matography over silica gel with ethyl acetate–hexane (1:1) produced a slower moving isomer that corresponded exactly to this material.

DL-**2-Amino-7-[bis(2-chloroethyl)amino]-1,2,3,4-tetrahydro-2-naphthoic Acid Hydrochloride Salt (6).** The hydantoin mustard **4b** (0.15 g) was dissolved in concentrated HCl and heated at 140 °C for 16 h in a sealed tube. The reaction mixture was evaporated in the cold and the residue dissolved in a small volume of 1 N HCl and extracted with ethyl acetate several times. The aqueous fraction was lyophilized to give 40 mg of the 7-isomer mustard **6** which was 87% pure by GC–MS when analyzed as a persilylated derivative: mass spectrum, m/z (relative intensity) 474 ($M^{\bullet+}$, 5.7), 459 (M – CH_3, 2.2), 431 (M – CH_3 – C_2H_4, 2.9), 402 (M – M_3Si + H, 3.7), 385 (M – Me_3SiNH_2, 1.9), 357 (M – Me_3SiO_2C, 100).

DL-**2-Amino-5-[bis(2-chloroethyl)amino]-1,2,3,4-tetrahydro-2-naphthoic Acid Hydrochloride Salt (7).** Following a similar procedure, the 5-isomer mustard **7** was isolated as a lyophilized powder, which was 92% pure by GC–MS when analyzed as a persilylated derivative: mass spectrum, m/z (relative intensity) 474 ($M^{\bullet+}$, 1.0), 459 (M – CH_3, 2.3), 431 (M – CH_3 – C_2H_4, 2.4), 402 (M – Me_3Si + H, 1.7), 357 (M – Me_3SiO_2, 100).

Transport Studies. Murine L1210 leukemia cells were grown in RPMI 1630 medium containing 16% heat-inactivated fetal calf serum and passaged every 2–3 days when cell densities approached 1×10^6 cells/mL. For experimental studies, cells were harvested from growth medium and washed twice in transport medium composed of, in mM, $CaCl_2 \cdot 2H_2O$ (0.7), $MgCl \cdot 6H_2O$ (0.5), choline chloride (125), HEPES (25), and salt-free bovine serum albumin (0.1). The final pH of the transport medium was 7.4. Cells were then incubated with 1.5 or 3.0 μM 2-aminobicyclo[2.2.1]heptane-2-carboxylic-^{14}C acid (BCH) along with the indicated concentration of either L-phenylalanine mustard or DL-2-amino-7-bis[(2-chloroethyl)amino]-1,2,3,4-tetrahydro-2-naphthoic acid (**6**). The initial rate of transport of BCH was terminated at 40 s by centrifugation of 1×10^6 cells through Versilube F-50 silicone oil. The cell pellets were solubilized in 0.2 N NaOH, acidified, and counted in a Packard 460 C liquid scintillation counter.

Evaluation of Antitumor and Myelosuppressive Activity. Murine L1210 leukemia cells were grown as described above under transport studies. Cells were harvested and washed twice in fresh growth medium. Bone marrow cells were removed from femurs of male CDF_1 mice and washed twice in RPMI 1630 containing 16% heat-inactivated fetal calf serum. A cell suspension containing *both* 100 tumor cells/mL and 100 CFU-C/mL (1.0×10^5 nucleated cells/mL) was prepared and the cells coexposed for 45 min to the respective drug in RPMI 1630 containing 16% heat-inactivated fetal calf serum. The cells were then harvested, washed twice in McCoy's 5A medium supplemented with 10% fetal bovine serum, 20 units/mL penicillin, and 20 μg/mL streptomycin. Cell survival was assessed following 1 week of growth in the same medium at 37 °C in a humidified atmosphere of 5% CO_2.[19] Experimental points represent the mean of three separate platings. Pregnant mouse uterine extract was used as a source of colony-stimulating factor for the bone marrow. The presence of either cell type has no effect on the plating efficiency of the other.

