Inactivation of Leukocyte Elastase by Aryl Azolides and Sulfonate Salts. Structure–Activity Relationship Studies

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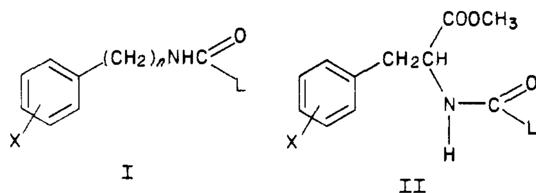
The inhibitory activity of a series of aryl azolides and sulfonate salts toward human leukocyte elastase is reported. Several of the compounds were found to be potent inhibitors of the enzyme. Active compounds were obtained only when the specificity group and the reactive moiety were separated by a two-carbon chain. The introduction of hydrophobic groups enhanced the inhibitory activity of these compounds, with the exception of the sulfonate salts. The nature of the leaving group had a profound effect on inhibitory activity, with compounds **23** and **26** being the most active ($k_{obsd}/[I]$ = 11 722 and 13 500 M^{-1} s^{-1}, respectively).

Human leukocyte elastase (HLE) has attracted considerable attention in recent years because of its likely involvement in the pathogenesis of pulmonary emphysema.[1] We have recently shown that latent isocyanates derived from appropriate amino acid esters, such as azolides[2] and sulfonate salts,[3] function as potent and selective inhibitors of HLE. We have extended our studies in this area by examining the inhibitory activity of several related compounds represented by structures I and II, in order to probe the makeup of the active site of HLE and to ascertain the structural features of the inhibitors that result in optimum inhibitory activity. Specifically, we have sought to determine the effect of substitution (X), chain length (*n*), and the nature of group L on inhibitory activity toward HLE. The results of our studies are presented herein.



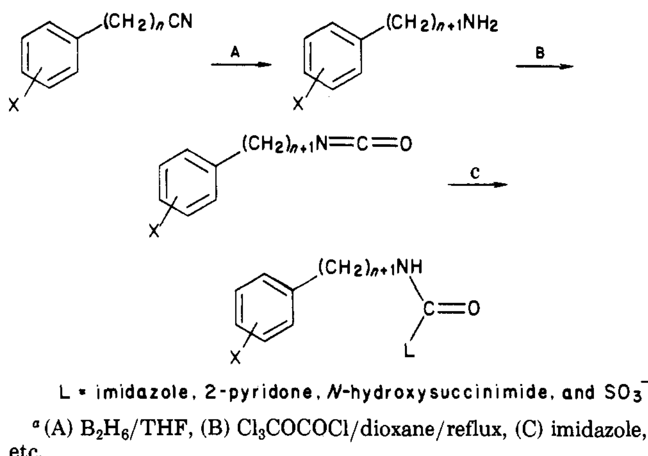
Chemistry. The compounds listed in Tables I and II were synthesized according to Schemes I and II, respectively. The reaction of the appropriate isocyanate with potassium metabisulfite in aqueous dioxane, imidazole, or other nucleophilic species yielded the desired compounds in high yields.

Results and Discussion

Compounds I and II were found to inactivate human leukocyte elastase irreversibly and in a time-dependent fashion (Figure 1). Substantial variations in inhibitory activity were observed upon changing the nature of X, L, and *n*.

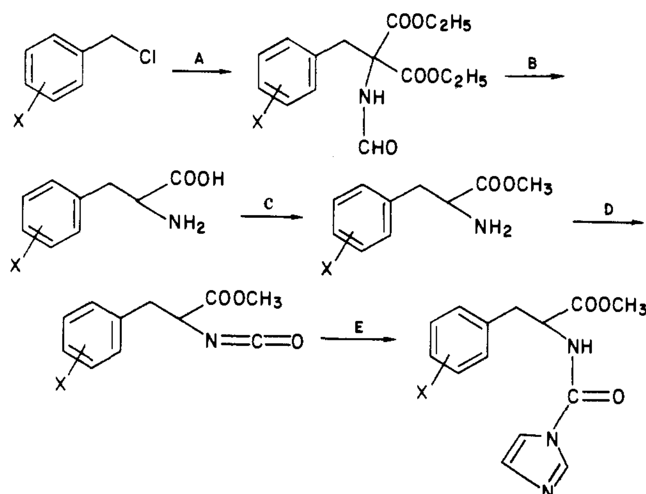
(a) Length of Spacer Group (*n*). Active compounds were obtained only when $n = 2$. No inhibition is observed when *n* is 0 or 1, irrespective of the nature of group L and substituent X. Assuming that the aromatic ring binds to the S_1 subsite of HLE, the reactive moiety NHCOL in the inactive compounds is probably located too far away from the catalytic residues for reaction to take place.

