EtOH–H_2O gave analytically pure **7d**·HCl, mp 242 °C dec. Anal. ($C_{16}H_{19}ClN_2O_2$·HCl) C, H, N.

Acknowledgment. This investigation was supported by Contract N01-CM-33743 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Eduction, and Welfare. The authors thank Dr. Harry B. Wood, Jr., and Mr. Robert B. Ing for their interest and to Mrs. Margaret Rounds for performing instrumental measurements.

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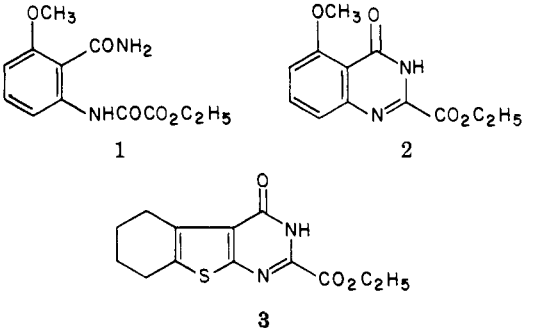
Synthesis of 3,4-Dihydro-4-oxothieno[2,3-*d*]pyrimidine-2-carboxylates, a New Series of Orally Active Antiallergy Agents

D. L. Temple, J. P. Yevich,* R. R. Covington, C. A. Hanning, R. J. Seidehamel, H. K. Mackey, and M. J. Bartek

Research Laboratories, Mead Johnson Pharmaceuticals, Evansville, Indiana 47721. *Received September 27, 1978*

A series of novel 3,4-dihydro-4-oxothieno[2,3-*d*]pyrimidine-2-carboxylic acid derivatives has been prepared and tested for antiallergenic activity. Members of the series, including both carboxylic acid salts and esters, have been found to exhibit oral activity in the rat passive cutaneous anaphylaxis (PCA) test. Activity is optimized by H or CH_3 substitution at the 5 position and lower alkyl groups at the 6 position. Ethyl 6-ethyl-3,4-dihydro-4-oxothieno-[2,3-*d*]pyrimidine-2-carboxylate and 3,4-dihydro-5-methyl-6-(2-methylpropyl)-4-oxothieno[2,3-*d*]pyrimidine-2-carboxylic acid dipotassium salt were the most potent of the esters and salts, respectively. Such compounds have been shown to have a duration of action of up to 4 h in the PCA test and to inhibit both histamine release from rat peritoneal mast cells in vitro and allergen-induced bronchospasm in the rat lung.

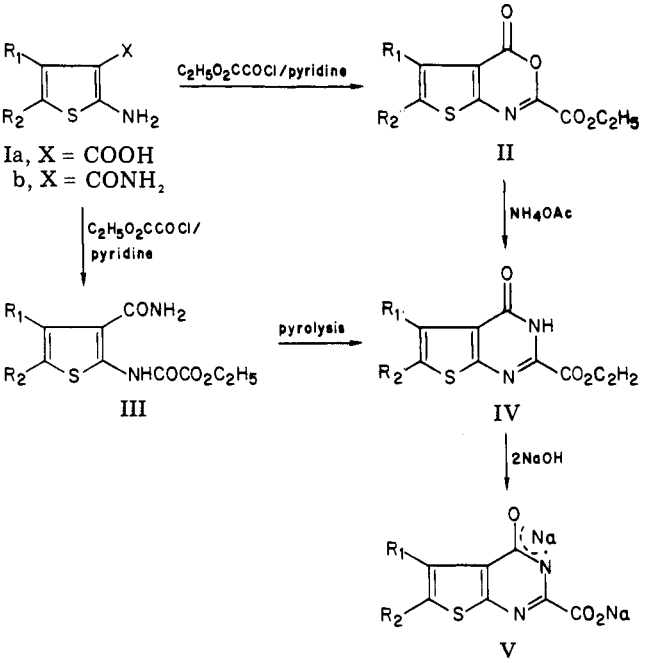
The discovery of the mediator release inhibitor disodium cromoglycate (DSCG) has opened a new approach to the therapy of bronchial asthma in man.[1] The fact that the drug must be administered topically by insufflation has spurred considerable work toward developing similar agents with oral activity. For example, a recent paper[2] has shown that *N*-aryloxamic acid esters (**1**) and the corresponding quinazolinone derivatives (**2**) were orally effective antiallergy agents.



The observation that alkoxy substituents on **1** and **2** conferred maximal activity to those molecules prompted us to undertake the synthesis of related compounds in which the alkoxy-substituted benzo group of **2** was replaced by a π-rich thieno moiety. This provided compounds of type **3** whose lipophilicity could readily be modified via appropriate substitution of the thiophene ring. Our objective, therefore, was to prepare compounds of type **3** with maximal intrinsic activity, oral absorption, and duration of drug action.

Compound **3** was described in the literature as having been prepared by the condensation of diethyl oxalate with the corresponding 2-aminothiophene-3-carboxamide.[3]

Scheme I



We prepared thieno[2,3-*d*]pyrimidine **3** both by pyrolysis of the oxamate intermediate **5** and ammonolysis of the thieno[2,3-*d*]oxazine intermediate **14** (Table I), and it was found that compounds **3**, **5**, and **14** all showed weak oral PCA activity. The disodium salt of the corresponding carboxylic acid **16** showed an oral PCA ED_{50} = 50 mg/kg.

