Design of Species- or Isozyme-Specific Enzyme Inhibitors. 3.[1] Species and Isozymic Differences between Mammalian and Bacterial Adenylate Kinases in Substituent Tolerance in an Enzyme–Substrate Complex

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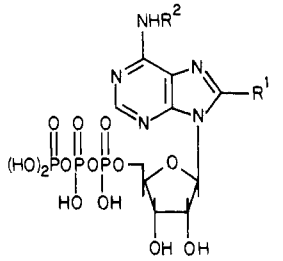
N^6- and 8-substituted adenosine 5′-triphosphate (ATP) derivatives have been synthesized and studied as potential species- or isozyme-selective inhibitors with *Escherichia coli* adenylate kinase (AK), the rat liver AK isozymes II and III, and the rat muscle AK isozyme. Substituent tolerance in the enzyme–ATP complexes was assessed from substrate properties, apparent enzyme–inhibitor dissociation constants (K_i values; for inhibitions competitive with respect to ATP), and I_{50} values (for noncompetitive inhibitions). 8-SCH_3-ATP and 8-S-*n*-C_3H_7-ATP were not substrates of muscle AK and gave I_{50} = 6.0 and 6.2 mM, respectively; 8-SC_2H_5-ATP and 8-S-*n*-C_3H_7-ATP gave I_{50} = 5.9 and 4.7 mM, respectively, with *E. coli* AK. In contrast, 8-SR-ATP (R = CH_3, C_2H_5, and *n*-C_3H_7) were substrates (V_{max} = 5–16% that of ATP; K_M = 0.04–0.18 mM, ATP = 0.09 mM) and gave K_i = 0.05–0.36 mM with AK II and III. 8-SR-ATP [R = *n*-C_4H_9, *n*-C_5H_{11}, CH_2CH_2OH, $(CH_2)_3OH$, and C_6H_5] gave K_i = 0.06–0.32 mM with AK II and III. N^6-$(CH_2)_5NHCOCH_2I$-ATP was a substrate of AK II and III (V_{max} = 21 and 9%, respectively, that of ATP) but not of the muscle AK, and with *E. coli* AK it gave I_{50} = 4.75 mM. N^6-$(CH_2)_6NHCOCH_3$-ATP gave K_i = 4.75 mM with AK III and I_{50} = 12.9 mM with muscle AK. These results, and previous findings with thymidine kinase variants, demonstrate the ability of simple substrate substituents to influence binding or lack of binding to a substrate site in a markedly species- or isozyme-selective manner.

Derivatives bearing a single substituent at any of six different positions of a substrate were recently analyzed kinetically as inhibitors of a bacterial and a mammalian form of the enzyme thymidine kinase.[1] It was found that certain substituents attached to these positions influenced binding or lack of binding to the substrate site in a manner which was markedly species selective. In the present work, the tendency of single substituents attached to a substrate to produce species-selective effects at the substrate site has been further explored, using bacterial and mammalian forms of a second enzyme, adenylate kinase (AK). This enzyme catalyzes transfer of phosphate from ATP to AMP to form ADP. We report the synthesis of ATP derivatives monosubstituted at either the N^6 or C-8 positions and describe their inhibitor and substrate properties with the rat muscle isozyme of AK, the rat liver isozymes AK II and AK III,[2,3] and *E. coli* AK.

A second function of the present studies was to determine if the substrate substituents could influence binding to the substrate site in an isozyme-selective manner: such effects were of interest to us in view of the possibility, discussed previously,[4] that fetal isozyme-selective inhibitors might constitute useful starting points in the design of antineoplastic agents. The evidence presented here indicates that the substituents attached at either N^6 or C-8 of ATP influence the extent of the enzyme inhibitions and the affinity for the ATP sites of the various adenylate kinases in a markedly species-selective and also isozyme-selective manner.