Scheme I[a]



[a] (A) B_2H_6/THF, (B) $Cl_3COCOCl$/dioxane/reflux, (C) imidazole, etc.

Scheme II[a]



[a] (A) NaH/THF/diethyl formamidomalonate, (B) H_2O/H^+, (C) $SOCl_2/CH_3OH$, (D) ClCOCl/toluene or TCF/dioxane, (E) imidazole, etc.

(b) Effect of Group L. As anticipated, the nature of group L had a profound effect on inhibitory activity (Table I, compounds **16**, **18**, **21**, and **24** and Table II, compounds **27–30**). Although no attempt was made to investigate the scope of the inhibition in relation to group L, it is clear that a diversity of latent isocyanates can function as inhibitors of HLE. This is highly desirable since group L is released into the surrounding milieu. Pulmonary emphysema is characterized by lung tissue destruction as well as acute inflammation and elevated levels of endogenous and exogenous oxidants.[4,5] Thus, it should be possible to

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Table I. Inhibition of Leukocyte Elastase by Azolides, Sulfonate Salts, and Other Related Compounds

$(CH_2)_nNHCOL$, X

I

compd[a]	mp, °C	X	n	L	$k_{obsd}/[I]$, $M^{-1}\,s^{-1}$
1	200 dec	H	0	SO_3^-	inactive[b]
2	225 dec	*m*-Cl	0	SO_3^-	inactive[b]
3	260 dec	*p*-Cl	0	SO_3^-	inactive[b]
4	200 dec	*m*-F	0	SO_3^-	inactive[b]
5	205 dec	*o*-CF_3	0	SO_3^-	inactive[b]
6	186–188	*m*-CF_3	0	SO_3^-	inactive[b]
7	165 dec	*p*-CF_3	0	SO_3^-	inactive[b]
8	107–108	*m*-F	0	imidazole	inactive[b]
9	162–164	*o*-CF_3	0	imidazole	inactive[b]
10	80–82	*m*-CF_3	0	imidazole	inactive[b]
11	130–132	*p*-CF_3	1	imidazole	inactive[b]
12	240 dec	*m*-F	1	SO_3^-	inactive[b]
13	103–106	*m*-CF_3	1	SO_3^-	inactive[b]
14	102–104	*m*-F	1	imidazole	inactive[b]
15	84–86	*m*-CF_3	1	imidazole	inactive[b]
16	270 dec	H	2	SO_3^-	4
17	265 dec	*m*-F	2	SO_3^-	18
18	101–103 dec	H	2	imidazole	35
19	106–108	*m*-F	2	imidazole	38
20	85–87	*m*-CF_3	2	imidazole	483
21	52–53	H	imidazole	2-pyridone	500
22	76–78	*m*-F	imidazole	2-pyridone	317
23	64–66	*m*-CF_3	imidazole	2-pyridone	11722
24	138–139	H	imidazole	*N*-hydroxysuccinimide	927
25	107–109	*m*-F	imidazole	*N*-hydroxysuccinimide	823
26	96–98	*m*-CF_3	imidazole	*N*-hydroxysuccinimide	13500

[a] All compounds gave correct elementary analyses. [b] No inhibition was observed when 243-fold excess of inhibitor over enzyme and a 10-min incubation time were used under comparable conditions.

Table II. Inhibition of Leukocyte Elastase by Derivatives of Phenylalanine

NHCO-L, X, $COOCH_3$

II

compd[a]	mp, °C	X	L	$k_{obsd}/[I]$, $M^{-1}\,s^{-1}$
27	127–130	H	SO_3^-	550[b]
28[a]	*c*	H	imidazole	2070
29[a]	*c*	H	2-pyridone	2329
30[a]	*c*	H	*N*-hydroxysuccinimide	6635
31	255 dec	*m*-F	SO_3^-	57
32	210 dec	*m*-CF_3	SO_3^-	13
33	*c*	*m*-F	imidazole	318
34	*c*	*m*-CF_3	imidazole	1096