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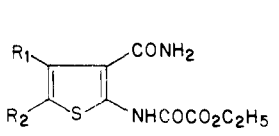
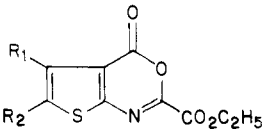
Table I. Ethyl *N*-[3-(Aminocarbonyl)thien-2-yl]oxamates and Ethyl 4-Oxo-4*H*-thieno[2,3-*d*][1,3]oxazine-2-carboxylates



4,5



6-14

compd	R_1	R_2	mp,[a] °C	yield, %	recrystn solvent	method	formula[b]
4	H	H	186.0-187.0	74	MeCN	A	$C_9H_{10}N_2O_4S$
5		$-(CH_2)_4-$	204.0-205.0	49	*i*-PrOH	A	$C_{13}H_{16}N_2O_4S$
6	H	C_2H_5	109.0-111.0	65	EtOAc-*n*-C_6H_{14}	B	$C_{11}H_{11}NO_4S$
7	H	*i*-C_3H_7	87.5-88.5	81	*i*-Pr_2O	B	$C_{12}H_{13}NO_4S$
8	H	*n*-C_6H_{13}	76.0-77.5	61	*i*-Pr_2O	B	$C_{15}H_{19}NO_4S$
9	CH_3	H	99.5-102.0	95	*i*-PrOAc-*i*-Pr_2O	B	$C_{10}H_9NO_4S$
10	CH_3	C_2H_5	97.5-99.5	34	*i*-Pr_2O	B	$C_{12}H_{13}NO_4S$
11	CH_3	*i*-C_4H_9	78.5-79.5	30	*n*-C_6H_{14}	B	$C_{14}H_{17}NO_4S$
12	CH_3	*n*-C_6H_{13}	56.5-57.0	93	Et_2O-*n*-C_6H_{14}	B	$C_{16}H_{21}NO_4S$
13	CH_3	CH_3CO	102.0-103.0	93	$CHCl_3$-*n*-C_6H_4	B	$C_{12}H_{11}NO_5S$
14		$-(CH_2)_4-$	148.5-180.5[c]	99	*i*-PrOAc	B	$C_{13}H_{13}NO_4S \cdot 0.25H_2O$

[a] Melting points are corrected according to USP XVII, class I, which requires immersion at 30 °C below the expected melting point. [b] All compounds listed gave satisfactory C, H, N analyses within ±0.4% of the theoretical values. [c] Melting point 150-153 °C when the bath temperature is taken up rapidly from 25 °C.

This paper describes the synthesis and biological activity of a series of thieno[2,3-*d*]pyrimidine-2-carboxylate antiallergy agents related to **3**.

Chemistry. Synthesis of 3,4-dihydro-4-oxothieno-[2,3-*d*]pyrimidine-2-carboxylates was carried out by the sequence shown in Scheme I. The required 2-aminothiophene-3-carboxylic acid derivatives Ia,b were prepared according to the versatile methods of Gewald.[4,5] The resulting 2-aminothiophene-3-carboxamides Ib were treated with 1 equiv of ethyloxalyl chloride in pyridine to yield the corresponding *N*-[3-(aminocarbonyl)thien-2-yl]oxamates III (Table II). Two equivalents of ethyloxalyl chloride was reacted with the 2-aminothiophene-3-carboxylic acid derivatives Ia in pyridine to give 4-oxo-4*H*-thieno[2,3-*d*][1,3]oxazine-2-carboxylates II (Table II). Either pyrolysis of the oxamate derivatives III at 260 °C or aminolysis of the oxazine derivatives II with ammonium acetate in acetic acid–ethanol gave the desired 3,4-dihydro-4-oxothieno[2,3-*d*]pyrimidine-2-carboxylates in reasonable yield. Saponification of the esters with 2 equiv of base in aqueous alcohol yielded the corresponding disodium salts V, which were generally isolated as stable hydrates as shown by elemental analysis. The level of hydration was confirmed by Karl Fischer water determination on representative compounds.

Nitration of compounds **17** and **29** with concentrated nitric acid in acetic anhydride–trifluoroacetic acid yielded, respectively, 5- and 6-nitro derivatives **35** and **31**; these were reduced catalytically to the corresponding amino derivatives **36** and **32**. The 5-iodo analogue **38** was prepared via the 5-chloromercury derivative according to the procedure of Gewald.[5]

Structure–Activity Relationships. Table III lists test results from the PCA screen. Where activity or inactivity is indicated by a single number, this represents the screening results at that particular dose.

Dose–response ED_{50} values were determined for selected compounds. These values are also listed in Table III along with the corresponding fiducial limits.

The unsubstituted thien-2-yloxamate **4** showed good PCA inhibition by both the oral and intravenous routes, indicating good intrinsic activity and oral absorption. Cyclization to the corresponding thienopyrimidine derivative **15** gave a much less active compound. Of the oxazine derivatives shown in Table III, most were orally inactive with the exception of the 5-methyl-6-hexyl derivative **12**, which showed an oral ED_{50} = 8.2 mg/kg.

Most of the thienopyrimidine esters showed oral PCA activity, with the 5-hydro-6-ethyl derivative **17** being the most noteworthy. A poorly defined intravenous ED_{50} value for the corresponding acid **18** made an accurate estimate of the intrinsic activity of this particular system difficult. (For the purposes of this discussion, it is assumed that the carboxylic acid is the active antiallergic agent and that the esters are simply latentiated derivatives thereof.) Increasing the length of the 6-alkyl chain (e.g., **19**) gave less active compounds, as did alkyl substitution in the 5 position (e.g., **23**). Interestingly, PCA activity was retained, although slightly reduced, by both 5 substituents with $+\sigma$ and $+\pi$ values (5-iodo, **38**) and $-\sigma$ and $-\pi$ values (5-NH_2, **36**). A 5-methyl group ($-\sigma$, $+\pi$) had a similar affect, as illustrated by compound **23**.