Determination of Alkylating Potency and Half-Life ($t_{1/2}$). The reaction of L-phenylalanine mustard and DL-2-amino-7-bis-[(2-chloroethyl)amino]-1,2,3,4-tetrahydro-2-naphthoic acid (**6**) with γ-(4-nitrobenzyl)pyridine[20] was used to determine both alkylating potency and stability of the drugs in aqueous solution containing physiological concentrations of chloride ion (Dulbecco's phosphate buffered saline). The drugs were made up as 65 mM stock solutions in 75% ethyl alcohol containing equimolar hydrochloric acid and diluted 200-fold into Dulbecco's phosphate buffered saline to initiate the study. One-milliliter aliquots were removed at time = 0, 7.5, 15, 60, and 135 min, the pH adjusted to 4.8 with 0.1 M sodium acetate, and residual alkylating activity determined exactly as described by Truhaut et al.[21] following reaction with γ-(4-nitrobenzyl)pyridine. Experimental points represent the mean of three separate determinations.

Acknowledgment. We thank Dr. James A. Kelley from this laboratory for the mass spectra determinations. We are also indebted to Yetta Buckberg for her valuable assistance in typing this manuscript.

Registry No. **1**, 6270-37-7; **2**, 106094-73-9; **3**, 106094-74-0; **4a**, 106094-75-1; **4a** (monoamine), 106094-81-9; **4b**, 106094-79-5; **5a**, 106094-76-2; **5a** (monoamine), 106094-82-0; **5b**, 106094-80-8; **6**, 106094-83-1; **6**·HCl, 106094-77-3; **7**, 106114-32-3; **7**·HCl, 106094-78-4; ethylene oxide, 75-21-8.

(19) Foster, R. S.; MacPherson, B. R.; Browdie, D. A. *Cancer Res.* **1977**, *37*, 1349.
(20) Epstein, J.; Rosenthal, R. W.; Ess, R. J. *Anal. Chem.* **1955**, *27*, 1435.
(21) Truhaut, R.; Delacoux, E.; Brule, G.; Bohuon, C. *Clin. Chim. Acta* **1963**, *8*, 235.

2-[(3-Pyridinylmethyl)thio]pyrimidine Derivatives: New Bronchosecretolytic Agents

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Received November 18, 1985

2-[(3-Pyridinylmethyl)thio]pyrimidine derivatives (**1a–n**) promote the excretion of phenol red into the mouse trachea, indicating an increased tracheobronchial secretion. Furthermore, 2-[(3-pyridinylmethyl)thio]pyrimidine (**1a**) (tasuldine) produces greater excretion of phenol red into the mouse trachea after systemic administration than the known bronchosecretolytic ambroxol. Compound **1a** also reduces the viscosity of canine bronchial mucus. Compound **1a** has been selected for clinical investigations.

In some diseases of the respiratory tract, mucus secretion is disturbed and the abnormal secretion often aggrevates the illness.[1] Therefore, much effort has gone into designing forms of treatment that change the quantity or quality of tracheobronchial mucus. In general, expectorants can be devided in two groups.[2] The first, the so-called mucolytics such as acetylcysteine[1] and 2-mercaptoethanesulfonate,[1] is capable of reducing sputum viscosity after local application (e.g., inhalation) by breaking disulfide bridges that link strands in mucus glycoproteins. The second group, the so called broncho-

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(2) Brune, K; Szelenyi, I. In *Lehrbuch der Pharmakologie*; Estler, C.-J., Ed.; Schattauer-Verlag: Stuttgart, 1983; p 217.

