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NOVEL 4-SUBSTITUTED PYRIDINE DERIVATIVES: PRACTICAL DERIVATIZATION AND BIOLOGICAL PROFILES OF REVERSIBLE H^+/K^+-ATPase INHIBITORS

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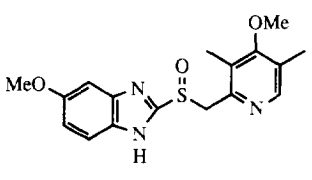
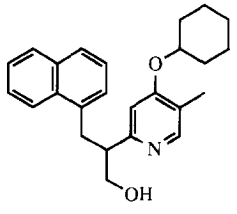
Abstract: An easy method to prepare novel 4-alkoxy-, 4-alkylthio- or 4-aryloxy-5-methyl-2-[1-(hydroxymethyl)-2-(1-naphthyl)-ethyl (or -ethenyl)]pyridine derivatives having reversible inhibitory activity against H^+/K^+-ATPase is described. Use of a methylsulfinyl- or methylsulfonyl group as a leaving group makes it possible to effectively introduce various alkoxy or alkylthio groups into the 4-position of the pyridine ring at the final stages of synthesis. Biological profiles of the prepared compounds are briefly mentioned.

Unlike histamine H_2 receptors, which are found in tissues throughout the body, H^+/K^+-ATPase is located predominantly in the parietal cells of the stomach and catalyzes the terminal step in gastric acid secretion;[1] therefore, it is expected that selective H^+/K^+-ATPase inhibitors have intrinsic advantages over H_2 antagonists as safe and potent antiulcer agents. Indeed, irreversible H^+/K^+-ATPase inhibitors, represented by omeprazole (**1**), have proved to be highly effective in the clinic for the treatment of acid-related gastrointestinal disorders.[2] However, it has been suggested that sustained suppression of gastric acid secretion caused by irreversible attack of such inhibitors against the enzyme is associated with the formation of gastric carcinoids in long-term carcinogenicity studies[3] and has led to sustained hypergastrinemia.[4] To overcome the disadvantage associated with the irreversible action of these inhibitors, several research groups have been trying to develop reversible H^+/K^+-ATPase inhibitors.[5,6] In the course of the chemical modification of **1**, we found that a series of novel 4-substituted pyridine derivatives, represented by AU-2064 (**2**), showed reversible inhibitory activity against H^+/K^+-ATPase.[7,8] To investigate the structure-activity relationships of substituents at the 4-position of the pyridine ring, we attempted to introduce various heteroatom nucleophiles into the position efficiently. In this communication, we describe an easy way to prepare novel 4-substituted pyridine derivatives having reversible inhibitory activity against H^+/K^+-ATPase by nucleophilic substitution of highly functionalized 4-methylsulfinyl- or 4-methylsulfonyl- pyridines. We also briefly describe their biological profiles.



omeprazole (**1**)

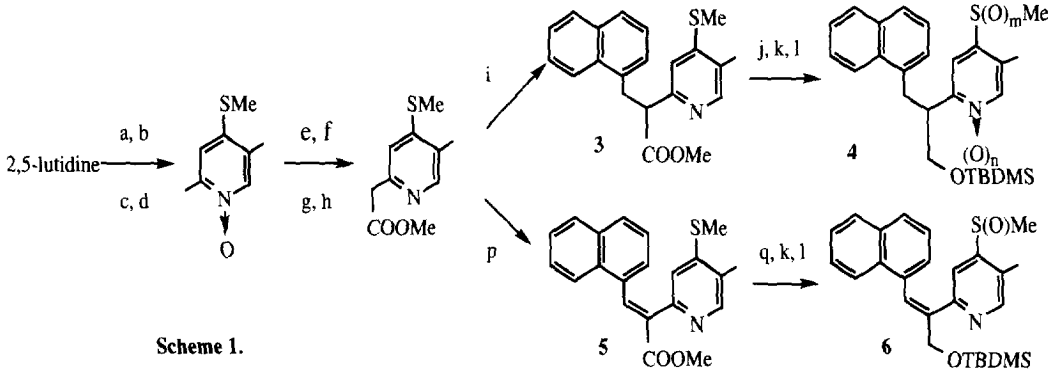


AU-2064 (**2**)