Syntheses. The N^6-substituted ATP derivatives **1i** and **1j** were prepared as previously reported.[4,5] For synthesis of the 8-substituted ATP derivatives **1a–h**, 8-Br-ATP was prepared by bromination of ATP[6] and treated in aqueous methanol with the appropriate alkylthio anion; formation of **1a–h** could be accelerated by catalytic amounts of dibenzo-18-crown-6. The products were isolated as their tetrasodium salts, which were homogeneous as indicated by ultraviolet extinction coefficient, paper electrophoresis, paper chromatography, high-pressure LC, anion-exchange column chromatography, and elemental analysis.

Adenylate Kinase Studies. The present series of 8- and N^6-substituted ATP derivatives were studied as inhibitors of the adenylate kinase catalyzed conversion of AMP to ADP. The inhibition constants obtained are presented in Table II. In those cases in which the inhibitions are competitive with respect to ATP, these constants are given as K_i values, and for noncompetitive inhibitions they are given as I_{50} values. The ATP derivatives were studied as their monomagnesium complexes in order to preclude possible contributions to inhibition caused by depletion of magnesium ions required for the catalyzed reaction. Some of the present compounds were also tested for their ability to substitute for ATP as a phosphate donor with the adenylate kinases, and these findings are given in Table III.



1a, R^1 = SCH_3; R^2 = H
b, R^1 = SC_2H_5; R^2 = H
c, R^1 = $S(CH_2)_2CH_3$; R^2 = H
d, R^1 = $S(CH_2)_3CH_3$; R^2 = H
e, R^1 = $S(CH_2)_4CH_3$; R^2 = H
f, R^1 = SCH_2CH_2OH; R^2 = H
g, R^1 = $S(CH_2)_3CH_2OH$; R^2 = H
h, R^1 = SC_6H_5; R^2 = H
i, R^1 = H; R^2 = $(CH_2)_5NHCOCH_2I$
j, R^1 = H; R^2 = $(CH_2)_6NHCOCH_3$

The 8-(methylthio)-ATP derivative **1a** was a substrate of AK II and AK III and was a competitive inhibitor with respect to ATP for both isozymes. The K_M values were only ca. twice the K_M of ATP and ca. half the respective enzyme–substrate dissociation constants (K_i values), suggesting that the methylthio group has little effect on affinity for the ATP sites of AK II and III. Compound **1a** inhibited the rat muscle AK isozyme 20-fold more weakly and in noncompetitive fashion, indicating that the methylthio group hinders or possibly prevents binding to the ATP site and that **1a** adsorbs to a form of the enzyme which is other than the free enzyme form to which ATP binds. This view is supported by the lack of detectable substrate activity of **1a** with the muscle isozyme (Table III).

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Table I. Physical Properties of 8-Substituted Derivatives of Adenosine 5′-Triphosphate

no.	yield, %	UV λ_{max} (pH 7), nm ($\epsilon \times 10^3$)	electrophoresis[a] pH 7.5	electrophoresis[a] pH 3.6	R_f system 1[b]	R_f system 2[b]	HPLC retn time, min[c]	formula	anal.
1a	44	281 (18.8)	0.80	1.0		0.34		$C_{11}H_{14}N_5O_{13}P_3SNa_2$	
1b	61	281 (18.5)	0.83	0.90		0.39		$C_{12}H_{16}N_5O_{13}P_3SNa_4 \cdot 4H_2O$	C, H, N, S
1c	60	281 (18.6)	0.80	0.90	0.29	0.44	16.0	$C_{13}H_{18}N_5O_{13}P_3SNa_4 \cdot 2H_2O$	C, H, N, S
1d	56	281 (18.9)	0.76		0.32	0.54		$C_{14}H_{20}N_5O_{13}P_3SNa_4 \cdot H_2O$	C, H, N
1e	55	281 (18.4)	0.76	0.85	0.44	0.61	19.0	$C_{15}H_{22}N_5O_{13}P_3SNa_4$	C, N, N
1f	39	281 (16.7)	0.82	0.90	0.12	0.26		$C_{12}H_{16}N_5O_{13}P_3SNa_4 \cdot 4H_2O$	C, H, N, S
1g	43	282 (17.3)	0.80	0.85		0.34	15.5	$C_{14}H_{20}N_5O_{13}P_3SNa_4 \cdot 2H_2O$	C, H, N
1h	57	284 (16.0)	0.80	0.90		0.44	18.25	$C_{16}H_{16}N_5O_{13}P_3SNa_4 \cdot H_2O$	C, H, N

