Antianaphylactic Agents. 1. 2-(Acylamino)oxazoles

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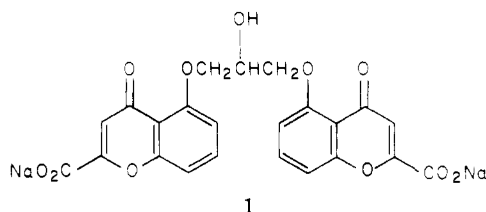
The synthesis and biological properties of 35 2-(acylamino)oxazoles are described. The majority of the compounds inhibit the release of slow-reacting substance of anaphylaxis (SRS-A) in vitro from sensitized guinea pig chopped lung. In addition, several of the compounds inhibited the release of SRS-A from passively sensitized human chopped lung and protected guinea pigs from the effects of anaphylaxis in a modified Herxheimer test.

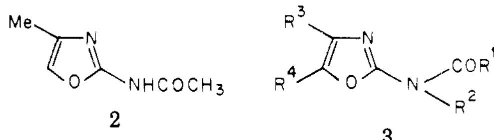
An allergic attack in man is generally considered to be the result of an antigen–antibody combination on the mast cell with subsequent release of the mediators of immediate hypersensitivity, which include histamine, slow reacting substance of anaphylaxis (SRS-A), and various kinins.[1] The mediators then interact with bronchial smooth muscle or mucous membranes eliciting the clinical manifestations of asthma, which include bronchospasm and the occlusion of airways by tenacious mucous plugs.[1] Traditionally, asthma has been treated with drugs which cause relaxation of the bronchial smooth muscle, examples being isoprenaline which is a β-adrenergic stimulant[2] or aminophylline which, being a phosphodiesterase inhibitor,[3] causes an increase in c-AMP levels. If this treatment fails, corticosteroids are generally used.[1] Classical antihistamines alone are not used much in the treatment of the disease, since they do not antagonize the bronchoconstriction induced by SRS-A in smooth muscles.[4] This mediator is probably of greater importance in causing the bronchospasm in asthma than is histamine.[4] However, antihistamines are often useful in the treatment of young asthmatics.[5] The introduction of sodium cromoglycate (1)



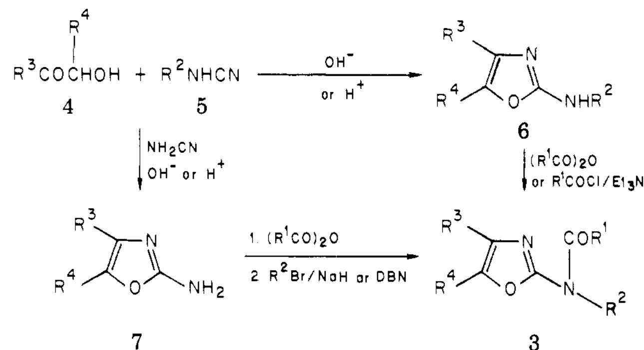
has demonstrated that an agent which inhibits the release of mediators will often give prophylactic protection to the asthmatic patient.[6] However, 1 suffers from the disadvantage of being ineffective when given orally, and our objective was to find compounds which inhibit the release of the mediators of anaphylaxis when taken by mouth. In this paper, we described a series of compounds, chemically unrelated to 1, which are orally active in guinea pigs.

The rat passive cutaneous anaphylaxis (PCA)[7] test is widely used as a primary screen for the detection of antiallergic activity. We have used that test and three others: the in vitro sensitized guinea pig chopped-lung (GPCL) test,[8,9] the passively sensitized human lung test,[10] and the modified Herxheimer test.[11]

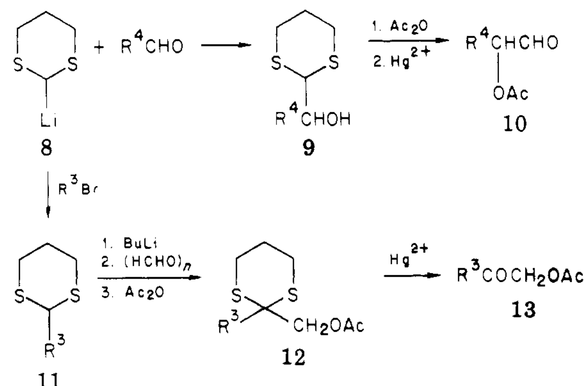
Our starting point was the observation that the 2-(acylamino)oxazole[12] (2) showed some activity in the rat



Scheme I



Scheme II



PCA test when dosed orally at 2 × 100 mg/kg. However, modification of the structure did not lead to other compounds having similar activity, but conversion of the secondary amide to a tertiary amide led to compounds of type 3 which inhibited the release of mediators in the guinea pig chopped-lung test.