The 6 position, in contrast, seemed more sensitive to substitution than the 5 position. Both strong electron-donating groups such as 5-NH_2 (e.g., **32**) and strong electron-withdrawing groups such as 5-CH_3CO (e.g., **33**) reduced PCA activity.

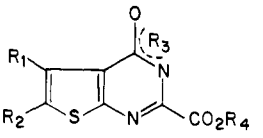
Of the corresponding carboxylic acid disalts, the 5-methyl-6-isobutyl derivative showed the best oral PCA activity. Good intrinsic activity for this particular system was indicated by the low intravenous ED_{50} = 0.14 mg/kg.

PCA Duration Studies. A good duration of action is necessary for a compound to be useful for the clinical prophylaxis of asthma. Comparative oral PCA duration studies for oxamate **4**, ester **17**, and carboxylic acid dipotassium salt **26** are shown in Figure 1. All compounds were dosed orally at 30 mg/kg, and inhibitions of the PCA response were measured at various time intervals following drug administration.

As may be seen from Figure 1, both the oxamate ester **4** and the thienopyrimidine ester **17** showed longer durations of action than the carboxylic acid disalt **26**. This was presumably due to a favorable absorption and distribution combined with a "latentiated drug" affect with the esters.

Blood drug-level studies (Figure 2, see Experimental Section) showed a parallelism between PCA activity and plasma levels of the corresponding carboxylic acid **18** following an oral dose of 50 mg/kg of thienopyrimidine ester **17**. Low plasma levels of ester per se indicated rapid

Table II. 3,4-Dihydro-4-oxothieno[2,3-*d*]pyrimidine-2-carboxylates



compd	R_1	R_2	R_3	R_4	mp,[a] °C	yield, %	recrystn solvent	method	formula[b]
15	H	H	H	C_2H_5	191.0-192.0	41	*i*-PrOH	C	$C_9H_8N_2O_3S$
3		$-(CH_2)_4-$	H	C_2H_5	207.0-209.0[c]	48	DMF-MeOH	C	$C_{13}H_{14}N_2O_3S$
16		$-(CH_2)_4-$	Na	Na	>355	73		D	$C_{11}H_8N_2O_3S\cdot 2Na\cdot 2H_2O$[d]
17	H	C_2H_5	H	C_2H_5	168.0-169.0	55	EtOH	E	$C_{11}H_{12}N_2O_3S$
18	H	C_2H_5	Na	Na	>350	51		D	$C_9H_6N_2O_3S\cdot 2Na\cdot 2H_2O$[d]
19	H	*i*-C_3H_7	H	C_2H_5	182.0-183.0	45	EtOH	E	$C_{12}H_{14}N_2O_3S$
20	H	*i*-C_3H_7	Na	Na	>350	98		D	$C_{10}H_8N_2O_3S\cdot 2Na\cdot 0.25\text{-}0.75H_2O$
21	H	*n*-C_6H_{13}	H	C_2H_5	114.0-115.0	85	EtOH	E	$C_{15}H_{20}N_2O_3S$
22	H	*n*-C_6H_{13}	Na	Na	>350	72		D	$C_{13}H_{14}N_2O_3S\cdot 2Na\cdot 2H_2O$[d]
23	CH_3	C_2H_5	H	C_2H_5	148.5-173.5[e]	56	EtOH	E	$C_{12}H_{14}N_2O_3S$
24	CH_3	C_2H_5	Na	Na	>350	100		D	$C_{10}H_8N_2O_3S\cdot 2Na\cdot 2H_2O$
25	CH_3	*i*-C_4H_9	H	C_2H_5	175.0-176.0	52	*i*-PrOH	E	$C_{14}H_{18}N_2O_3S$
26	CH_3	*i*-C_4H_9	K	K	>350	96		D	$C_{12}H_{12}N_2O_3S\cdot 2K\cdot 0.25\text{-}0.75H_2O$
27	CH_3	*n*-C_6H_{13}	H	C_2H_5	134.0-135.0	33	*i*-PrOH	C	$C_{16}H_{22}N_2O_3S$
28	CH_3	*n*-C_6H_{13}	Na	Na	>350	76		D	$C_{14}H_{16}N_2O_3S\cdot 2Na\cdot 0.25H_2O$[d]
29	CH_3	H	H	C_2H_5	151.5-162.0	65	EtOH	C	$C_{10}H_{10}N_2O_3S$
30	CH_3	H	Na	Na	>350	70		D	$C_8H_4N_2O_3S\cdot 2Na\cdot 0.25\text{-}0.75H_2O$
31	CH_3	NO_2	H	C_2H_5	229.0-229.5[f]	82	EtOH	F	$C_{10}H_9N_3O_5S$
32	CH_3	NH_2	H	C_2H_5	199.5-215.0	61	MeOH	G	$C_{10}H_{11}N_3O_2S$
33	CH_3	CH_3CO	H	C_2H_5	236.0-242.0	78	DMF-EtOH	E	$C_{12}H_{12}N_2O_4S$
34	CH_3	CH_3CO	Na	Na	>350	86		D	$C_{10}H_6N_2O_4S\cdot 2Na\cdot 1.5H_2O$
35	NO_2	C_2H_5	H	C_2H_5	200.0-212.0	47	$CHCl_3$-EtOH	F	$C_{11}H_{11}N_3O_5S$
36	NH_2	C_2H_5	H	C_2H_5	181.5-184.5	37	MeOH-*i*-PrOH	G	$C_{11}H_{13}N_3O_3S$
37	NH_2	C_2H_5	Na	Na	>350	52		D	$C_9H_7N_3O_3S\cdot 2Na\cdot 1.5H_2O$
38	I	C_2H_5	H	C_2H_5	188.0-190.0	16	MeOH	H	$C_{11}H_{11}IN_2O_3S$