[a] Mobilities relative to ATP (= 1.0). [b] Compositions given under the Experimental Section. [c] For conditions see Experimental Section; HPLC = high-pressure liquid chromatography.

Table II. Inhibition of *E. coli* Adenylate Kinase and of Rat Adenylate Kinase Isozymes by ATP Derivatives[a]

	rat AK II[b]		rat AK III[b]		rat muscle[c]		*E. coli*[c]	
no.	K_i,[d] mM	compd concns, mM	K_i, mM	compd concns, mM	I_{50},[e] mM	compd concns, mM	I_{50}, mM	compd concns, mM
1a	0.32	0.40, 0.55	0.36	0.33, 0.46	5.95	2.7, 4.1		
1b	0.08	0.27, 0.81	0.05	0.81, 3.24			5.9	1.7, 3.8
1c	0.055	0.30, 0.89	0.065	0.30, 0.89	6.15	2.5, 3.7	4.7	1.5, 3.9
1d	0.075	0.23, 0.34	0.06	0.22, 0.56	6.2	2.9, 5.1		
1e	0.08	0.20, 0.30	0.06	0.20, 0.30				
1f	0.25	0.33, 0.55	0.22	0.54, 1.09				
1g	0.145	0.22, 0.34	0.13	0.34, 0.56				
1h	0.32	0.78, 1.67	0.32	0.31, 0.43				
1i			6.2	2.5, 3.4			4.75	2.0, 8.1
1j			4.75	3.8, 5.7	12.9	3.8, 5.7		

[a] In all cases, the plot of inhibitor level vs. slope of the Lineweaver-Burk plot was linear. [b] Inhibitions were competitive with respect to ATP. [c] Inhibitions were noncompetitive with respect to ATP. [d] Enzyme–inhibitor dissociation constant. [e] Inhibitor concentration giving 50% inhibition.

Table III. Substrate Constants of ATP Derivatives with Rat Adenylate Kinase Isozymes

	rat AK II			rat AK III			rat muscle		
compd	enz per assay[a]	K_M,[b] mM	V_{max},[c] rel %	enz per assay	K_M, mM	V_{max}, rel %	enz per assay	K_M, mM	V_{max}, rel %
ATP	1	0.09	100	1	0.09	100	1	0.65	100
1a	10	0.18	5	20	0.15	8.3	10		0
1b				20	0.07	11.4			
1c	10	0.06	15.7	20	0.04	10.4	20		0
1f				20	0.12	12.5			
1i	20	1.00	21.0	20	0.40	8.9	20		0

[a] Amount of enzyme relative to the normal assay level (= 1). [b] K_M (Michaelis constant) = substrate concentration for half-maximal velocity. [c] Maximal velocity.

Successive addition of four methylenes to the methylthio group of **1a** to give **1b–e** did not diminish the substrate activity with AK II and III and produced a uniform four- to sevenfold increase in affinity for the ATP sites. The additional methylenes did not alter the character or extent of the noncompetitive inhibition observed with the muscle isozyme, nor did they induce detectable substrate activity. Compounds **1b** and **1c** behaved as noncompetitive inhibitors of the *E. coli* AK; the I_{50} values were similar to those observed with rat muscle AK. Introduction of a terminal hydroxyl into the 8-(ethylthio) and 8-(butylthio) substituents (**1f** and **1g**) decreased affinity by factors of 3 and 2, respectively, for both AK II and III and produced a parallel change in K_M in the one case of substrate activity examined (**1f** with AK III). Substitution of the alkyl groups of **1b–e** by a phenyl group (**1h**) decreased affinity for the ATP sites of AK II and III by a factor of 4–6.