None of the compounds of type 3 demonstrated activity in the rat PCA test, although they showed good activity in the other tests. Consequentially, we undertook a systematic investigation of the structural requirements for biological activity.

Chemistry. The substituted 2-aminooxazoles were prepared by acid- or base-catalyzed condensation of α-hydroxy ketones, α-bromo aldehydes, or α-hydroxy aldehydes with cyanamide or substituted cyanamides (Scheme I). The latter compounds were prepared by reaction of excess cyanogen bromide with the appropriate amine, whereas the α-hydroxy ketones and α-hydroxy aldehydes were either purchased or prepared as in Scheme II.

The mechanism involved in the reaction of cyanamides with α-keto alcohols or α-hydroxy aldehydes has been

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Table I. Effect of 2-(Acylalkylamino)oxazoles on Mediator Release from Sensitized Guinea Pig Chopped Lung upon Antigen Challenge

no.	R^1	R^2	yield, %	bp, °C (mmHg)	formula anal.[a]	% inhibn[d] SRS-A	% inhibn[d] histamine
18	Me	Me	54.6	27-29[c]	$C_7H_{10}N_2O_2$	9	0
19	*i*-Pr	Me	87.4	49-50 (0.35)	$C_9H_{14}N_2O_2$	25	61
20	*n*-Pr	Et	41.0	63-64 (0.1)	$C_{10}H_{16}N_2O_2$	13	30
21	*n*-Bu	*n*-Pr	71.9	83-84 (0.2)	$C_{12}H_{20}N_2O_2$	55	48
22	*n*-Pent	*n*-Pr	76.5	96-98 (0.4)	$C_{13}H_{22}N_2O_2$	47	44
23	Me	*n*-Bu	75.4	58-60 (0.1)	$C_{10}H_{16}N_2O_2$	39	26
24	*n*-Pr	*n*-Bu	45.4	96-98 (1.0)	$C_{12}H_{20}N_2O_2$	52	33
25	*n*-Bu	*n*-Bu	78.9	88-91 (0.2)	$C_{13}H_{22}N_2O_2$	49	21
26	*i*-Pr	*n*-Bu	81.0	75-76 (0.15)	$C_{12}H_{20}N_2O_2$	54	38
27	*t*-Bu	*n*-Bu	40.8	89-91 (0.35)	$C_{13}H_{22}N_2O_2$	23	0
28	$CHEt_2$	*n*-Bu	40.3	127 (2.5)	$C_{14}H_{24}N_2O_2$	55	29
29	c-Pr	*n*-Bu	75.6	99-101 (0.7)	$C_{12}H_{18}N_2O_2$	46	22
30	c-Bu	*n*-Bu	68.4	107-108 (0.2)	$C_{13}H_{20}N_2O_2$	74	34
31	c-Pent	*n*-Bu	49.5	109-110 (0.15)	$C_{14}H_{22}N_2O_2$	66	47
32	c-Hex	*n*-Bu	59.5	46.5-48.5[c]	$C_{15}H_{24}N_2O_2$	32	27
33	$PhCH_2$	*n*-Bu	46.8	126-130 (0.2)	$C_{16}H_{20}N_2O_2$	31	0
34	c-Hept	*n*-Bu	46.5	138-141 (1.0)	$C_{16}H_{26}N_2O_2$	49	0
35	*n*-Hex	*n*-Bu	59.1	106-108 (0.5)	$C_{15}H_{26}N_2O_2$	8	nt[b]
36	CH_2-*c*-Pent	*n*-Bu	46.1	124-126 (0.8)	$C_{15}H_{24}N_2O_2$	22	0
37	DL-CH(Me)Et	*n*-Bu	46.2	82-85 (0.2)	$C_{13}H_{22}N_2O_2$	76	54
38	D-(-)-CH(Me)Et	*n*-Bu	36.1	88-92 (0.6)	$C_{13}H_{22}N_2O_2$	63	8
39	L-(+)-CH-(Me)Et	*n*-Bu	43.4	88-91 (0.6)	$C_{13}H_{22}N_2O_2$	79	30
40	Et	*i*-Pr	50.0	65 (0.5)	$C_{10}H_{16}N_2O_2$	17	nt[b]
41	*n*-Bu	*i*-Pr	34.5	77 (0.3)	$C_{11}H_{16}N_2O_2$	0	0
42	*i*-Pr	*i*-Pr	39.5	60-62 (0.4)	$C_{10}H_{14}N_2O_2$	10	nt[b]
43	*n*-Pr	CH(Me)Et	41.0	87 (0.5)	$C_{11}H_{16}N_2O_2$	34	6
44	*i*-Pr	CH(Me)Et	40.0	83 (0.5)	$C_{11}H_{16}N_2O_2$	13	3
45	*n*-Pr	c-Hex	35.0	118 (0.7)	$C_{13}H_{18}N_2O_2$	29	37
46	Et	*n*-Pent	75.6	68 (0.05)	$C_{11}H_{16}N_2O_2$	41	16
47	*i*-Pr	*n*-Pent	74.0	86-87 (0.4)	$C_{12}H_{18}N_2O_2$	66	28
48	Me	*n*-Hex	83.9	90-92 (0.08)	$C_{12}H_{20}N_2O_2$	8	nt[b]
49	*i*-Pr	*n*-Hex	73.8	106-109 (1.0)	$C_{13}H_{20}N_2O_2$	42	18
50	*n*-Pr	$CH_2CH(Me)Et$	58.6	87 (0.5)	$C_{12}H_{18}N_2O_2$	33	35
51	*i*-Pr	$CH_2CH(Me)Et$	70.0	82 (0.8)	$C_{12}H_{18}N_2O_2$	37	29
52	*n*-Pr	$CH_2CH{=}CH_2$	70.0	76 (0.6)	$C_{10}H_{12}N_2O_2$	31	51
53	*i*-Pr	$CH_2CH{=}CH_2$	79.8	68 (0.5)	$C_{10}H_{12}N_2O_2$	23	42
KHF[e]						38	37