[a] Footnote *a*, Table I. [b] Footnote *b*, Table I. [c] Lit. (ref 3) mp 219-220 °C. [d] Level of hydration confirmed by Karl Fischer water determination. [e] Melting point 148-150 °C when the bath temperature is taken up rapidly from 25 °C. [f] Uncorrected.

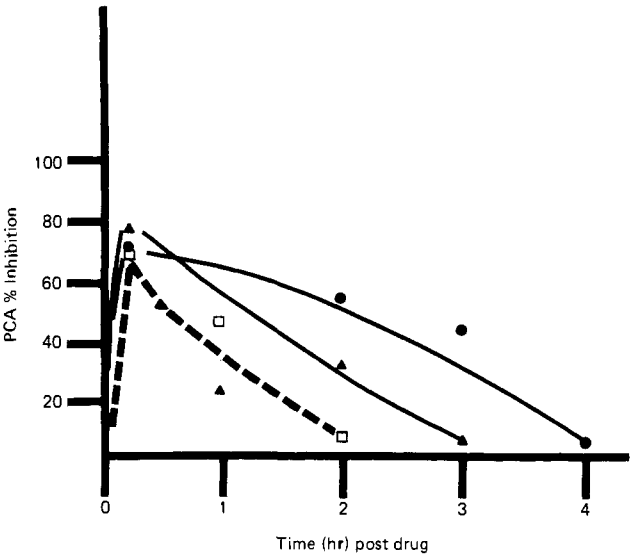


Figure 1. Comparative time–response curves in the rat PCA test for thiophene derivatives administered orally at 30 mg/kg: oxamate 4 (▲), ester **17** (●), and carboxylic acid disalt **26** (□).

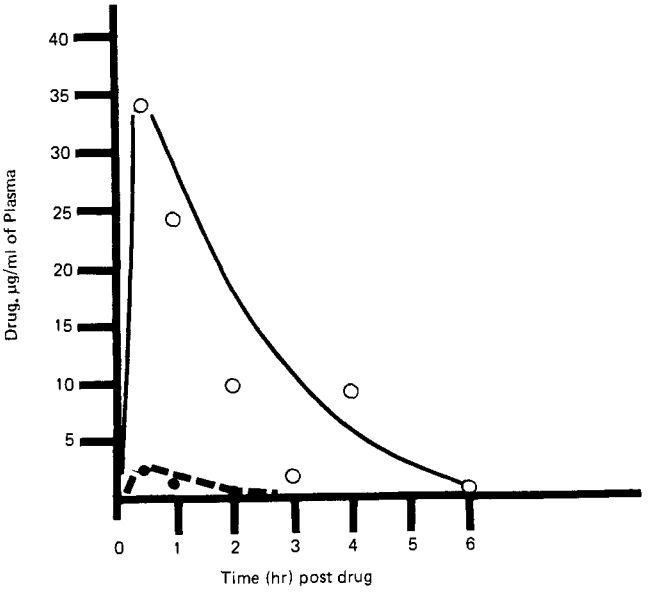
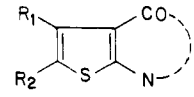


Figure 2. Plasma levels of **17** (●) and **18** (○) in rats following a 50 mg/kg oral dose of **17**.

metabolic conversion of the labile ester to the active carboxylic acid species.

Ester **17** and the corresponding disodium salt **18** were evaluated for in vitro inhibition of histamine release from

Table III. Antiallergic Activity of Thiophene Derivatives [compound, PCA ED_{50}[a-c] (mg/kg PO)]



				thienopyrimidinones	
R_1	R_2	oxamates	oxazines	esters	salts
H	H	4, 4.5 (3.3-6.1), 0.2 (0.09-0.26) iv		15, >25[d]	
H	C_2H_5		6, 34.4 (15.4-76.7)	17, 2.4 (1.5-3.9)	18, 13.0 (4.5-37.9), 1.2 (0-38) iv
H	i-C_4H_9		7, >10[d]	19, 17.9 (7.0-47.8)	20, >10, 1.0 (0.57-1.9) iv
H	n-C_6H_{13}		8, I at 10[d]	21, >5[d]	22, 17.7 (8.0-41.0)
CH_3	H			29, ~50[d]	30, ~50[d]
CH_3	C_2H_5		10, I at 5[d]	23, 11.3 (4.8-26.3)	24, 10.0 (3.0-31.0)
CH_3	i-C_4H_9		11, I at 5[d]	25, 8.1 (3.0-26.0)	26, 2.7 (1.3-5.6), 0.14 (0.1-0.2) iv
CH_3	n-C_6H_{13}		12, 8.2 (4.0-18.0)	27, ~50[d]	
CH_3	NH_2			32, <50[d]	
CH_3	CH_3CO		13, I at 5[d]	33, ~25[d]	34, I at 50[d]
NH_2	C_2H_5			36, ~10[d]	37, I at 25[d]
I	C_2H_5			38, 8.1 (4.0-19.0)	

[a] Approximate ED_{50} values are listed for compounds which gave a statistically significant inhibition by Dunnett's test ($p < 0.05$) in the single-dose experiment employed. [b] Test compound was administered 15 min prior to antigen challenge of the sensitized animals. [c] Under these test conditions, the intravenous ED_{50} for disodium cromoglycate was 0.6 (0.15-2.6) mg/kg. [d] Indicates single-dose determinations.