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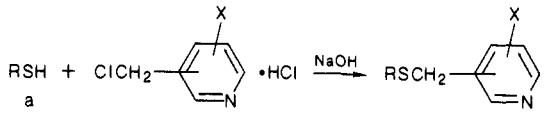
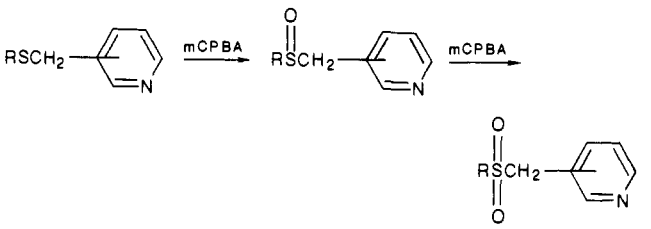
Scheme I



Scheme II



secretolytics, is effective after systemic administration. Most of these drugs act, however, by vagal reflexes; examples include potassium iodide,[3] emetine,[4] ammonium salts,[4] and guaiacol.[4] On the other hand, the mechanism of the bronchosecretolytic agent such as bromhexine[5] and ambroxol[6] is still unknown. They do not appear to depend on reflex or nervous mechanism. The clinical efficacy of these drugs, however, remains controversial.[1]

Our aim was to develop new bronchosecretolytics that change the viscoelasticity of the respiratory mucus after systemic administration, resulting in an improvement of the clinical status.

Synthesis

Compounds **1a–4b**[7] may be produced by a process utilizing known methods in which a mercaptan corresponding to the general formula **a** as described in Scheme I is reacted with an appropiate chloromethyl heterocycle such as 3-picolyl chloride. The reaction is best carried out by dissolving the mercaptan in an aqueous–ethanolic sodium hydroxide solution in which the sodium hydroxide is present preferably in at least twice the equimolar quantity and subsequently reacting the resulting solution at room temperature for 4 h with an equimolar quantity of the (chloromethyl)pyridine hydrochloride compound dissolved in aqueous–ethanolic solution. Because the sodium hydroxide is used in a stoichiometric excess, the free base is obtained and may be subsequently converted into a therapeutically acceptable salt in the usual way by reaction with a pharmaceutically acceptable acid. For extended structure–activity-relationship studies and for metabolic questions it was necessary to oxidize some (pyridinylmethyl)thio derivatives with *m*-chloroperbenzoic acid in methylene chloride at room temperature to the corresponding sulfinyl and sulfonyl compounds **5a–i**, as described in Scheme II.[8]

Biological Results and Discussion

Mouse Assay. To compare the bronchosecretory activity of the compounds, phenol red concentration in the trachea was used as marker for tracheobronchial secretion. The method of this assay is described in the Experimental Section. Within 30 min, the basal secretion of phenol red into the trachea was 0.61 ± 0.32 μg/mL of wash fluid. Carbachol (0.03–0.125 mg/kg sc) had a significant effect on tracheal phenol red output in a dose-dependent manner.

Table I. Effect of Carbachol, Norfenefrine, and Salbutamol on Intratracheal Phenol Red Concentration

drug	administration form	ED_{50} (50% increase in phenol red concentration compared to basal secretion)[a]
carbachol	subcutaneous	0.2 (0.16–0.24) μmol/kg
norfenefrine	subcutaneous	1.3 (1.0–1.5) μmol/kg
salbutamol	intragastric	12.5 (9.8–14.4) μmol/kg
ambroxol	intragastric	603.21 (597.62–613.46) mmol/kg

[a] ED_{50} values were calculated by linear regression analysis; see test method C;[9] the 95% confidence limits are in parentheses.

After maximum stimulation the phenol red concentration was increased to 105% compared to basal secretion. Norfenefrine also stimulated intratracheal phenol red secretion. Maximal phenol red concentration was comparable with that found after carbachol stimulation. Following salbutamol stimulation, however, the maximum phenol red output was significantly lower than that which was observed, following carbachol or norfenefrine stimulation. The secretagogue effect was blocked by the appropriate antagonists. The ED_{50} values for the reference compounds are shown in Table I.