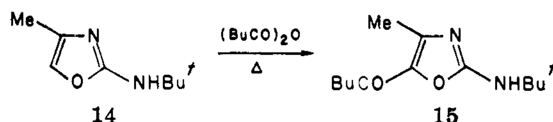
[a] All compounds were analyzed for C, H, and N. [b] Not tested. [c] Melting point. [d] Of release from guinea pig chopped lung at 10 μg/mL. [e] Ketotifen hydrogen fumarate.

discussed in detail in an earlier publication.[13]

The primary aminooxazoles **7** were acylated under standard conditions and the derived amides alkylated using sodium hydride or 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) and an alkyl bromide or iodide. The same products were obtained from the secondary amines, **6**, when acylated under standard conditions (Table I).

The secondary amines were generally only characterized by IR and NMR because of their instability to light and air. Examples of the various methods of preparation of the compounds described in this paper are given under the Experimental section.

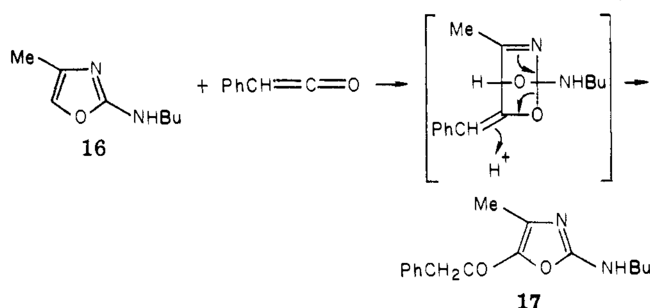
When R^2 was bulky, e.g., *tert*-butyl (**14**), then acylation



of the 5 position of the oxazole ring supervened, yielding mainly the ketone **15**, the structure of which was confirmed by NMR.