Table IV. Effects of Compounds 17, 18, and DSCG on the Allergic Release of Histamine from Rat Peritoneal Mast Cells in Vitro

	% inhibn		
compd concn (μM):	1.25	5.0	20
17	19	55	82
18	22	51	73
disodium cromoglycate	20	50	75

rat peritoneal mast cells. These compounds showed activity comparable to that of DSCG in this test (Table IV), indicating that the antiallergenic activity of the thieno[2,3-*d*]pyrimidine-2-carboxylates may be due to inhibition of mediator release.

While the rat PCA test affords a measure of a compound's ability to block allergic reactions in the skin, a more relevant indicator of a drug's potential as a respiratory disease agent is its protective effect against allergic bronchospasm. Several thienopyrimidine derivatives were evaluated for inhibition of allergen-induced bronchospasm in sensitized, spontaneously breathing, anesthesized rats. As shown by the data in Table V, compounds **17** and **28** both displayed dose-related activity following id administration with ED_{50} values of 1.0 and 3.8 mg/kg, respectively. The maximal inhibition by both compounds is statistically equivalent to that of DSCG given by the iv route. Active compounds also inhibited an antigen-induced hypotensive effect in this test.

Experimental Section

Chemistry. Melting points were taken in capillaries utilizing a Thomas-Hoover melting point apparatus and were corrected according to USP XVII, class I. Uncorrected melting points are so indicated. Infrared (IR) spectra were determined on a Beckman IR-18A or IR-9 infrared spectrophotometer, ultraviolet spectra (UV) were obtained on a Beckman DK-2A. Nuclear magnetic resonance (NMR) spectra were determined on a Perkin-Elmer R-32 90-MHz instrument using tetramethylsilane as an internal standard.

Method A. Ethyl *N*-[3-(Aminocarbonyl)-4,5,6,7-tetrahydrobenzo[*b*]thien-2-yl]oxamate (5). A suspension of 72.92 g (0.405 mol) of ethyl 2-amino-3-(aminocarbonyl)-4,5,6,7-tetrahydrobenzo[*b*]thiophene in 200 mL of dry pyridine was stirred at 25 °C, and the addition of 55.25 g (0.405 mol) of ethyloxalyl

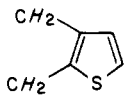
Table V. Inhibitory Effects of Test Drugs (or Vehicle) on Allergic Responses Induced by Egg Albumin (EA) Challenge in EA-Sensitized, Spontaneously Breathing, Anesthetized Rats

					allerg resp			
					bronchospasm	hypotension	% inhibn of control allerg resp,[c] mean ± SE	
drug	dose, mg/kg	route[a]	time,[b] min	no. of anim	% drop of pulmon arter blood press., mean ± SE	% drop in mean arter blood press., mean ± SE	bronchospasm	hypotension
saline[d]	1[h]	iv	1	15	36.4 ± 3.5	49.7 ± 1.9		
methocel[e]	2[h]	id	15	9	32.2 ± 4.7	46.3 ± 3.5		
DSCG	2	iv	1	5	13.0 ± 2.9	38.8 ± 2.4	63.8 ± 8.0[f]	22.4 ± 4.7[f]
28	50	id	15	5	14.0 ± 1.6	45.0 ± 5.7	61.2 ± 4.5[f]	14.0 ± 9.3
28	12.5	id	15	5	21.6 ± 4.6	55.2 ± 3.1	41.0 ± 11.8[f]	0
28	6.25	id	15	5	28.0 ± 8.2	38.4 ± 8.2	34.2 ± 12.7	28.8 ± 4.6
28	3.125	id	15	7	27.6 ± 4.7	47.6 ± 6.6	28.3 ± 10.2[g]	15.4 ± 9.4
28	1.56	id	15	5	42.0 ± 7.6	63.8 ± 5.2	9.4 ± 5.8	0
17	3.125	id	15	7	17.1 ± 4.0	42.4 ± 4.8	53.6 ± 9.9[f]	18.9 ± 7.8
17	0.78	id	15	5	29.2 ± 6.5	51.8 ± 4.0	27.8 ± 11.1	5.2 ± 4.7
17	0.39	id	15	4	48.8 ± 5.6	48.7 ± 0.9	4.3 ± 4.3	2.7 ± 1.8

[a] Intraduodenal (id) and intravenous (iv). [b] Interval between drug administration and EA challenge. [c] Control allergic response is the response after vehicle administration. [d] Saline served as vehicle for DSCG. [e] Methocel served as vehicle for all drugs except DSCG. [f] Significant difference from zero to $p < 0.01$. [g] Significant difference from zero to $p < 0.05$, respectively. [h] In mL/kg.

chloride was initiated. The reaction mixture warmed and was placed in an ice bath during the remainder of the addition and for 30-min thereafter. (A solution formed, followed by a precipitate.) The resulting mixture was stirred at 25 °C for 1 h, and then 150 mL of dry CH_3CN was added to dilute the thick slurry which was stirred overnight. The mixture was further diluted with 1 L of *i*-PrOH, filtered, and dried to give 58.80 g (49%) of yellow powder. Recrystallization from *i*-PrOH gave yellow crystals: mp 204.0–205.0 °C; IR (KBr) 1678 (C=O, ester), 1710 (C=O, amide), 1750 (C=O, oxamate ester) cm^{-1}. Anal. ($C_{13}H_{16}N_2O_4S$) C, H, N.