N^6-[5-(Iodoacetamido)pentyl]-ATP (**1i**) was a substrate of AK II and AK III but not of rat muscle AK under the conditions used. The affinity (K_i = 6.2 mM) of **1i** for AK III was ca. 100 times less than that of the 8-(alkylthio)-ATP derivatives **1b–e**. N^6-(6-Acetamidohexyl)-ATP (**1j**) inhibited AK III competitively and rat muscle AK noncompetitively, thus providing additional evidence that the N^6 substituents of **1i** and **1j** selectively hinder binding to the ATP site of the muscle isozyme.

E. coli AK was inhibited noncompetitively by compound **1i**. Compounds **1i** and **1j** were not examined as substrates of *E. coli* AK. However, in the course of other studies[7] with this enzyme and N^6-substituted ATP derivatives, weak substrate activity which disappeared with increasing substituent length was observed; the tetramethylene homologue of **1i**, with a V_{max} = 0.12% that of ATP and a K_M of 2.5 mM (ATP, 0.06 mM), was a very weak substrate.[7] This suggests that the N^6 substituent of **1i** may not totally exclude **1i** from the ATP site of the *E. coli* enzyme, although the inhibition data show that this substituent does impose large steric constraints on binding to that ATP site.

The substituents introduced at either N^6 or C-8 of ATP in the present work thus appear to influence affinity for the enzymatic ATP sites in both a species- and an isozyme-selective manner. The magnitude of the selective

effect is relatively difficult to assess in the case of the N^6-substituted compounds, since they produced a relatively high K_M value as well as K_i and I_{50} values which were similar to each other. In the case of the 8-substituted ATP derivatives, the selective effects can be seen to be quite marked, as exemplified by **1c** which showed no substrate activity with the muscle AK at a concentration 20 times its K_M with AK III and which showed no affinity for the ATP site of the muscle or *E. coli* adenylate kinases at concentrations which were 100 and 80 times, respectively, its K_i value with AK II or III.

In previous work with a bacterial and a mammalian thymidine kinase,[1] examples of species-selective substituent tolerance at the thymidine site were found as a result of attaching various substituents to any of six different atoms of thymidine. The additional examples reported herein indicate that species-selective substituent tolerance at substrate sites of ATP-utilizing phosphokinases is apparently not a rarity; however, data are not yet available to suggest how often this effect may be encountered among enzymes in general.

Experimental Section

Chemical Synthesis. General. Ethanethiol, propanethiol, butanethiol, pentanethiol, 2-mercaptoethanol, and thiophenol were purchased from Aldrich Chemical Co. 4-Mercapto-1-butanol was purchased from MCB and methyl sulfide from Matheson. *N,N*-Dimethylformamide was distilled from calcium hydride and stored over molecular sieves. Paper chromatography (Table I) was carried out by the ascending technique on Whatman No. 1 paper in (1) 1-butanol–acetic acid–water (5:2:3) and (2) 1-propanol–NH_4OH–water (55:10:35). Electrophoresis was carried out on Whatman No. 1 paper at pH 3.5 (0.05 M citrate) and at pH 7.5 (0.05 M triethylammonium bicarbonate). Ultraviolet spectra were obtained on Cary Model 15 and Varian Model 635 spectrophotometers. Elemental analyses were performed by Galbraith Laboratories Inc., Knoxville, Tenn. High-pressure liquid chromatography was performed on a Waters Model 204 chromatograph, equipped with a dual solvent delivery system (Model M-6000 A) and a Waters (Model 660) programmer. Compounds were analyzed on a μBondapak NH_2 column (30 cm × 4 mm) utilizing a 3 mL/min flow rate with a linear gradient of ammonium dihydrogen phosphate (0.03 M, pH 3, to 0.3 M, pH 6) over a 30-min period. The column eluent was monitored at 254 nm.