[a] All compounds gave correct elementary analyses. [b] Compounds derived from L-phenylalanine methyl ester. The remainder of the compounds were made from DL-*m*-fluoro- and DL-*m*-(trifluoromethyl)phenylalanine methyl esters. [c] Oil.

develop compounds that have high inhibitory activity as well as the ability to release an antioxidant and/or an antiinflammatory agent during the inactivation process. We have observed that simple sulfonate salts such as $RNHCOSO_3^-\,K^+$ where R is *n*-propyl, *n*-butyl, *tert*-butyl, *n*-hexyl, cyclohexyl, *m*-chlorophenyl, and *p*-chlorophenyl are devoid of any inhibitory activity under comparable conditions.

(c) Effect of X. Leukocyte elastase is a serine protease that shows an affinity for hydrophobic substrates.[6] Thus,

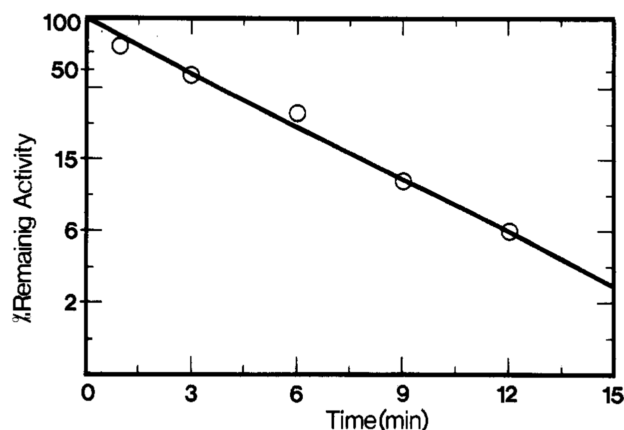

Figure 1. Time-dependent inactivation of leukocyte elastase by inhibitor **23**. The enzyme (34 nM) was incubated with the inhibitor (340 nM) in 0.1 M Tris-buffer, pH 7.2.

we have sought to enhance the inhibitory activity of these compounds through the use of hydrophobic substituents. The presence of the CF_3 group resulted in a marked increase in the inhibitory activity of compounds **20**, **23**, and **26** (Table I). The magnitude of the enhancement in compounds **23** and **26** is indicative of the involvement of other factors besides hydrophobicity. Studies carried out by Bieth[7-13] and others[14] have shown that inhibitors bearing

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a CF_3CO group exhibit enhanced binding toward HLE and porcine pancreatic elastase (PPE). The highly hydrophobic trifluoroacetyl group has been suggested to bind to a unique binding pocket that is close to the S_1 subsite. Thus, an alternative mode of binding, with proper juxtaposition of the reactive moiety in the inhibitor and the catalytic residues, is possible in this series of compounds. Compounds **23** and **26** are two of the most potent inhibitors that have ever been reported for leukocyte elastase. The $k_{obsd}/[I]$ values of these compounds compare very favorably with those reported for peptidyl chloromethyl ketones (1560 M^{-1} s^{-1})[15] and arenesulfonyl fluorides (2300 M^{-1} s^{-1}).[14] In sharp contrast, the introduction of the CF_3 group resulted in a drastic reduction in the inhibitory activity of the sulfonate salts (Table II, compounds **27** and **32**). This is probably the result of nonproductive binding.[14] Surprisingly, the phenylalanine and substituted phenylalanine compounds such as **29** and **30** (Table II) were less active than the corresponding achiral compounds (**23** and **26**, Table I). The converse was observed in the case of imidazole and the sulfonate salts. Compound **27** was as active as some of the previously reported[3] amino acid derived sulfonate salts and is currently being used as a prototype for the development of inhibitors of cathepsin G. None of the active compounds showed any inhibitory activity toward PPE.

In summary, these results confirm our earlier findings,[2,3] namely, that latent isocyanates can function as inhibitors of leukocyte elastase, and demonstrate that a variety of blocked isocyanates can be used. The flexibility afforded by this approach portends well as far as the development of chemotherapeutic agents for emphysema is concerned.