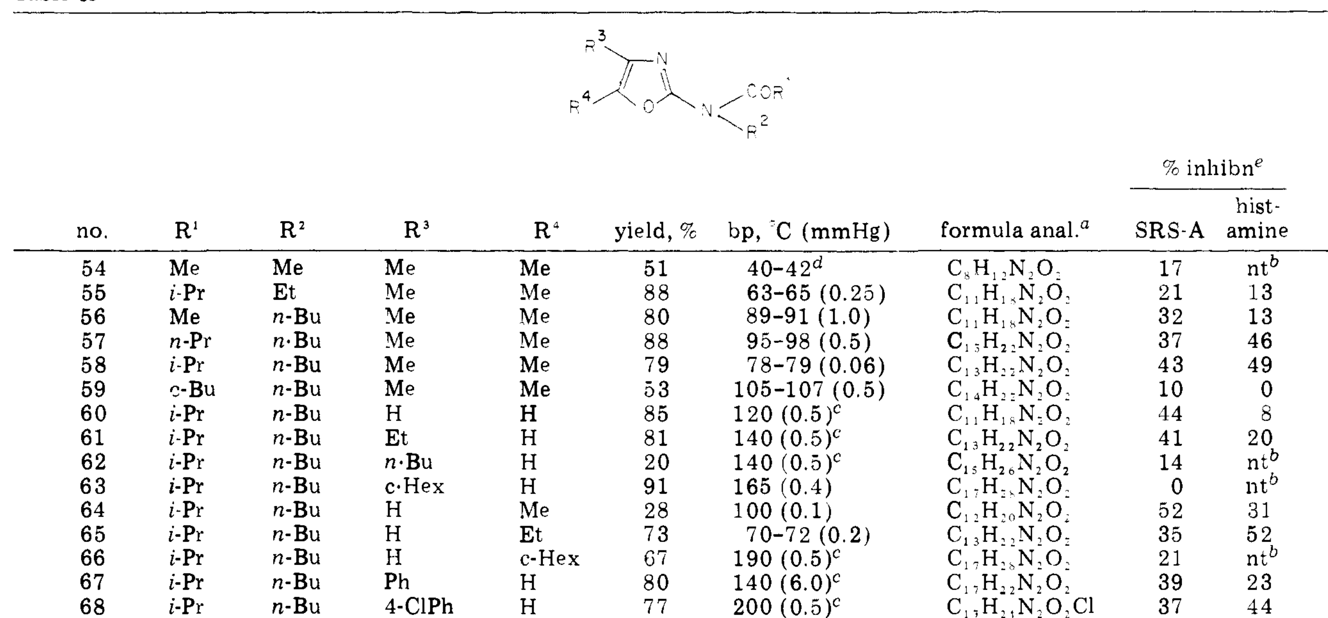
This is not altogether unexpected, since the presence of a 2-amino function activates the 5 position of the ring to electrophilic attack.[14]

More surprising, since steric factors are unlikely to be involved, was the formation of ketone **17** when 2-(butyl-



amino)-4-methyloxazole (**16**) was acylated using phenylacetyl chloride in the presence of triethylamine (TEA). The compound may arise by a mechanism postulated by Crank[15] for the formation of a 5-[*N*-(methylthio)carbamyl]oxazole from a 2-aminooxazole and methyl