In our original procedures to prepare **2** and its related derivatives, substituents at the 4-position of the pyridine ring were introduced by replacing a nitro or halo group of the corresponding pyridine N-oxides with an alcohol or amine before introducing a naphthylmethyl unit.[9] However, in this case, individual intermediates, each bearing different 4-position substituent, must be independently converted to a final product via

several steps, a process which was tedious and inefficient for the present purpose. We therefore devised another synthetic strategy to modify the 4-position substituent. An appropriate masked leaving group is introduced into the 4-position of the pyridine at an earlier stage of the synthesis. After building up the fundamental skeleton, the masked leaving group is activated and efficiently replaced with various heteroatom nucleophiles. A preferable leaving group must have the following features: (1) the group should be stable in the masked form during both construction of the skeleton and transformation of a functional group(s), (2) the masked form should be easily convertible to the real leaving group without impairing the skeleton and functional groups, (3) the real leaving group should be readily replaceable with a desired nucleophile. With reference to Barlin and Brown's kinetic investigations,[10] followed by Furukawa et al.'s synthetic studies on the *ipso*-substitution reaction of simple pyridine derivatives,[11] we selected a methylsulfinyl or methylsulfonyl group as the leaving group and introduced a methylthio group into the 4-position of the pyridine ring as the masked leaving group.

5-Methyl-2-[2-(1-naphthyl)-1-*tert*-butyldimethylsiloxymethylethyl (or ethenyl)]pyridines and pyridine N-oxides possessing a 4-sulfinyl or 4-sulfonyl group (**4** and **6**) were prepared from 2,5-lutidine in 12 steps in the manner described in Scheme 1. The methylsulfenyl group was introduced by treating 4-bromo-2,5-lutidine N-oxide with sodium methylmercaptide. Four steps later, 5-methyl-4-methylthio-2-pyridylacetic acid methyl ester was alkylated with 1-bromomethylnaphthalene or condensed with 1-naphthylaldehyde to produce the saturated and unsaturated esters (**3** and **5**), which were converted to the corresponding alcohols by reducing the ester group with LAH and DIBAL, respectively. In the case of **5**, DIBAL reduction afforded the desired allylic alcohol accompanied by the saturated ester **3**, even though **5** was treated with an equimolecular amount of DIBAL in toluene or THF at -78 °C. Protection of the hydroxy group as a *tert*-butyldimethylsilyl



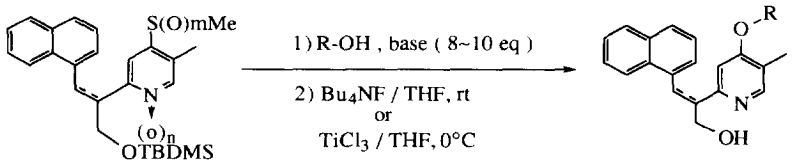
Scheme 1.

a: H_2O_2-Na_2WO_4, AcOH-H_2O, 80 °C, 5 h, 97%; b: HNO_3-H_2SO_4, 80 - 90 °C, 3 h, 78%; c: AcBr, rt, 12 h, 94%; d: CH_3SNa, H_2O-MeOH, reflux, 3 h, 95%; e: i) Ac_2O, 100 °C, 30 min, ii) NaOH, H_2O-MeOH, rt, 2 h, 80%; f: $SOCl_2$, $CHCl_3$, 0 °C, 1 h, 98%; g: i) NaOH, H_2O-EtOH, 0 °C, ii) NaCN, H_2O-EtOH, reflux, 1 h, 71%; h: HCl-MeOH, 1.5 h, 86%; i: i) LDA, THF, -78 °C, ii) bromomethylnaphthalene, 0 °C, 1.5 h, 77%; j: LAH, THF, 0 °C, 45 min, 99%; k: ^{t}BuMe$_2$SiCl, imidazole, DMF, rt, 2 h, 98%; l: mCPBA, CH_2Cl_2; p: 1-naphthylaldehyde, piperidine-AcOH, benzene, reflux, 5 h, (E)-77%, (Z)-23%; q: DIBAL, THF, -78 °C, 2 h, 52% (the saturated ester, 38%).