Experimental Section

Melting points were recorded on a Mel-Temp apparatus and are uncorrected. The infrared and proton NMR spectra were recorded with a Perkin-Elmer 1330 infrared spectrophotometer and a Hitachi Perkin-Elmer R-24B NMR spectrometer, respectively. (*tert*-Butoxycarbonyl)alanyl-*p*-nitrophenol and (methoxysuccinyl)-Ala-Ala-Pro-Val-*p*-nitroanilide were purchased from Sigma Chemical Co. Leukocyte elastase was obtained from Elastin Products, Co., St. Louis. A Gilford UV/visible "Response" spectrophotometer was used in the enzyme assays and inhibition studies.

Synthesis. Representative syntheses are described below.

Diethyl (*m*-(Trifluoromethyl)benzyl)formamidomalonate (35). A solution of diethyl formamidomalonate (24.4 g, 0.12 mol) in 50 mL of dry toluene was added to a slurry of sodium hydride (5.2 g, 0.13 mol) in 5 mL of toluene under nitrogen with stirring. *m*-(Trifluoromethyl)benzyl chloride (23.35 g, 0.12 mol) was then added and the reaction mixture was refluxed for 5 h. After the mixture was cooled to room temperature, 10 mL of absolute ethanol was added and the reaction mixture was poured into 500 mL of ice-water. Extraction with ethyl acetate (4 × 100 mL) followed by drying with anhydrous sodium sulfate and evaporation of the solvent left an oily residue, which solidified upon triturating with acetone. A 26.8-g (62%) sample of compound **35** was obtained: mp 64–66 °C; IR (KBr) 3270 cm^{-1} (NH), 1700 (C=O); NMR ($CDCl_3$) δ 8.2 (s, 1 H), 7.4 (m, 4 H), 4.3 (q, 4 H), 3.8 (s, 2 H), 1.3 (t, 6 H). Anal. ($C_{16}H_{19}F_3NO_5$) C, H, N, F.

***m*-(Trifluoromethyl)phenylalanine Hydrochloride (36).** A mixture of compound **35** (67.2 g, 0.186 mol) and 234 mL of 10% HCl was refluxed for 4 h. After cooling to 0 °C, the precipitated solid was collected by suction and washed repeatedly with acetone. Compound **36** was obtained in 37% yield (18.6 g): mp 227–230 °C; IR (KBr) 3300–2700 cm^{-1} (NH), 1710 (COOH); NMR (Me_2SO-d_6/Me_4Si) δ 7.7 (s, 4 H), 4.3 (t, 1 H), 3.4 (d, 2 H). Anal. ($C_{10}H_{11}NO_2ClF_3$) C, H, N, Cl, F.

***m*-(Trifluoromethyl)phenylalanine Methyl Ester Hydrochloride (37).** Thionyl chloride (5.5 mL) was added dropwise to dry methanol (50 mL) kept at -5 °C under nitrogen. A 18.6-g (0.069 mol) sample of compound **36** was then added and the temperature raised to 65 °C. After the mixture was stirred for 2 h, removal of the excess methanol on the rotary evaporator left a solid, which was collected and washed thoroughly with ethyl ether. A 16.0-g (82%) sample of pure **37**, mp 165–166 °C, was obtained: IR (KBr) 3200–2600 cm^{-1} (NH), 1730 (C=O); NMR (Me_2SO-d_6/Me_4Si) δ 7.7 (s, 4 H), 4.4 (t, 1 H), 3.7 (s, 3 H), 3.3 (t, 2 H). Anal. ($C_{11}H_{13}F_3NO_2Cl$) C, H, N, Cl, F.

***m*-(Trifluoromethyl)phenylalanine Methyl Ester Isocyanate (38).** A 5.0-mL (0.042 mol) sample of trichloromethyl chloroformate was added to a mixture of compound **37** (8.51 g, 0.03 mol) in 75 mL of dry dioxane under nitrogen. The reaction mixture was refluxed overnight under an efficient hood and with use of a gas trap (300 mL of 20% aqueous NaOH). Removal of the solvent in vacuo, followed by vacuum distillation of the oily residue, yielded 6.9 g (84%) of pure isocyanate: IR (neat) 2230 cm^{-1} (N=C=O), 1730 (C=O); NMR ($CDCl_3$/Me_4Si) δ 7.5 (s, 4 H), 4.4 (d, 1 H), 3.8 (s, 3 H), 3.2 (d, 2 H). Anal. ($C_{12}H_{10}NO_3F_3$) C, H, N, F.