Method B. Ethyl 5,6,7,8-Tetrahydro-4-oxobenzothieno-[2,3-*d*][1,3]oxazine-2-carboxylate (14). Ethyloxalyl chloride (6.92 g, 0.051 mol) was added dropwise to a partial solution of 2-amino-4,5,6,7-tetrahydrobenzothiophene-3-carboxylic acid (5.0 g, 0.025 mol) in dry pyridine (25 mL) at 0 °C. The mixture was stirred at 25 °C for 1 h and then poured into cold water (1 L). Filtration and air-drying provided a yellow solid (7.00 g, 99%). Recrystallization (*i*-PrOAc) gave a yellow solid, which slowly melted over a broad range (148.5–180.5 °C, corrected). When heated rapidly, the material melts 150–153 °C, resolidifies, and slowly remelts: NMR ($CDCl_3$) δ 4.26 (q, 2 H, OCH_2), 2.82 (m, 4 H, i), 1.78 [m, 4 H, $(CH_2)_2$], 1.36 (t, 3 H, CH_3); IR (KBr) 1640



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(C=C), 1700 (C=N), 1728 (C=O, ester), 1750 (C=O lactone) cm^{-1}. Anal. ($C_{13}H_{13}NO_4S \cdot 0.25H_2O$) C, H, N.

Method C. Ethyl 3,4,5,6,7,8-Hexahydro-4-oxobenzothieno[2,3-*d*]pyrimidine-2-carboxylate (3). A melt of oxamate **5** (8.89 g, 0.03 mol) was magnetically stirred and heated at 260 °C in an oil bath until bubbling (H_2O evolution) was no longer evident. The hot residue was dissolved in DMF (reflux) and the solution was poured into MeOH. The resulting solid was collected and recrystallized from DMF–MeOH to give fine, yellow needles (4.02 g, 48%): mp 207.0–209.0 °C; NMR ($CDCl_3$) δ 12.20 (s, 1 H, NH), 4.63 (q, 2 H, OCH_2), 3.00 (m, 4 H, i), 1.90 [m, 4 H, $(CH_2)_2$], 1.50 (t, 3 H, CH_3); IR (KBr) 1670 (C=O, quinazolinone), 1740 (C=O, ester) cm^{-1}; UV λ_{max} (0.1 N HCl) 255 nm (ε 6700), 348 (17 800); λ_{max} (0.1 N NaOH) 275 nm (ε 10 200), 310 (13 700). Anal. ($C_{13}H_{14}N_2O_3S$) C, H, N.

Method D. 3,4,5,6,7,8-Hexahydro-4-oxobenzothieno[2,3-*d*]pyrimidine-2-carboxylic Acid Disodium Salt Dihydrate (16). A solution of the ester **3** (12.00 g, 0.043 mol) and NaOH (4.0 g, 0.10 mol) in H_2O (440 mL) and EtOH (160 mL) was heated on a steam bath until solution was complete (there is an initial solution followed by the precipitation of quinazoline ester monosodium salt). The solution was stirred at room temperature for 6 h (the product began to precipitate after 2 h) and then filtered to give **16** (10.40 g, 73%) after air-drying: mp >355 °C (note: the addition of *i*-PrOH may be necessary to induce precipitation of the disodium salts of pyrimidine-2-carboxylic acids); NMR (Me_2SO-d_6, CF_3COOH) δ 2.87 (m, 4 H, i), 1.76 [m, 4 H $(CH_2)_2$]; IR (KBr) 1500–1640 (complex pattern of bands, C=O, C=N, C=C, CO_2^-). Anal. ($C_{11}H_8N_2O_3S \cdot 2Na \cdot 2H_2O$) C, H, N.

Method E. Ethyl 3,4-Dihydro-5-methyl-6-(2-methylpropyl)-4-oxothieno[2,3-*d*]pyrimidine-2-carboxylate (25). A mixture of ethyl 5-methyl-6-(2-methylpropyl)-4-oxothieno-[2,3-*d*]oxazine-2-carboxylate (**11**; 5.0 g, 0.017 mol), ammonium acetate (1.1 g, 0.015 mol), and acetic acid (420 mg, 0.007 mol) (excess NH_4OAc is preferred) in EtOH (50 mL) was refluxed for 1 h with stirring. Pouring into ice–water precipitated a light tan solid, which was collected and dried. Recrystallization (*i*-PrOH) gave **25** (2.0 g, 40%): mp 174–175 °C (uncorrected); NMR (Me_2SO-d_6) δ 0.9 [d, 6 H, $(CH_3)_2$], 1.3 (t, 3 H, CH_3), ~1.8 (m, 1 H, CH), 2.4 (s, 3 H, CH_3C=C), 2.7 (d, 2 H, CH_2C=C), 4.4 (q, 2 H, OCH_2), ~12.4 (br, 1 H, NH); IR (KBr) 1660 (C=O, quinazolone), 1725 (C=O, ester) cm^{-1}. Anal. ($C_{14}H_{18}N_2O_3S$) C, H, N.