In the mouse assay the test compounds **1a–5i** and ambroxol were active. The effect on tracheal phenol red secretion did not depend on reflex or nervous mechanisms since it was not specifically inhibited by anticholinergic or sympatholytic drugs (data not shown). As shown in Table II, 2-[(3-pyridinylmethyl)thio]pyrimidine (**1a**, hydrochloride and succinate salt) is distinguished by a pronounced secretory activity. Its effect in promoting the secretion of phenol red into the mouse trachea was approximately 120 times stronger than that of the known bronchosecretolytic ambroxol.

In the mouse assay the increased phenol red corresponds to an increased hexose concentration in the fluid.[9] This indicates a stimulated mucin secretion. But taking into consideration that α-adrenergic and cholinergic agonists only increase water and mucin secretion while β-adrenergic agonists only increase mucin secretion[10–12] and due to the fact that phenol red secretion is more enhanced by carbachol and norfenefrine than by salbutamol, it can be concluded that phenol red is capable of acting as a marker for both mucin and water secretion.

Various substituents, methyl, amino, or hydroxy groups, at position 4 (**1b**, **1d**, **1e**) or at both position 4 and 6 (**1c**, **1g**) of the pyrimidine ring led to a reduction of bronchosecretolytic potency. The exceptions were the 4-OH, 6-NH_2 compounds (**1f**, **1m**), which had activity comparable to that of **1a**.

Substitution with lipophilic groups such as methyl at the mercaptomethylene bridge (**1i**) and/or with methyl or chloro in position 2 or 6 at the pyridine ring (**1j**, **1k**, **1l**, **1n**) caused an evident reduction of biological activity. But with the introduction of two more polar groups, e.g., hydroxy and amino groups, into compound **1k** (resulting in **1m**), the bronchosecretolytic potency is again comparable

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(4) Perry, W. F.; Boyd, E. M. *J. Pharmacol. Exp. Ther.* **1941** *73*, 65.
(5) Dr. Karl Thomae GmbH, D-7950 Biberach DE-AS 1 169 939.
(6) Dr. Karl Thomae GmbH, D-7950 Biberach DE-PS 1 593 579.
(7) Heumann-Pharma, D-8500 Nuremberg EP Application 0 093 252.
(8) Heumann-Pharma, D-8500 Nuremberg EP Application 0 124 630.
(9) Engler, H.; Szelenyi, I. *J. Pharmacol. Methods* **1984**, *11*, 151.
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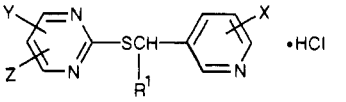
(11) Phipps, R. J.; Nadel, J. A.; Davis, B. *Am. Rev. Respir. Dis.* **1980**, *121*, 359.
(12) Borson, D. B.; Chinn, R. A.; Davis, B.; Nadel, J. A. *J. Appl. Physiol.* **1980**, *49*, 1027.

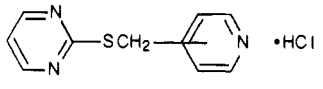
Table II. Influence of 2-[(3-Pyridinylmethyl)thio]pyrimidine Derivatives and Ambroxol on the Tracheal Secretion of Phenol Red in the Mouse