***N*-(1*H*-Imidazol-1-ylcarbonyl)-*m*-(trifluoromethyl)phenylalanine Methyl Ester (34).** A 2.73-g (0.01 mol) sample of compound **38** was mixed with imidazole (0.68 g, 0.01 mol) in 20 mL of anhydrous ethyl ether under nitrogen. After the mixture was refluxed for 2 h, the solvent was evaporated in vacuo, leaving an oily residue (3.3 g, 97%). The oily material was homogeneous by TLC (silica gel/$CHCl_3$/EtOAc, 1:1): IR (neat) 3130 cm^{-1} (NH), 1715 (C=O); NMR ($CDCl_3$/Me_4Si) δ 8.3 (t, 1 H), 7.2 (m, 6 H), 4.4 (t, 1 H), 3.7 (s, 3 H), 3.2 (q, 2 H). Anal. ($C_{15}H_{14}N_3F_3O_3$) C, H, N, F.

***N*-(Sulfocarbonyl)-DL-*m*-(trifluoromethyl)phenylalanine Methyl Ester, Monopotassium Salt (32).** A 2.73-g (0.01 mol) sample of compound **38** in 5 mL of dioxane was added to potassium metabisulfite (1.11 g, 0.01 mol) in 5 mL of water at room temperature. After the mixture was stirred overnight, the precipitated solid was collected by suction and washed thoroughly with acetone. Compound **32** was obtained in 92% yield (3.6 g): IR (KBr) 3360 cm^{-1} (NH), 1710 (C=O); NMR (D_2O/DSS) δ 7.6 (s, 4 H), 6.8 (d, 1 H), 4.6 (t, 1 H), 3.7 (s, 3 H), 3.2 (d, 2 H). Anal. ($C_{12}H_{11}F_3NO_6KS$) C, H, N, S.

L-Phenylalanine Methyl Ester Isocyanate (39). A 10.7-g (0.05 mol) sample of L-phenylalanine methyl ester hydrochloride salt was mixed with 200 mL of a 13% phosgene solution in toluene and refluxed for 4 h. **Caution:** This reaction should be carried out under an efficient hood with a gas trap of 20% aqueous NaOH. Evaporation of the solvent in vacuo left an oily residue, which was vacuum distilled, yielding 8.97 g (88%) of a colorless oil: IR (neat) 2250 cm^{-1} (N=C=O), 1735 (C=O); NMR ($CDCl_3$/Me_4Si) δ 7.3–6.8 (m, 5 H), 4.2 (t, 1 H), 3.5 (s, 3 H), 2.9 (d, 2 H). Anal. ($C_{11}H_{11}NO_3$) C, H, N.

***N*-(1*H*-Imidazol-1-ylcarbonyl)-L-phenylalanine Methyl Ester (28).** A 1.94-g (0.01 mol) sample of isocyanate **39** was added to imidazole (0.68 g, 0.01 mol) in 10 mL of anhydrous THF under nitrogen. After refluxing for 2 h and removal of the solvent in vacuo, a viscous colorless oil was obtained (2.40 g, 88%), homogeneous by TLC (silica gel chloroform/ethyl acetate, 1:1): IR (neat) 1735 cm^{-1} (C=O); NMR ($CDCl_3$/Me_4Si) δ 8.3 (s, 1 H), 7.0–7.5 (m, 5 H), 5.9 (s, 1 H), 5.0 (br s, 1 H), 3.7 (s, 3 H), 3.3 (m, 2 H). Anal. ($C_{14}H_{15}N_3O_3$) C, H, N.

***N*-(Sulfocarbonyl)-L-phenylalanine Methyl Ester, Monopotassium Salt (27).** Isocyanate **39** (4.1 g, 0.02 mol) in 10 mL of dry dioxane was added to potassium metabisulfite (2.22 g, 0.01 mol) in 10 mL of water with stirring. The precipitated product was collected and washed thoroughly with acetone to yield 3.6 g (92%) of pure product: IR (KBr) 3200 (NH), 1740 and 1700 (C=O), 1220 (SO_3^-); NMR Me_2SO-d_6/Me_4Si) 8.2 (d, 1 H), 7.3 (s, 5 H), 4.5 (q, 1 H), 3.7 (s, 3 H), 3.15 (d, 2 H). Anal. ($C_{11}H_{12}SO_6NK$) C, H, N, S.