ether followed by oxidation with mCPBA yielded 4-methylsulfinyl or 4-methylsulfonylpyridines and pyridine N-oxide (**4** and **6**). In both cases, the methylsulfenyl group was intact until the oxidation step and the presence of the group had no detrimental influence on the other reactions. To oxidize the sulfur atom, the

saturated 4-methylthiopyridine was allowed to react with 2.5 equimolecular amounts of mCPBA (adding mCPBA in four portions over a period of an hour at 0 °C, and stirring the solution at 0 °C for an hour and at room temperature for 2 hr) to afford 4-methylsulfonylpyridine **4a** (m=2, n=0; 26% yield)[12] and 4-methylsulfonylpyridine N-oxide **4b** (m=2, n=1; 66% yield). Meanwhile, the treatment of the 4-methylthiopyridine (**3**) with 2.4 equimolecular amounts of mCPBA over a period of 23 hr at -20 °C gave a mixture of **4a** (31%), **4b** (15%), **4c** (m=1, n=0; 24%) and **4d** (m=1, n=1; 25%). Since monitoring the reaction by thin layer chromatography (TLC) showed that sulfoxide **4c** was initially formed, it was thought that treating 4-methylthiopyridine with slightly less than an equimolecular amount of mCPBA would yield the methylsulfinylpyridine as a single product. Indeed, the unsaturated 4-methylthiopyridine (**5**), when allowed to react with a 0.94 equimolecular amount of mCPBA (by adding mCPBA in 5 portions over a period of 2.5 hr at -15 °C and stirring the solution at -15 °C for an additional hour) yielded the desired methylsulfinylpyridine **6** as a single product in a 96% yield. The literature indicated that the methylsulfinyl group of a pyridine ring can be replaced with an alkoxide and alkyl sulfide but not with a phenoxide.[10a] Therefore, **4a**, **4b**, and **4d**, the methylsulfonyl and methylsulfinyl groups of which are expected to be more reactive than those of **4c** and **6**, could also be used as a starting material if a phenoxide or the less nucleophilic and the more bulky alkoxide were used as a nucleophile.

Table1. Reaction of pyridine derivatives with various alcohols.



4, 6 **7**

7[a]	R	**4** or **6** (m, n)	Base	Conditions	Saturated or Unsaturated	Yield (%)[c]
a	2-PyCH_2-	**4a** (2, 0)	NaH	80°C, 4 h	sat.	90
b	^{t}BuCH_2-		^{t}BuOK	80°C, 3.5 h	sat.	84
c	3-Me-2-butenyl		NaH	80°C, 3.5 h	sat.	78
d	2-(HO)PhCH_2-		^{t}BuOK	100°C, 19.5 h	sat.	72
e	Ph	**4b** (2, 1)	^{t}BuOK	reflux, 17.5 h[b]	sat.	45
f	2-butynyl		^{t}BuOK	80°C, 1 h[b]	sat.	65
g	$(F_3C)_2$CH-		^{t}BuOK	60°C, 6 h	sat.	no reaction
h	c-HexCH_2-	**4c** (1, 0)	NaH	80°C, 8 h	sat.	68
i	Ph$O(CH_2)_2$-		NaH	80°C, 6 h	sat.	78
j	2-(HO)PhCH_2-	**6** (1, 0)	^{t}BuOK	80°C, 1.5 h	unsat.	60
k	2-(MeO)PhCH_2-		^{t}BuOK	80°C, 1.5 h	unsat.	32

a: All new compounds showed satisfactory 300 MHz ^{1}H NMR and HR FAB-MS spectra supporting the described structures; b: ^{t}BuOH was used as a solvent; c: Overall isolated yields were based on **4** or **6**, not optimized.

To confirm the reactivity of the leaving group and the stability of the other structures and functional

groups under the substitution conditions, **4c** was first treated with sodium methylmercaptide in ^{t}BuOH at temperature ranging from room temperature to 60 °C. The reaction occurred smoothly and the desired product was obtained in an 80% yield after deprotection of the TBDMS group. Next, the pyridines or pyridine N-oxide were treated with various alkoxides: **4a-d** and **6** were heated in an appropriate alcohol (1ml) in the presence of 8-10 equimolecular amounts of base such as ^{t}BuOK or NaH. When the alcohol could not be used as a solvent, the reaction was carried out in ^{t}BuOH or DMSO. After the usual work-up, the residue was treated with Bu_4NF in THF, or with $TiCl_3$ in THF if the pyridine N-oxide **4b** was used as a starting material (in this case, reduction of N-oxide and deprotection of the TBDMS group occur at the same time) to afford the desired 4-alkoxy- or 4-aryloxy-pyridine derivatives **7** in moderate-to-good yields as summarized in Table 1.