compd[a]	X	Y	Z	R^1	yield, %	formula	mp, °C	ED_{50},[e] mmol/kg
1a	H	H	H	H	45	$C_{10}H_{10}ClN_3S$	134–135	5.34 (4.98–5.62)
	H	H	H	H	60	$C_{10}H_9N_3S$[b]	53	4.82 (4.26–5.20)
	H	H	H	H	55	$C_{14}H_{15}N_3O_4S$[c]	97–98	4.67 (4.08–5.26)
1b	H	4-CH_3	H	H	43	$C_{11}H_{12}ClN_3S$	154	39.40 (38.31–40.76)
1c	H	4-CH_3	6-CH_3	H	38	$C_{12}H_{14}ClN_3S$	155–156	44.81 (43.0–46.32)
1d	H	4-NH_2	H	H	57	$C_{10}H_{10}N_4S$[b]	119–120	22.90 (21.73–24.28)
1e	H	4-OH	H	H	32	$C_{10}H_9N_3OS$[b]	167–168	456.0 (454.70–459.32)
1f	H	4-OH	6-NH_2	H	57	$C_{10}H_{10}N_4OS$[b]	210–211	8.93 (7.54–10.24)
1g	H	4-NH_2	6-NH_2	H	43	$C_{14}H_{17}N_5O_4S$[c]	149–150	272.18 (269.34–276.30)
1h	H	4-OH	6-CH_3	H	68	$C_{11}H_{11}N_3OS$[b]	168–169	64.30 (62.49–67.0)
1i	H	H	H	CH_3	31	$C_{14}H_{14}N_3O_6S$[d]	115–116	42.60 (40.97–43.23)
1j	6-CH_3	H	H	H	37	$C_{15}H_{17}N_3O_4S$[c]	110–111	298.20 (296.14–301.43)
1k	6-Cl	H	H	H	42	$C_{10}H_8ClN_3S$[b]	89–90	84.14 (82.76–83.62)
1l	2-Cl	H	H	H	64	$C_{10}H_8ClN_3S$[b]	96–97	84.14 (81.90–84.23)
1m	6-Cl	4-OH	6-NH_2	H	41	$C_{10}H_9ClN_4OS$[b]	227–228	8.30 (7.16–9.03)
1n	6-CH_3	H	H	CH_3	58	$C_{12}H_{13}N_3S$[b]	oil	432.30 (422.7–461.8)
ambroxol								603.21 (597.62–613.46)

[a] Synthetic method A. [b] Free base. [c] Isolated as succinate salt. [d] Isolated as racemate 1.5-oxalate salt. [e] ED_{50} values were calculated by linear regression analysis; see test method C;[9] route of administration po; the 95% confidence limits are in parentheses.

Table III. Effect of 2-[(Pyridinylmethyl)thio]pyrimidine Derivatives on the Tracheal Secretion of Phenol Red in the Mouse after Oral Administration



compd[a]	yield, %	formula	mp, °C	ED_{50},[d] mmol/kg po
2a[b]	60	$C_{10}H_{10}ClN_3S$	163–164	1668.80 (1660.20–1672.40)
2b[c]	43	$C_{10}H_{10}ClN_3S$	170–173	41.72 (39.26–42.41)

[a] Synthetic method A. [b] Substituted in position 2 of the pyridine ring. [c] Substituted in position 4 of the pyridine ring. [d] See test method C;[9] the 95% confidence limits are in parentheses.

to that of **1a**. No preference for a substitution pattern to reach the activity of **1a**, could be observed.

A clear loss of bronchosecretolytic activity is also obtained by changing the linkage of the mercaptomethylene chain to the positions 4 and especially 2 of the pyridine ring (Table III).

Further, compounds in which the pyrimidine ring (i.e., R) is exchanged for benzoxazole or 2-pyridine rings and the mercaptomethylene chain is bonded in position 2 of the pyridine ring (Table IV) are also less bronchosecretolytically active than **1a**. In general, exchange of the pyrimidine ring for substituted or unsubstituted aryl groups, five- or six-membered heterocyclic group which may be condensed with a substituted phenyl group or with substituted heterocycles leads to strongly diminised secretolytic activities in the phenol red excretion model of the mouse. Only **4e** and **4d** appeared to be exceptions (Table V).

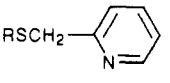
Oxidation of the sulfur atom in the connecting link to sulfinyl and sulfonyl derivatives also resulted in less active substances (Table VI). Only compound **5** had similar activity to that of the parent compound **1a**.