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N-[2(1H)-Pyridon-1-ylcarbonyl]-L-phenylalanine Methyl Ester (29). A mixture of isocyanate **39** (1.02 g, 5 mmol) and 2-pyridone (0.48 g, 5 mmol) in 5 mL of dioxane was refluxed for 2 h. Removal of the solvent in vacuo left a viscous oil (1.4 g, 93%): IR (neat) 1720 cm^{-1} (C=O); NMR ($CDCl_3/Me_4Si$) δ 8.3 (d, 1 H), 7.2 (s, 7 H), 6.5 (d, 1 H), 6.3 (d, 1 H), 4.8 (t, 1 H), 3.6 (s, 3 H), 3.1 (d, 2 H). Anal. ($C_{16}H_{16}N_2O_4$) C, H, N.

N-(Succinimid-1-ylcarbonyl)-L-phenylalanine Methyl Ester (30). A 1.02-g (5 mmol) sample of isocyanate **39** and 0.58 g (5 mmol) of N-hydroxysuccinimide were refluxed in 5 mL of dioxane for 2 h. Evaporation of the solvent left a viscous oil (1.52 g, 95%): IR (neat) 3300 cm^{-1} (NH br), 1770 (C=O), 1725 (C=O); NMR ($CDCl_3/Me_4Si$) δ 7.3 (s, 5 H), 4.5 (d, 1 H), 3.7 (s, 3 H), 3.2 (d, 2 H), 2.8 (s, 4 H). Anal. ($C_{15}H_{16}N_2O_6$) C, H, N.

N-(1H-Imidazol-1-ylcarbonyl)-2-phenethylamine (18). A 2.94-g (20 mmol) sample of 2-phenylethyl isocyanate and imidazole (1.36 g, 20 mmol) in 20 mL of dioxane were refluxed under nitrogen for 2 h. Removal of the solvent in vacuo left a white residue, which was recrystallized from CH_2Cl_2/hexane to give 3.7 g (86%) pure product: mp 101–103 °C; IR (KBr) 3200 cm^{-1} (NH), 1700 (C=O); NMR ($CDCl_3/Me_4Si$) δ 8.1 (t, 1 H), 7.8 (s, 1 H), 7.3 (s, 1 H), 7.1 (s, 5 H), 6.7 (s, 1 H), 3.3 (t, 2 H), 2.7 (t, 2 H). Anal. ($C_{12}H_{13}N_3O$) C, H, N.

Succinimido N-(2-Phenylethyl)carbamate (24). A 2.94-g (0.02 mol) sample of 2-phenylethyl isocyanate, 2.30 g (0.02 mol) of N-hydroxysuccinimide, and 20 mL of dioxane were refluxed under nitrogen for 2 h. Removal of the solvent left a solid residue, which was recrystallized from CH_2Cl_2/hexane to yield 4.62 g (88%) pure product: mp 138–139 °C; IR (KBr) 3260 cm^{-1} (NH), 1730 (C=O, br); NMR ($CDCl_3/Me_4Si$) δ 7.3 (s, 5 H), 5.6 (br s, 1 H), 3.4 (q, 2 H), 2.8 (t, 2 H), 2.7 (s, 3 H). Anal. ($C_{13}H_{14}N_2O_4$) C, H, N.

1-[(2-Phenylethyl)carbamoyl]-2(1H)-pyridone (21). A 2.94-g (20 mmol) sample of 2-phenylethyl isocyanate and 1.90 g (20 mmol) of 2-pyridone in 20 mL of dioxane were refluxed for 2 h. Recrystallization of the solid residue yielded 4.2 g (87%) of product: mp 52–53 °C; IR (KBr) 3100 cm^{-1} (NH br), 1700 and 1650 (C=O); NMR ($CDCl_3/Me_4Si$) δ 8.3 (d, 1 H), 7.2 (s, 6 H), 6.4 (d, 1 H), 6.2 (d, 1 H), 3.5 (t, 2 H), 2.7 (t, 2 H). Anal. ($C_{14}H_{14}N_2O_2$) C, H, N.

Potassium Oxo[(2-phenylethyl)amino]methanesulfonate Salt (16). 2-Phenylethyl isocyanate (2.94 g, 20 mmol) in 10 mL of dioxane was mixed with potassium metabisulfite (2.22 g, 10 mmol) in 10 mL of water and the mixture stirred overnight. The precipitated solid was collected by suction and washed with acetone. A 5.13-g (96%) sample of pure salt was obtained: IR (KBr) 3300 cm^{-1} (NH), 1660 (C=O); NMR (Me_2SO-d_6) δ 7.3 (s, 5 H), 3.5 (t, 2 H), 2.8 (t, 3 H). Anal. ($C_9H_{10}NSO_4K$) C, H, N, S.

