Abs._{sample}$ is the absorbance of the tested compound.

The SOD activity was performed by measuring inhibition of the photoreduction of nitro blue tetrazolium (NBT). The indirect assay is comprised of several reactions. Briefly, the photochemically excited riboflavin was first reduced by methionine into a semiquinone, which donated an electron to oxygen to form the superoxide source. The superoxide readily converted NBT into a purple formazan product which was detected by spectrophotometer at 550 nm.

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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 μg/mL. Twenty one strains of microorganisms (Prachayasittikul et al., 2009a), cultured in MH broth at 37 °C for 18-24 h, were diluted with 0.9 % normal saline solution to adjust the cell density to 1×10^8 cells/mL compared with 0.5 McFarland. The organisms were inoculated onto each plate and further incubated at 37°C for 24-48 h. Compounds which possessed high efficacy to inhibit bacterial cell growth were analyzed.

RESULTS AND DISCUSSION

Chemistry

1-Adamantylthio analogs of tetrahydropyridines **15-18** were prepared from the deoxydative substitution reaction of 3-picoline or 3- and 4-phenylpyridine 1-oxides with 1-AdmSH in refluxing acetic anhydride (Prachayasittikul et al., 1985, 1991). 1,2,3,4-Tetrahydropyridines **15a** and **15b** were obtained from the reaction of 3-picoline and 3-phenylpyridine 1-oxides, respectively. Whereas analog **16** was achieved from 3-picoline 1-oxide under the identical condition in the presence of triethylamine. Under the similar condition with triethylamine, 4-phenylpyridine 1-oxide furnished 1,2,3,6-tetrahydropyridine **17**. When the reaction of 4-phenylpyridine 1-oxide with 1-AdmSH in acetic anhydride was performed without addition of triethylamine, 1,2,3,6-tetrahydropyridine **18a** was isolated. Structures of these tetrahydropyridines **15-17** were confirmed by ^{1}H-NMR, IR and UV spectral data and melting points. However, 1-acetyl-2,6-dihydroxy-3-(1-adamantylthio)-4-phenyl-1,2,3,6-tetrahydropyridine **18a** was quite analogous to tetrahydropyridine **18b** which was isolated from 4-*t*-butylpyridine 1-oxide with *t*-butyl mercaptan (Kokosa et al., 1976). The stereochemistry at C-2 and C-3 of **18a** was determined using coupling constant between H-2 and H-3 and the Karplus relationship. The coupling constant of 1.2 Hz suggested that these two protons are *trans*-quasidiequatorial. Therefore, hydroxyl at C-2 and sulfide at C-3 are *trans*-quasidiaxial. Upfield methine proton (H-3) appears at δ 3.95 ppm suggested that the sulfide presents at C-3 position. This assignment is supported by ^{13}C-NMR of C-3 at δ 44.7 ppm, which is in the range found for carbon bearing sulfide group. Based on the known tetrahydropyridines (Egan et al., 1969), thus, the stereochemistry of the hydroxyl group at C-6 is quasi-axial in twist chair form. The presence of phenyl group at C-4 of **18a** resulted in no longer observed of allylic coupling. Due to the similarity of chemical shift and coupling constant, the hydroxyl group at C-6 is assigned to have the same stereochemistry as C-2. The two hydroxyl groups at C-2 and C-6 are *cis*-quasidiaxial. Mass spectra of **18a** did not show the molecular ion, but instead of low intensity of m/z 381, due to the loss of one mole of water from the molecular ion. Usually 1-Adm substituent shows m/z 135 as a base peak. UV spectra showed λ_{max} at 243 nm of alkene. Its IR spectra confirmed the presence of OH and CO of amide groups.

The tetrahydropyridines **15-18** are by-products from the reaction of 3-picoline 1-oxide or phenylpyridine1-oxides with 1-AdmSH. The structure of analog **18a** was identified by comparison of its spectral data; ^{1}H-, ^{13}C-NMR, IR, UV and mass spectra with the previously well established (Prachayasittikul et al., 1991). The formation of tetrahydropyridine **18a** was proposed to be involved episulfonium ion intermediate (Prachayasittikul et al., 1991).