Table II



no.	R¹	R²	R³	R⁴	yield, %	bp, °C (mmHg)	formula anal.[a]	% inhibn[e] SRS-A	% inhibn[e] histamine
54	Me	Me	Me	Me	51	40-42[d]	$C_8H_{12}N_2O_2$	17	nt[b]
55	*i*-Pr	Et	Me	Me	88	63-65 (0.25)	$C_{11}H_{18}N_2O_2$	21	13
56	Me	*n*-Bu	Me	Me	80	89-91 (1.0)	$C_{11}H_{18}N_2O_2$	32	13
57	*n*-Pr	*n*-Bu	Me	Me	88	95-98 (0.5)	$C_{15}H_{22}N_2O_2$	37	46
58	*i*-Pr	*n*-Bu	Me	Me	79	78-79 (0.06)	$C_{13}H_{22}N_2O_2$	43	49
59	c-Bu	*n*-Bu	Me	Me	53	105-107 (0.5)	$C_{14}H_{22}N_2O_2$	10	0
60	*i*-Pr	*n*-Bu	H	H	85	120 (0.5)[c]	$C_{11}H_{18}N_2O_2$	44	8
61	*i*-Pr	*n*-Bu	Et	H	81	140 (0.5)[c]	$C_{13}H_{22}N_2O_2$	41	20
62	*i*-Pr	*n*-Bu	*n*-Bu	H	20	140 (0.5)[c]	$C_{15}H_{26}N_2O_2$	14	nt[b]
63	*i*-Pr	*n*-Bu	c-Hex	H	91	165 (0.4)	$C_{17}H_{28}N_2O_2$	0	nt[b]
64	*i*-Pr	*n*-Bu	H	Me	28	100 (0.1)	$C_{12}H_{20}N_2O_2$	52	31
65	*i*-Pr	*n*-Bu	H	Et	73	70-72 (0.2)	$C_{13}H_{22}N_2O_2$	35	52
66	*i*-Pr	*n*-Bu	H	c-Hex	67	190 (0.5)[c]	$C_{17}H_{28}N_2O_2$	21	nt[b]
67	*i*-Pr	*n*-Bu	Ph	H	80	140 (6.0)[c]	$C_{17}H_{22}N_2O_2$	39	23
68	*i*-Pr	*n*-Bu	4-ClPh	H	77	200 (0.5)[c]	$C_{17}H_{21}N_2O_2Cl$	37	44

[a] All compounds were analyzed for C, H, N. [b] Not tested. [c] Air bath temperature. [d] Melting point. [e] Of release from guinea pig chopped lung at 10 μg/mL.

isothiocyanate. In our case, phenylketene is formed from phenylacetyl chloride and TEA and reacts with the aminooxazole in a Diels-Alder–retro-Diels-Alder fashion to give the observed ketone.

Small amounts of the N-acylated products were formed in both cases.

Discussion

The structure **3** can be varied at the four points R^1, R^2, R^3 and R^4.

Initially, in order to simplify the problem, we kept R^3 constant as a 4-methyl substituent on the oxazole ring and varied the acyl and alkyl functions on the amino group. In varying R^1 and R^2 we used the Topliss operational scheme[16] for side chains but included a somewhat larger sample than strictly necessary.

All the compounds listed in Tables I and II were tested at 2 × 100 mg/kg po in the rat PCA test but none were active. However, the compounds exhibited activity in the guinea pig chopped-lung test (GPCL) described below, and observations and conclusions are drawn from those results. In this test, the ability of a compound to inhibit the release of slow-reacting substance of anaphylaxis (SRS-A) and histamine from sensitized guinea pig lung fragments upon antigen challenge is assessed.

The first three compounds, **18–20**, showed marginal activity, but increasing R^2 to *n*-propyl and R^1 to butyl (**21**) or amyl (**22**) caused a significant increase in the activity.

Changing R^2 to butyl and altering R^1 to methyl to give **23** caused a reduction in activity.

Increasing the R^1 function to propyl or butyl and keeping R^2 as butyl gave **24** and **25**, which had a similar activity to **21** and **22**. Activity was not improved by altering R^1 to isopropyl (**18**). However, compounds **21**, **22**, and **24–26** would have similar π values and, based on the improvement of activity of this group over compounds **18–20** and **23**, it would appear that a $+\pi$ effect plays an important role in the biological activity.

With this in mind, we kept R^2 constant as butyl and varied R^1. Further branching of R^1 to give the *tert*-butyl derivative **27** reduced activity. This implied that a steric factor involving R^1 had come into play but the 1-ethylpropyl analogue **28** was equiactive with **24–26**. In accordance with the Topliss scheme, we decided to investigate compounds where R^1 is a cycloalkyl function, since these groups have the advantage of maximizing the possibility of hydrophobic bonding while minimizing unfavorable steric influences.