Enzyme Assays and Inhibition Studies. Human leukocyte elastase was assayed by mixing 10 μL of a 3.4 × 10^{-5} M enzyme solution (in 0.05 M sodium acetate buffer, pH 5.5), 50 μL of dimethyl sulfoxide, and 940 μL of Tris–buffer, pH 7.2, in a thermostated test tube. After equilibration at 25 °C, a 100-μL aliquot was transferred to a thermostated cuvette containing 890 μL of Tris–buffer and 10 μL of a 3.15 × 10^{-2} M solution of (methoxysuccinyl)-Ala-Ala-Pro-Val-p-nitroanilide. The change in absorbance was monitored at 410 nm for 2 min. In a typical inhibition run, 50 μL of a 3.4 × 10^{-4} M solution of the appropriate inhibitor in dimethyl sulfoxide was mixed with 10 μL of a 3.4 × 10^{-5} M enzyme solution and 940 μL Tris–buffer in a constant temperature bath. One hundred microliter aliquots were withdrawn at different time intervals and transferred to a cuvette containing substrate, 10 μL of a 3.15 × 10^{-2} M solution, and 890 μL of Tris–buffer. After incubation for 30 s, the absorbance was monitored for 2 min at 410 nm. The inhibitor to enzyme ratio varied between 10 and 50, depending on the potency of the inhibitor. The method of Kitz and Wilson[16] was used to analyze the data, and the results were expressed in terms of the bimolecular rate constant, $k_{obsd}/[I]$ M^{-1} s^{-1}.

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Analogues of Caffeine and Theophylline: Effect of Structural Alterations on Affinity at Adenosine Receptors

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A variety of analogues of caffeine and theophylline in which the 1-, 3-, and 7-methyl substituents have been replaced with n-propyl, allyl, propargyl, and isobutyl and, in a few cases, with chloroethyl, hydroxyethyl, or benzyl were assessed for potency and selectivity as antagonists at A_1- and A_2-adenosine receptors in brain tissue. Caffeine and theophylline are nonselective for these receptors. Nearly all of the 22 analogues of caffeine are more potent than caffeine itself at adenosine receptors. Replacement of the 1-methyl moiety with n-propyl, allyl, or propargyl substituent has little effect on potency at the A_1 receptor while enhancing potency about 7- to 10-fold at the A_2 receptor. 3,7-Dimethyl-1-propylxanthine is only slightly (1.4-fold) more potent than caffeine at the A_1 receptor while being 10-fold more potent at the A_2 receptor. 1,3-Di-n-propyl-7-methylxanthine is also selective for the A_2 receptor, being 8-fold more potent than caffeine at the A_1 receptor and 40-fold more potent at the A_2 receptor. A number of other caffeine analogues including 3,7-dimethyl-1-n-propylxanthine, 7-allyl-1,3-dimethylxanthine, and 1,3-dimethyl-7-propargylxanthine are also somewhat selective for the A_2 receptor. The most potent caffeine analogue was 1,3-di-n-propyl-7-propargylxanthine, which was about 100-fold more potent than caffeine at both A_1 and A_2 receptors. The 10 theophylline analogues were relatively nonselective except for the 1-ethyl analogue and the 1,3-diallyl analogue, which were selective for the A_2 receptor, and the 1,3-di-n-propyl, 1,3-diisobutyl, and 1,3-dibenzyl analogues, which were somewhat selective for the A_1 receptor. 1,3-Di-n-propylxanthine was 20-fold more potent than theophylline at the A_1 receptor and 5-fold more potent at the A_2 receptor.

Adenosine has a variety of roles in modulating the function of the cardiovascular, endocrine, and nervous system.[1] Definition of the nature of the receptors involved in such roles has been hindered by the lack of selective antagonists for the two proposed classes of adenosine receptors, the so-called A_1 (Ri) and A_2 (Rs) receptors. Caffeine (**1**) and theophylline (**24**) represent prototypes of a wide range of xanthine antagonists for adenosine receptors. But caffeine, theophylline, and most of the other

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