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Antioxidant activity

The antioxidant activity of thiotetrahydropyridines **15-18** was performed and found that (Table 1) all exhibited NBT superoxide scavenging (10.91-19.95 %) and DPPH free radical scavenging (1.70-22.39 %) activities. The tetrahydropyridine **15a** was the strongest antioxidant both in SOD (19.95 %) and DPPH (22.39 %) assays. However, such activities of analogs **15-18** have not been reported. Therefore, 1,2,3,4-thiotetrahydropyridines (**15** and **16**) and 1,2,3,6-thiotetrahydropyridines (**17** and **18**) are found to be new antioxidants. It is notable that the most potent antimicrobials **(15a)** also exerts the strongest antioxidant in scavenging superoxide and free radical.

Antimicrobial activity

The 1,2,3,4- and 1,2,3,6-tetrahydropyridines bearing 1-adamantylthio moiety (**15-18**) were investigated for antimicrobial activity using agar dilution method against 21 strains of microorganisms. Results (Table 2) showed that all the tested compounds exhibited no growth inhibition against yeast. Only 1,2,3,4-tetrahydropyridines **15a** and **16** displayed growth inhibition against gram-positive and gram-negative bacteria; *Streptococcus pyogenes*, *Corynebacterium diphtheriae* NCTC 10356 and *Moraxella catarrharis*. In addition, the tetrahydropyridine **15a** also exhibited antigrowth activity against *Vibrio cholerae* and *Micrococcus flavas*. Significantly, the analog **15a** was the most potent antimicrobial that inhibited the growth of *S. pyogenes* and *M. catarrharis* with MIC of 32 μg/mL, of *C. diphtheriae* NCTC 10356 and *V. cholerae* with MIC of 64 μg/mL including of *M. flavas* with MIC of 128 μg/mL. Interestingly, it is noted that the active antimicrobials **15a** and **16** are group of tetrahydropyridines derived from 3-picoline having methyl group at 5- and 3-positions, respectively. Such notion was not observed for analogous tetrahydropyridine **15b** bearing phenyl substituent at position 5. Our previous studies showed that fully aromatic; pyridyl sulfides such as 3-(1-adamantylthio)-4-phenylpyridine was the most potent antimicrobials among the tested compounds (Prachayasittikul et al., 2009a). So far bioactivities of thiotetrahydropyridines **15a** and **16** have not been reported. Thus, 1-acetyl-2-(1-adamantyl thio)-3,4-diacetoxy-5-methyl-1,2,3,4-tetrahydropyridine (**15a**) and 1-acetyl-2-(1-adamantylthio)-3-hydroxy-3-methyl-4-acetoxy-1,2,3,4-tetrahydropyridine (**16**) represent a novel group of antimicrobials.

Table 1: Antioxidative activity of tetrahydropyridines **15**-**18**.

Compound[a]	**NBT superoxide scavenging activity**[b] (%)	**DPPH free radical scavenging activity**[c] (%)
15a	19.95	22.39
15b	14.21	9.39
16	14.80	13.45
17	14.58	1.70
18a	10.91	4.90

[a] Compounds were tested at 300 μg/mL.
[b] Superoxide dismutase (SOD, 4140 U/mg protein) from bovine erythrocytes was used as a standard.
[c] α-Tocopherol was used as a control.

Table 2: Antimicrobial activity of tetrahydropyridines **15-18**.

Compound	Activity	Microorganism	MIC (μg/mL)
15a*	active	*M. catarrhalis*, *S. pyogenes*	32
		C. diphtheriae NCTC *10356*, *V. cholerae*	64
		M. flavas	128
15b	inactive	—	—
16	active	*M. catarrhalis*	64
		S. pyogenes, C. *diphtheriae* NCTC *10356*	256
17	inactive	—	—
18a	inactive	—	—
Ampicillin	active	*P. shigelloides*	10

MIC: Minimum inhibitory concentration was the lowest concentration to inhibit the growth of microorganisms.
*At 256 μg/mL showed 75 % inhibition against *M. catarrhalis*.

CONCLUSION

The investigation demonstrates a new and unique 1-adamantylthio analog of 1,2,3,4- and 1,2,3,6-thiotetrahydropyridines (**15a-b**, **16**, **17** and **18a**) as antioxidants whereas the analog **15a** is the strongest one to scavenge superoxide and free radical. Furthermore, thiotetrahydropyridines **15a** and **16** also exert antimicrobial actions that the analog **15a** is the most potent. Significantly, the 1,2,3,4-tetrahydropyridine **15a** is the most potent antioxidant and antimicrobial derived from 3-picoline. In addition, 1,2,3,6-tetrahydropyridine **18a** was isolated from the reaction of 4-phenylpyridine 1-oxide with 1-AdmSH. As a results, such new bioactive compounds display benefit potential for further development as medicinal applications.

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