General Method for the Synthesis of 8-(Alkylthio)- and 8-[(Hydroxyalkyl)thio]adenosine 5′-Triphosphates (1b–h). 8-Bromoadenosine 5′-triphosphate tetralithium salt was prepared as reported by Ikehara[6] to afford the nucleotide in 42% yield (calcd from 260 nm, pH 1, ε 16000). The high-pressure LC retention time was 14.0 min, and ATP (12.25 min) was not detectable. The thiol (1 mmol) and sodium methoxide (1.3 mmol) were stirred with 1 mg of dibenzo-18-crown-6 and 15 mL of absolute methanol for 15 min at ambient temperature. 8-Br-ATP·Li_4 (0.255 mmol), dissolved in a minimum amount of water, was added to the methanol solution and additional water was added to dissolve all solids. The mixture was warmed to 70 °C for 0.1–1.5 h, and the reaction was followed by the change in UV absorption maxima from 273 to 280 nm. The solution was evaporated in vacuo at 30 °C. The residue was dissolved in 100 mL of water and applied to a column (2.5 × 20 cm) of DEAE bicarbonate. The column was washed with water and then eluted with a linear gradient of 0.0–0.3 M triethylammonium bicarbonate (1 L + 1 L). The fractions corresponding to the thioether were pooled and evaporated in vacuo. The residue was evaporated several times with ethanol to give the triethylammonium salt. This was converted to the sodium salt by dissolving the white solid in methanol (2 mL) and adding 1.0 M NaI in acetone (1 mL), followed by acetone (35 mL). The precipitate was washed with acetone (3 × 20 mL) by centrifuging at 2000 rpm and then dried in vacuo.

8-(Methylthio)adenosine 5′-Triphosphate (1a). To prepare sodium methyl mercaptide, methyl mercaptan (~40 mL, 0.8 mol) was cooled to a liquid with a dry ice condenser. Sodium methoxide (0.5 mol) in methanol was added dropwise, and the reaction mixture was stirred at −80 °C for 1 h and then allowed to warm to room temperature. Toluene (100 mL) was added and the mixture distilled until the boiling point of toluene was reached. The resulting slurry was filtered, washed with diethyl ether, and dried, to afford 34 g (0.49 mol, 97%) of the sodium salt. Sodium methyl mercaptide (50 mg, 0.71 mmol) and dibenzo-18-crown-6 (1 mg) were dissolved in water (3 mL). Tetralithium 8-bromoadenosine 5′-triphosphate (175 mg, 0.255 mmol), dissolved in a minimum amount of water, was added. Methanol was added until the solution became slightly turbid, and then the reaction mixture was stirred at ambient temperature for 2 h. The solution was evaporated in vacuo at 30 °C and purified as for compounds **1b–h** to afford 67 mg (0.105 mmol, 42%) of the sodium salt.

Enzyme Kinetic Studies. Adenosine 5′-monophosphate, adenosine 5′-triphosphate, lactate dehydrogenase (type II, rabbit muscle), and phosphoenolpyruvate were from Sigma Chemical Co. The pyruvate kinase was purchased from Boehringer Mannheim and the NADH was from PL Biochemicals.