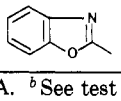
Canine Bronchial Mucus. Consequently some of the most active compounds were selected to test their action on the physical properties of the canine bronchial mucus. Table VII shows that these compounds (**1a,f,m**) produced a reduction in the viscosity of the canine bronchial mucus. This process was not blocked by cholinergic or sympathomimetic antagonists. The mechanism that leads to the reduced viscosity, however, is not yet clear. Further studies including possible changes in the chemical composition of the mucus must follow. These compounds, however, proved to have bronchosecretolytic activity in the dog.

Conclusion

The pharmacological studies reported here show that the 2-[(3-pyridinylmethyl)thio]pyrimidine derivatives have superior bronchosecretolytic properties in the phenol red screening model of the mouse in comparison to the known and therapeutically widely used drug ambroxol. Compounds **1a–n** exhibited bronchosecretolytic activities in the phenol red screening model of the mouse. Compound **1a** (isolated and tested as the hydrochloride or succinate salt) proved to have the greatest activity and, after systemic

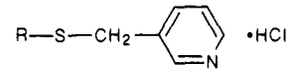
Table IV. Effect of 2-[(Pyridinylmethyl)thio] Derivatives on the Tracheal Secretion of Phenol Red in the Mouse

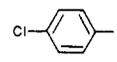
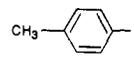
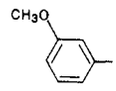
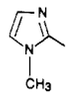
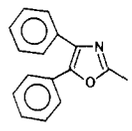
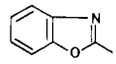
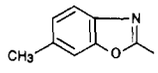
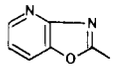
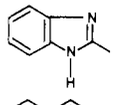
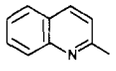
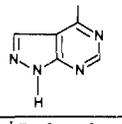


compd[a]	R	yield, %	formula	mp, °C	ED_{50},[b] mmol/kg po
3a		28	$C_{11}H_{10}N_2S \cdot 2HCl$	106–108	21.82 (18.74–24.01)
3b		43	$C_{13}H_{10}N_2OS \cdot {}^1/_2(COOH)_2$	98–102	156.62 (151.39–161.78)

[a] Synthetic method A. [b] See test method C;[9] the 95% confidence limits are in parentheses.

Table V. Influence of Various Substituents of 3-[(Pyridinylmethyl)thio] Derivatives on the Tracheal Secretion of Phenol Red in the Mouse



compd[a]	R	yield, %	formula	mp, °C	ED_{50},[d] mmol/kg po (confidence limits)
4a		68	$C_{12}H_{11}Cl_2NS$	152–153	22.04 (20.93–23.78)
4b		47	$C_{13}H_{14}ClNS$	148–149	39.71 (37.82–41.63)
4c		56	$C_{13}H_{14}ClNOS$	130–131	181.70 (183.12–192.41)
4d		25	$C_{11}H_{12}Cl_2N_2S$[b]	149–152	7.63 (6.24–9.17)
4e		43	$C_{10}H_{15}Cl_2N_3S$[b]	187–189	5.35 (4.46–6.28)
4f		47	$C_{21}H_{17}ClN_2OS$	156–157	13.13 (11.81–15.27)
4g		56	$C_9H_{11}ClN_2S_2$	130–131	20.26 (18.43–23.10)
4h		46	$C_{13}H_{10}N_2OS$[c]	88	61.90 (55.73–70.10)
4i		63	$C_{14}H_{13}ClN_2OS$	182–183	51.23 (47.81–53.99)
4j		55	$C_{12}H_{11}Cl_2N_3OS$[b]	183–185	37.96 (36.02–38.95)
4k		58	$C_{13}H_{13}Cl_2N_3S$[b]	211–213	31.83 (29.11–33.64)
4l		52	$C_{15}H_{14}Cl_2N_2S$[b]	173–175	21.52 (18.26–24.73)
4m		66	$C_{11}H_9N_5S$[c]	148–149	41.10 (37.90–44.11)

[a] Synthetic method A. [b] Isolated as dihydrochloride. [c] Isolated as free base. [d] See test method C.[9]

administration, produced the greatest reduction in the viscosity of the canine bronchial mucus. According to our present results, 2-[(3-pyridinylmethyl)thio]pyrimidine derivatives represent a new structural class of broncho-secretolytic drugs that are different from known structures of bronchosecretolytic and mucolytic agents. Compound **1a** was selected for clinical investigations.