Method F. Ethyl 3,4-Dihydro-5-methyl-6-nitro-4-oxothieno[2,3-*d*]pyrimidine-2-carboxylate (31). Ethyl 3,4-dihydro-5-methyl-4-oxothieno[2,3-*d*]pyrimidine-2-carboxylate (**29**; 1.0 g, 0.0042 mol) was dissolved in CF_3COOH (10 mL) and Ac_2O (5 mL) was added. The solution was cooled to −15 °C and concentrated HNO_3 (1.2 mL) in CF_3COOH (4 mL) was added dropwise. After stirring the solution at −12 to −15 °C for 10 min, a yellow solid precipitated and H_2O (100 mL) was added. The pale yellow solid was filtered and air-dried to give **31** (1.10 g, 92%), mp 228–229 °C. Recrystallization from EtOH gave 0.98 g (82%) of yellow solid, mp 229–229.5 °C (uncorrected). Anal. ($C_{10}H_9N_3O_5S$) C, H, N.

Method G. Ethyl 6-Amino-3,4-dihydro-5-methyl-4-oxothieno[2,3-*d*]pyrimidine-2-carboxylate (32). A solution of 2.10 g (0.0074 mol) of **31** in dry DMF (100 mL) was hydrogenated in the presence of 1.0 g of 10% Pd/C at 3 atm. The catalyst was removed by filtration and the filtrate added to H_2O (1 L). The yellow solution was extracted with $CHCl_3$ and the $CHCl_3$ layer washed repeatedly with saturated NaCl solution. The $CHCl_3$ solution was dried ($MgSO_4$) and evaporated in vacuo to give **32** as an orange solid (1.13 g, 61%), mp 218–218.5 °C (uncorrected). Recrystallization (MeOH) gave yellow needles in low yield, mp 199.5–215.0 °C. Anal. ($C_{10}H_{11}N_3O_3S$) C, H, N.

Method H. Ethyl 6-Ethyl-3,4-dihydro-5-iodo-4-oxothieno[2,3-*d*]pyrimidine-2-carboxylate (38). The method of Gewald et al.[5] was followed. A mixture of ethyl 6-ethyl-3,4-dihydro-4-oxothieno[2,3-*d*]pyrimidine-2-carboxylate (**17**; 2.65 g, 0.0105 mol) and mercuric acetate (10.60 g, 0.034 mol) in acetic acid (40 mL) was heated at 100 °C for 6 h. The mixture was poured into saturated NaCl solution (200 mL) and filtered to give the 5-chloromercury derivative (4.10 g, 80%), mp 237 °C dec. This material was dissolved in H_2O (150 mL) containing I_2 (4.0 g) and KI (10.0 g), stirred for 3 days, and then filtered to give a dark solid. The solid was washed with EtOH and dried to give 0.27 g of tan solid which exhibited a broad melting point above 240 °C. Recrystallization (MeOH) gave yellow crystals (**38**; 0.50 g, 16%), mp 188.0–190.0 °C. Anal. ($C_{11}H_{11}IN_2O_3S$) C, H, N.

Determination of Blood Levels of Compound 17. Mature Sprague–Dawley rats (150–200 g) were fasted overnight prior to receiving a single 50 mg/kg oral dose of compound **17**. Blood samples were obtained from the orbital sinus at 0.5, 1, 2, 3, 4, and 6 h and at 4 and 6 h by exsanguination following decapitation (two animals/time period). One milliliter of the plasma sample was adjusted to pH 1–2 with 1 N HCl and then extracted twice with 10 mL of $CHCl_3$ for 30 min/extraction. Following centrifugation, the combined extracts for a given sample were taken to dryness under a stream of nitrogen, and the residue was dissolved in 50 μL of methanol and analyzed by high-pressure liquid chromatography using a Waters μBondapak® C-18 reverse-phase column. Adequate separation of compounds **17** and **18** from each other and from endogenous contaminants was achieved with a mobile phase of 51% absolute methanol/49% KH_2PO_4 (0.01 M, v/v). The compounds were detected at 315 mm with a Varian Varicord® variable-wavelength detector. Recovery of these compounds from the control was linear from 1 to 20 μg/mL.

Rat Passive Cutaneous Anaphylaxis (PCA) Test. This test was performed by adaptations of literature procedures.[6,7] Adult male Sprague–Dawley or Wistar rats were injected with egg albumin (EA) im (10 mg/kg) and with killed β-pertussis vaccine (2×10^{10} organisms/rat) iv. The animals were bled 10 to 12 days later to afford antisera containing predominantly IgE antibody.

Male Sprague–Dawley or Wistar rats (100–150 g) were passively sensitized with 0.1-mL id injections of two dilutions of the antiserum in saline. The lower dilution should yield a 20–25-mm diameter spot and the higher one a 10–15-mm diameter spot.

After a 48-h latent period, the rats were treated with either test compound or vehicle and then challenged at an appropriate time. At least five animals were used for each test compound. The PCA response was induced by iv injection of EA (25 mg/kg) and Evans Blue dye (25 mg/kg) in saline. The PCA response was scored by measuring the spot diameter on the excised and reflected skin 20 to 30 min after challenge. Ten minutes prior to sacrifice, histamine was injected id at a dose of 0.1 mg/0.1 mL saline in order to rule out antihistaminic activity of the test compound. Percent inhibition was calculated as the ratio of the difference between mean spot diameters for test drug and control to that of control.