For preparation of the rat isozymes, an adult male Sprague–Dawley rat was sacrificed by cervical dislocation, and the liver and skeletal muscle were quickly dissected and placed in ice. Liver tissue was homogenized as reported,[2] except that one-third the amount of buffer was used. A portion of the centrifuged homogenate (3.5 mL) was subjected to Sephadex slab electrofocusing at 8 °C with an LKB Model 2117-501 apparatus, using an ampholine mixture (1:1) of pH ranges 6–8 and 7–9 in the presence of 20% sucrose and the methods described in the LKB instrument manual. The homogenate was applied to the anode end of the gel. Well-separated zones corresponding to AK II (p*I* = 6.6) and AK III (p*I* = 7.3) were obtained; these were centrifuged through glass wool and then dialyzed for 3 days against 0.01 M Tris buffer, pH 7.5, containing 20% sucrose, 1 mM EDTA; and 1 mM $MgSO_4$. The two enzyme preparations were then stored at 4 °C. The isoelectric points were similar in value to those previously reported[2] for rat liver adenylate kinases (AK II, p*I* = 7.0; AK III, p*I* = 7.6). As reported,[2] AK III was the major peak. A trace zone, p*I* = 6.4, was seen in some runs. Rat skeletal muscle (10 g) was homogenized in a blender with 25 mL of a buffer containing $KHPO_4$ (20 mM), glucose (10 mM), EDTA (0.5 mM), dithioerythritol (0.5 mM), and glycerol (5%). The homogenate was centrifuged at 2 °C for 1 h at 90000*g*. A portion of the filtered homogenate (3.5 mL) was electrofocused as above with an ampholine of pH range 6–8. A single sharp zone of AK activity, p*I* = 6.4, was usually observed; appropriate fractions were dialyzed under the conditions used for AK II and AK III and stored at 4 °C. In some runs a minor, well-separated zone of p*I* = 6.0 was seen.

E. coli B adenylate kinase, which has not previously been described, was obtained in partially purified form after fractional precipitation with ammonium sulfate and DEAE-cellulose chromatography. Details of this purification will be reported elsewhere.[7] Electrofocusing of the preparation at pH 3.5–10 or 4–6 revealed seven proteins, only one of which (p*I* = 4.9) showed AK activity. The K_M of ATP was 0.06 mM in the AMP range 0.13–0.29 mM using the assay system given below.

The enzyme-catalyzed reactions were followed at 23 °C by measuring the rate of change of optical density at 340 nm for a period of 5 min in a Cary Model 15 spectrophotometer using 1-cm cells containing a final volume of 1 mL. Initial velocities were linear and proportional to the concentration of primary enzyme and independent of the level of secondary enzymes in the assay system. Each kinetic study employed five or more concentrations of substrate, and the substrate constants were determined from Lineweaver–Burk double-reciprocal plots of velocity vs. substrate level, all of which were linear. Compounds 1 were tested initially for substrate activity at a concentration of ca. 0.8 mM. All four adenylate kinases were studied in 1 mL of 0.1 M Tris-HCl (pH 7.6) containing $MgSO_4$ (2 mM), KCl (0.12 M), PEP cyclohexylammonium salt (0.3 mM), NADH (0.38 mM), pyruvate kinase (8.6 units), lactate dehydrogenase (8.6 units), and AMP (0.25 mM). In the presence of 0.35 mM ATP, the K_M value of AMP was 0.09 mM for AK II and AK III and 0.65 mM for the muscle AK. The K_M values of ATP are given in Table III; by coincidence, they are identical with those of AMP.

Inhibition studies used, for each of two levels of inhibitor, five or more levels of ATP in the range 0.1–0.8 mM for AK II and AK III and 0.15–1.1 mM for muscle AK. The stock solution of each

ATP compound 1 contained an equimolar amount of $MgSO_4$. Apparent enzyme–inhibitor dissociation constants (K_i values; for competitive inhibitors) and I_{50} values (for noncompetitive inhibitors) were obtained from replots of inhibitor concentrations vs. slopes of the Lineweaver–Burk plots. The K_i or I_{50} values were reproducible to within ±12%. The amount of inhibition remained unchanged when the levels of pyruvate kinase and lactate dehydrogenase were increased twofold, showing that the inhibitory effect was exerted solely on the adenylate kinase.