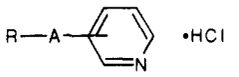
Experimental Section

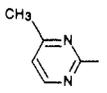
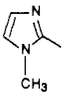
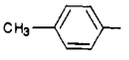
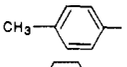
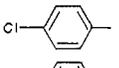
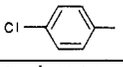
Melting points were determined by means of a Mettler FP5 melting point apparatus and are uncorrected. Infrared spectra were recorded as KBr disks on a Perkin-Elmer Model 257 spectrophotometer. ^1H NMR spectra were run on a Varian EM 390 spectrometer either in $CDCl_3$ or Me_2SO-d_6 tetramethylsilane as the internal standard. All compounds had NMR and IR spectra consistent with their structures and gave satisfactory C, H, and N analyses (± 0.3% of the calculated values).

A. General Synthetic Methods for the Preparation of (Pyridinylmethyl)thio Derivatives. A 210-mmol sample of sodium hydroxide dissolved in 120 mL of water is added dropwise at room temperature to a solution of 100 mmol of mercaptan in 250 mL of ethanol. A 100-mmol sample of the picolyl chloride hydrochloride dissolved in 100 mL of water is then slowly added at 25 °C, followed by stirring for 4 h at room temperature. The reaction solution is concentrated in vacuo and taken up in 500 mL of ether, the organic phase is washed three times with 100 mL of water, dried over sodium sulfate, and filtered, and the filtrate is concentrated in vacuo. The solid accumulating, which represents the base, was treated with ethereal hydrogen chloride to give the product as the hydrochloride salt.

B. General Synthetic Methods for the Preparation of (Pyridinylmethyl)sulfinyl (a) and (Pyridinylmethyl)-sulfonyl (b) Derivatives. A 18.1-g (110 mmol) sample (**a**) or 36.2-g (220 mmol) sample (**b**) of *m*-chloroperbenzoic acid dissolved in 300 mL (or 600 mL) of methylene chloride is added dropwise in 10–15 min at room temperature to a solution of 100 mmol of 3-[(pyridinylmethyl)thio] derivative (base) in 500 mL of methylene chloride. After a reaction time of 15 min (**a**) or 30 min (**b**), 120 mL of a 3% sodium hydrogen carbonate solution is added to the reaction solution and the methylene chloride phase is separated off. The organic phase is washed with water until neutral, dried over sodium sulfate, and concentrated in vacuo. A 200-mL sample of an equimolar solution of HCl in ethanol is added to the base accumulating, and then the hydrochloride is precipitated with ether. The (pyridinylmethyl)sulfinyl (**a**) or (pyridinlymethyl)-

Table VI. Effect of (Pyridinylmethyl)sulfinyl and (Pyridinylmethyl)sulfonyl Derivatives on the Tracheal Secretion of Phenol Red in the Mouse