Alcohols possessing normal nucleophilicity were readily introduced into the pyridine (**7h** - **j**) using the 4-methylsulfinyl pyridine **4c** or **6** as a starting material. The alcohols with diminished nucleophilicity caused by increasing acidity (**7a**, **c** and **f**) or steric hindrance (**7b**)[13] were introduced effectively using the 4-methylsulfonylpyridine **4a** and the 4-methylsulfonyl pyridine N-oxide **4b** as starting materials. In contrast, the methylsulfinyl group of **4c** could not be replaced with 2-pyridylmethanol under the same conditions with complete recovery of **4c**. In the case of 2-methoxyphenylmethanol, the reduced nucleophilicity of the potassium alkoxide resulting from steric hindrance of the 2-methoxy group probably lowered the yield of **7k**, since using **4a** instead of **6** did not improve the yield of the corresponding saturated 4-(2-pyridylmethoxy)-pyridine. Using **4b** could conduct the phenoxy group into the ring (**7e**), a finding which is of interest when compared with the description in the literature.[10a] However, the methylsulfonyl group of **4b** could not be replaced by the highly acidic hexafluoroisopropanol. The double bond of **6** was intact during the replacement of the methylsulfinyl group with an alkoxide to afford the desired product.

Brief biological profiles of these prepared pyridine derivatives are summarized in Table 2. All prepared compounds showed moderate-to-high activities against H^+/K^+ ATPase using hog stomach membrane fractions as enzyme sources in the same manner described in our previous studies.[7,8] Since these derivatives bound to the potassium site of H^+/K^+ ATPase in a competitive manner, the inhibitory activity of the compounds depended upon the medium's pH conditions. The stability of the prepared enzyme fractions under highly acidic conditions did not permit us to carry out the enzyme assay below pH 6.4, but all of the prepared compounds showed more potent activities at pH 6.4 than at pH 7.4, as expected. These *in vitro* inhibitory profiles of the pyridine derivatives indicated that they would be very suitable for clinical use as antiulcer drugs because the target enzymes were distributed predominantly over the stomach in highly acidic conditions. Concerning individual substituents at the 4-position of a pyridine ring, the stronger electron-withdrawing groups decreased the enzyme inhibitory activities of the compounds (**7a**, **7e**, **7f**, **7i**). This meant that a more basic pyridine, that is, an easily protonated compound, competed more effectively with a proton and/or a potassium cation at the potassium site of the enzyme than a less basic pyridine. Among the obtained pyridines, **7b** and **7j** showed *in vitro* potency almost equal to **2**.

The *in vivo* efficacies of the selected compounds were evaluated using their inhibitory activities toward tetragastrin-induced acid secretion in acute fistula rats (iv dosing) or pylorus-ligated rats (id dosing).[7,8] The compounds **7b** and **7d** had highly inhibitory effects on gastric acid secretion in acute fistula rats with 69% and 77% inhibition respectively of the control level of acid secretion at 3 mg/kg iv dosing. These data suggested that **7b** and **7d** were slightly less active than **2** in this evaluation system. However, in the case of id dosing in pylorus-ligated rats, **7d** showed effect almost comparable to **2**, and **7b** showed greater efficacy than **2**.

The prepared pyridine derivatives possessed relatively hydrophobic profiles which rendered the compounds easily metabolized by first pass effects and/or less absorbable in id dosing. In the case of **7b**, replacement of a cyclohexyloxy group at the 4-position of the pyridine ring with a neopentyloxy group diminished its hydrophobicity, while in the case of **7d**, introducing an *ortho*-hydroxyphenylmethoxy group on the pyridine ring improved the hydrophilicity of the parent compound **2**. Improvement of the physicochemical properties of **7b** and **7d** (calculated log P values of **7b** (5.87) and **7d** (5.65) were less than that of **2** (6.50)) probably reduced their metabolic instability and changed their bioavailability. That is the reason why **2** showed a relatively large discrepancy between iv and id dosing whereas **7b** did not. Detailed pharmacokinetic studies of the representative compounds are now in progress.