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Notes

Syntheses and Activities of Sulfur and Selenium Isosteric Substitution Analogues of Retinol

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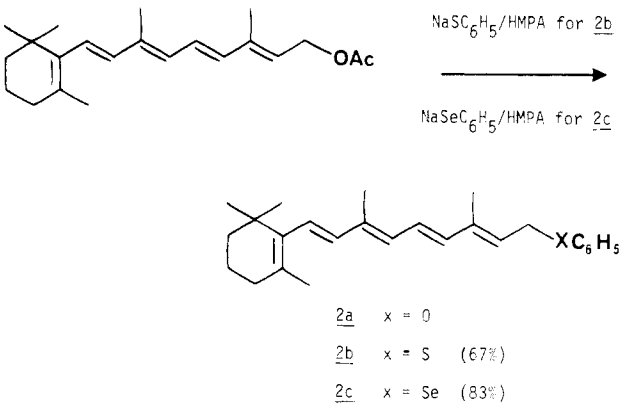
The syntheses of sulfur and selenium isosteric substitution analogues of retinol, namely, retinyl phenyl thioether (**2b**), retinyl phenyl selenoether (**2c**), and retinyl thioacetate (**2e**) are described. These retinoid derivatives were examined for activity in terms of "chemoprevention" of cancer by measuring the reverse keratinization of epithelial cells in vitro. Retinoid analogues **2b**, **2c**, and **2e** were found to be active in 20, 80, and 33.3% of the cultures, respectively, as compared to 72.7% activity for *trans*-retinol.

The active form of vitamin A (retinol) appears to differ depending on the target tissues.[1] Retinol, which is required for healthy reproductive functions,[2] is reversibly oxidized to retinal, which is utilized in visual proteins as photoreceptor molecules.[3] Retinal is then further oxidized, irreversibly, to retinoic acid which exhibits hormonal-like properties in the control of the normal growth, development, and differentiation of epithelial tissues.[4-6] These epithelial tissues make up the membranes that cover, enclose, and protect the major organs of the body. Well over half of cancer begins in these epithelial tissues. Natural retinoids, as well as synthetic retinoid analogues, have been shown to prevent or delay the onset of certain forms of epithelial cancer, such as, bladder, breast, lung, and skin cancer in animals, which previously were given doses of chemical carcinogens.[7-12] Natural retinoids have limited usefulness for "chemoprevention"[13] of cancer, because of excessive toxicity and inadequate tissue distribution. Therefore, it would be advantageous to explore the possibility of utilizing new synthetic retinoid derivatives with better therapeutic indexes and pharmokinetics in order to prevent or delay the onset of epithelial malignancies. With this background information in mind, sulfur and selenium analogues, retinyl phenyl thioether (**2b**), retinyl phenyl selenoether (**2c**), and retinyl thioacetate (**2e**) depicted in Schemes I and II, were synthesized and examined by tracheal organ culture in order to determine what effect isosteric substitution would have on activity.

Both retinyl phenyl thioether (**2b**) and retinyl phenyl selenoether (**2c**) are conveniently prepared (Scheme I) by substitution nucleophilic bimolecular reactions of sodium thiophenoxide or sodium phenylselenide, respectively,

Scheme I



upon retinyl acetate (**1**) in hexamethylphosphoric triamide. Both sodium thiophenoxide and sodium phenylselenide are good nucleophiles, and hexamethylphosphoric triamide is an excellent solvent for S_N2 reactions.[14]

The synthesis of *trans*-retinyl thioacetate (**2e**) employs the Wittig reaction[15] (Scheme II). Treatment of the Wittig salt **3**[16] with 1 equiv of *n*-butyllithium in anhydrous tetrahydrofuran, followed by the addition of freshly prepared 2-methyl-4-(thioacetyl)-2-(*E*)-butenal (**5**), affords both 11-*cis*-retinyl thioacetate (**2d**) and 11-*trans*-retinyl thioacetate (**2e**). Aldehyde **5** was prepared from 2-methyl-4-chloro-2-(*E*)-butenal[17] by displacement of the chlorine substituent with potassium thioacetate in ethanol.

These sulfur and selenium isosteric substitution analogues of retinol were examined for activity by an in vitro hamster tracheal organ culture assay.[18] This tracheal organ

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