compd[a]	R	A[b]	yield, %	formula	mp, °C	ED_{50},[e] mmol/kg po
5a		$SOCH_2$ (3)	32	$C_{10}H_{10}ClN_3OS$	168–169	7.82 (5.91–9.17)
5b		$SOCH_2$ (4)	30	$C_{10}H_{10}ClN_3OS$	165–166	391.10 (384.23–401.86)
5c		$SOCH_2$ (3)	35	$C_{13}H_{13}N_3O_5S$[c]	132–134	30.94 (26.73–33.86)
5d		$SOCH_2$ (3)	35	$C_{14}H_{15}N_3O_9S$[d]	135–136	49.83 (46.92–51.73)
5e		SO_2CH_2 (3)	57	$C_{14}H_{15}N_3O_{10}S$[d]	154–156	95.83 (93.28–98.11)
5f		$SOCH_2$ (3)	46	$C_{13}H_{14}ClNOS$	184–185	149.42 (144.16–153.22)
5g		SO_2CH_2 (3)	57	$C_{13}H_{14}ClNO_2S$	190–191	140.90 (132.81–148.96)
5h		$SOCH_2$ (3)	41	$C_{14}H_{12}ClNO_5S$[c]	181–184	43.90 (40.27–46.82)
5i		SO_2CH_2 (3)	67	$C_{14}H_{12}ClNO_6S$[c]	195–197	27.91 (24.76–30.72)

[a] Synthetic method B. [b] Right end of side chain A is linked to the pyridine in position described. [c] Isolated as oxalate salt. [d] Isolated as dioxolate salt. [e] See test method C.[9]

Table VII. Influence of **1a**, **1f**, and **1m** on the Viscosity of Canine Bronchial Mucus[a]

compounds	dose, mg/kg iv	% of the initial viscosity (=100%) 1 h after compound administration ($x \pm SD$)
control 0.9% NaCl		116.2 ± 23.6
1a·HCl	0.5	5.5 ± 3.0
	0.2	28.0 ± 16.0
	0.1	109.6 ± 38.8
1f	0.5	15.8 ± 2.6
	0.2	76.3 ± 22.4
1m	0.5	19.7 ± 8.2
	0.2	84.2 ± 10.6

[a] The viscosity of the mucus before drug application ranged between 2.61 and 29.58 mPa·s[b] and was taken as 100% compared to the corresponding viscosity 1 h after drug application. [b] Unit of measurement for dynamic viscosity; dimension mPa·s or $(N \cdot s/m^2) \times 10^{-3}$.

sulfonyl (**b**) derivatives are recrystallized from 2-propanol.

Biological Testing Methods. Compound Preparation, Administration, and Animals. Compounds for screening investigations were suspended by homogenization in a 0.5% (carboxymethyl)cellulose vehicle just prior to use. Control vehicle or compound suspensions were administered by gavage at a volume of 5 mL/kg to male mice (NMRI, Fa. Savo, Kisslegg, West Germany). The animals were deprived of food 24 h prior to dosing but allowed free access to water.

For extended studies, mongrel dogs weighing 18–24 kg were used. After deprivation of food (16 h prior to experiments), they were anesthetized by sodium phenobarbiturate (Nembutal, Ceva AG, Paris, France) (40 mg/kg iv and an additional infusion of 0.1 mg kg^{-1} min^{-1}). Test compounds were prepared in isotonic NaCl solution and given intravenously. Isotonic solution (0.9% w/v NaCl) served as a control.

C. Excretion of Phenol Red into the Trachea of the Mouse. The method used has been described elsewhere.[9] Briefly, the phenol red given intraperitoneally was measured photometrically in the tracheal lavage fluid of the mouse 50 min after oral administration of test compounds. Changes in basal phenol red output were calculated by comparing mucus output during stimulus with the means of that of control mice. The ED_{50} (dose with 50% increase of tracheal phenol red concentration compared to the control) was evaluated by using linear regression. The 95% confidence limits are in parentheses.

D. Measurement of the Viscosity of Canine Bronchial Mucus. Samples of bronchial mucus were taken by a bronchoscope (Model FB-170, Pentax AG, Hamburg, West Germany). To eliminate electrolytes occurring in mucus, samples were dialyzed against water at 0 °C over 24 h. After dialyzation and freeze-drying, the mucus samples were taken up in 0.1 M Tris-buffer (pH 7) (final concentration 1% w/v). After continuous stirring over 24 h, the viscosity of the samples was determined by a rotary viscometer (Rheoscan low shear-30, Contraves AG, Zurich, Switzerland).