In conclusion, we established an easy way to prepare various 4-alkylthio, 4-alkoxy or 4-aryloxypyridine derivatives using a 4-methylsulfenyl group as a masked leaving group. Although the yields of the substitution reaction have not been satisfactory to date, selecting the suitable oxidation stage of the starting material **4** or **6**, based on the nucleophilicity of the alcohols, made it possible to introduce an appropriate alkoxy or an aryloxy group into the 4-position of the pyridine effectively. All of the prepared compounds described in Table 1 showed expected reversible inhibitory activity against H^+/K^+-ATPase with IC_{50} values ranging from 0.5 to 30 μM at pH 6.4 (Table 2). Some of these compounds showed reasonable *in vivo* activities in an acute fistula rat or a pylorus-ligated rat model. Among the compounds in Table 2, **7b** was revealed as the most potent derivative in a pylorus-ligated rat model. Detailed structure-activity relationships of these derivatives and related analogues, and a further evaluation of the representative compounds will be published in due course.

Table 2. Biological profiles of 4-alkoxypyridine derivatives 7.

	H^+/K^+-ATPase	Acute fistula rats	Pylorus-ligated rats
	IC_{50} (μM) at pH 6.4	% inh. at 3 mg/kg iv	% inh. at 10 mg/kg id
2	0.5	83	39
7a	21% inh. at 4 μM	Not tested	Not tested
7b	0.6	69	56
7c	3.0	55	15
7d	1.8	77	33
7e	36% inh. at 10 μM	53*	24
7f	22% inh. at 4 μM	Not tested	Not tested
7h	3.1	31	Not tested
7i	27% inh. at 8μM	Not tested	Not tested
7j	0.9	55	18
7k	1.7	49	20

* This datum was % inhibition at 10 mg/kg iv, not 3 mg/kg iv.

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12. Spectral data of **4a** are as follows: ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): -0.04 (3H, s, Me of TBDMS), -0.02 (3H, s, Me of TBDMS), 0.85 (9H, s, ^{t}Bu of TBDMS), 2.63 (3H, s, Me of 5-Py), 2.87 (3H, s, Me of $MeSO_2$-), 3.41 (1H, dd, J = 8.3 Hz, 12.7 Hz, CHH'-OH), 3.49 (1H, m, CH of 2-Py), 3.69 (1H, dd, J = 5.3 Hz, 12.7 Hz, CHH'-OH), 3.95 (2H, d, J = 6.1 Hz, CH_2-Np), 7.09 (1H, m, aromatics), 7.26 (1H, m, aromatics), 7.38 (1H, s, Py-3), 7,46 (2H, m, aromatics), 7.67 (1H, d, J = 8.3 Hz, aromatics), 7.82 (1H, m, aromatics), 8.05 (1H, m, aromatics), 8.63 (1H, s, Py-6); IR (neat, cm^{-1}): 2932, 2860, 1476, 1320, 1254, 1149, 1107, 1068; High Resolution FAB-MS (m/e for $(C_{26}H_{35}NO_3SSi + H)^+$): Calcd: 470.2185; Found:470.2183.
13. Spectral data of a representative H^+/K^+ ATPase are as follows: **7b**: ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 0.97 (9H, s, CH_3 x 3 of neopentyl), 2.14 (3H, s, CH_3 of 4-Py), 3.10 - 3.16 (1H, m, CH of 2-Py), 3.12 (1H, d, J = 8.6 Hz, CHH' of neopentyl), 3.34 (1H, d, J = 8.6 Hz, CHH' of neopentyl), 3.46 (1H, dd, J = 7.8 Hz, 13.6 Hz, CHH'-OH), 3.60 (1H, dd, J = 7.2 Hz, 13.6 Hz, CHH'-OH), 3.93 (1H, dd, J = 4.5 Hz, 10.9 Hz, CHH'-Np), 4.06 (1H, dd, J = 2.8 Hz, 10.9 Hz, CHH'-Np), 6.02 (1H, s, Py-3), 7.02 (1H, d, J = 8.2 Hz, aromatics), 7.36 (1H, dd, J = 7.6 Hz, 8.2 Hz, aromatics), 7.48 (2H, m, aromatics), 7.70 (1H, d, J = 8.2 Hz, aromatics), 7.85 (1H, m, aromatics), 8.01 (1H, m, aromatics o), 8.16 (1H, s, Py-6); IR (neat, cm^{-1}): 3310, 2956, 2872, 1605, 1575, 1506, 1401, 1314, 1242, 1047, 1023; High Resolution FAB-MS (m/e for $(C_{24}H_{29}NO_2 + H)^+$): Calcd: 346.2277; Found: 364.2268.

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