Inhibition of Anaphylactic Histamine Release from Rat Peritoneal Mast Cells. Male Sprague–Dawley rats (200–300

g) were decapitated and injected ip with 10 mL of Hank's balanced salt solution (HBSS) containing 0.1% human albumin and 5 mM potassium phosphate. The peritoneum was massaged for 1 min and the lavage fluid aspirated and centrifuged at 350*g*. The supernatant was discarded and the cell pellets were suspended in 2 mL of rat antisera (prepared as in the PCA test) containing 0.1 mg of heparin. After sensitization by shaking the suspension at 37 °C for 2 h, the cells were centrifuged. The resulting cell pellets were resuspended and diluted to the final working volume in buffered HBSS. Aliquots (1.5 mL) of the sensitized cell suspension were challenged with 0.5 mL of EA (80 mg/mL). Drugs were tested at several different concentrations by adding them simultaneously with the antigen challenge. After antigen and drug additions, the cells were incubated for 10 min and histamine was assayed fluorimetrically. Percent inhibition was determined by comparison with histamine release in the absence of drug.

Inhibition of Allergic Bronchospasm in Sensitized Rats. The methods employed were similar to those previously described for this model.[8,9] Male Harlan–Wistar rats (225–275 g) were sensitized by treatment with egg albumin intramuscularly (1 mg/rat) and β pertussis vaccine ip (2×10^{10} organisms/rat). This procedure generates an immediate hypersensitivity reaction with maximum IgE antibody.[7]

Thirteen to fifteen days after sensitization, the rats were anesthesized with urethane (1.5 g/kg, ip) and the duodenum was exposed through a small abdominal incision. For id administration, drugs in solution or suspension were injected directly into the lumen of the duodenum. A cannulated jugular vein was used for iv drug or EA administration. A cannulated carotid artery was connected to a Statham P23D6 pressure transducer for blood-pressure measurements. The trachea was cannulated and connected to a Statham P23-BB pressure transducer to obtain measurements of pulmonary ventilation.

Rats were challenged with a 2 mg/rat dose of EA (iv), and the changes in pulmonary ventilation pressure and mean arterial blood pressure arising from the ensuing anaphylactic syndrome were measured. Drugs were administered (iv or id) at various time intervals prior to antigen challenge. Drug effects on the antigen-induced syndrome were derived by comparison of responses in individual drug-treated animals to a mean response obtained in a separate group of non-drug-treated (control) animals.

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Antiviral Activity of Aliphatic Nucleoside Analogues: Structure–Function Relationship

E. De Clercq*

Rega Institute for Medical Research, University of Leuven, B-3000 Leuven, Belgium

and A. Holy

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Science, 166 10 Prague, Czechoslovakia.
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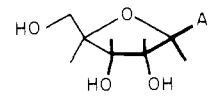
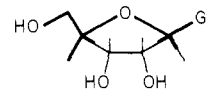
Of a series of 58 aliphatic nucleoside analogues, (*S*)-9-(2,3-dihydroxypropyl)adenine [(*S*)-DHPA] proved to be the most active congener, when assayed for antiviral activity in primary rabbit kidney cell cultures challenged with either vaccinia or vesicular stomatitis virus. Whereas most analogues derived from substituted purine and pyrimidine bases and bearing various hydroxy- or amino-substituted alkyl chains did not show evidence of antiviral activity at a concentration of 2 mM, (*S*)-DHPA inhibited both vaccinia and vesicular stomatitis virus replication at 0.05–0.1 mM. For 9-[(*RS*)-2,3-diazidopropyl]adenine and some di- and trihydroxybutyl analogues of DHPA, viz., 9-[(2*RS*,3*SR*)-2,3-dihydroxybutyl]adenine, 9-[(*RS*)- or 9-[(*S*)-3,4-dihydroxybutyl]adenine, 9-[(2*S*,3*R*)-2,3,4-trihydroxybutyl]adenine, and 3-(adenin-9-yl)-(*RS*)-alanine, an antiviral effect was noted at a concentration of 0.5–1 mM.

Two nucleoside analogues, in which the cyclic carbohydrate moiety was replaced by an acyclic side chain, have recently been reported to possess marked antiviral activity in both cell culture systems and animal models. (*S*)-DHPA, the *S* enantiomer of 9-(2,3-dihydroxypropyl)-adenine, was found to inhibit the replication of a number of DNA and RNA viruses (vaccinia, herpes simplex, vesicular stomatitis, and measles).[1] The related compound 9-[(2-hydroxyethoxy)methyl]guanine (acycloguanosine) proved selectively inhibitory to herpes viruses; acycloguanosine would owe its antiherpes activity to the fact that it is specifically phosphorylated to the monophosphate derivative in herpes-infected cells and that, upon further conversion to the triphosphate, it would inhibit herpes virus DNA polymerization more effectively than cellular DNA synthesis.[2,3]

Both compounds are derived from the natural nucleosides, of which DHPA has preserved the base-C(1)-



(*S*)-DHPA



acycloguanosine

C(2)-C(3) portion, whereas acycloguanosine retained the base-C(1)-O-C(4)-C(5) fragment.

In studies aimed at delineating the structural requirements that underlie the broad-spectrum antiviral activity of (*S*)-DHPA, various aliphatic nucleoside analogues of nucleic acid bases were synthesized and their antiviral potentials were assessed in primary rabbit kidney cell cultures challenged with either an RNA virus (vesicular

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