The cyclopropyl compound **29**, as expected, had similar activity to **26**, but the cyclobutyl compound **30** and cyclopentyl compound **31** were somewhat more active. However, the activity of cyclohexyl compound **32** was rather low, as was the benzyl compound **33**, although the cycloheptyl derivative **34** possessed similar activity to **26**. It thus appeared that optimum activity in this group resides in **30**.

In order to determine whether or not chirality played a role in the activity, compounds **37–39** were prepared but found to be essentially equiactive with **30**.

We now turned our attention to the R^2 substituent and prepared a number of derivatives in which this function was branched. However, the compounds **40–44** displayed only marginal activity. It would appear from the group **45–48** that when R^2 is a long straight chain alkyl group then the best activity is observed with R^1 as isopropyl. Terminal branching of R^2 or the introduction of an olefinic function did not improve activity, as illustrated with **49–52**.

We examined compounds where R^3 and R^4 were altered (Table II). In the examples with R^3 and R^4, both methyl (**54–59**), and choosing suitable R^1 and R^2 functions from Table I, it can be seen that only moderate activity was observed. It thus appears that fully substituting the oxazole ring while not abolishing activity entirely has a detrimental effect upon it. When R^3 and R^4 are hydrogen (**60**), activity is retained, as is the case when R^3 is extended to ethyl (**61**), but falls dramatically when R^3 is altered to butyl (**62**) or cyclohexyl (**63**).

Three compounds, **64–66**, were prepared with R^3 as H and R^4 an alkyl substituent. These compounds tended to confirm the observation that the methyl group is the best alkyl ring substituent whether placed in the 4 or 5 position of the oxazole ring.

Only two 4-aryl substituted oxazoles, **67** and **68**, were prepared and both possessed moderate activity. However, due to difficulties in the synthesis of such compounds, this approach was not pursued.

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Table III. Effect of 2-(Acylamino)oxazoles on Mediator Release from Passively Sensitized Human Lung upon Antigen Challenge

	% inhibn[b]			% inhibn[b]	
no.	SRS-A	histamine	no.	SRS-A	histamine
21	38	0	**36**	0	0
22	47	44	**37**	36	5
24	88	14	40	0	0
26	93	23	41	0	0
27	23	37	42	0	0
29	72	0	**47**	47	14
30	55	0	48	15	13
31	69	0	**58**	43	49
32	26	29	Ket[a]	100	23

[a] Ket, ketotifen. [b] Of release from human lung at 10 μg/mL.

Although the figures for the reduction in release of histamine are given in Tables I and II, no consistent pattern is discernible for this mediator, and our conclusions, based on the SRS-A results in the GPCL test, are the following: (a) the oxazole ring should be substituted in the 4 or 5 position by a small alkyl group, preferably methyl; (b) the 2-amino function should carry a moderately long straight chain *n*-alkyl substituent, preferably butyl; (c) the *N*-acyl function should be small and branched, preferably isopropyl or cyclobutyl. We attempted to correlate the activity of compounds in the GPCL test with the lipophilic and steric parameters of R^1 and R^2, but without success.

In the guinea pig lung test, the immunoglobulin IgG, (γ_1) is involved in the release of mediators on antigen challenge, whereas it is generally accepted that in bronchial asthma the immunoglobulin concerned is IgE. In order to ascertain the activity of the aminooxazoles in a system involving IgE, we passively sensitized, with human serum containing antibodies to grass pollen, surgical specimens of human lung[10] and tested the ability of the compounds to inhibit the release of mediators upon antigen challenge. The results are shown in Table III. Although it is difficult to draw conclusions on structure–activity relationships, it is clear that the compounds follow the general trends seen in the GPCL test. The variation in results probably reflects the quality of the lung tissue rather than an intrinsic defect of the test system.

Finally, to enable us to demonstrate antiallergic activity in an in vivo situation, we tested a number of the compounds in a modified Herxheimer test, the details of which are described below. Table IV lists the results obtained. The index $(D + M)/M$ gives a rough order of potency for the compounds, and it is considered that those compounds with an index >1.3 demonstrate useful activity. (See Experimental Section for definition of terms.) Because some animals are fully protected in this test procedure, it is not possible to assign confidence limits to the data.

However, it should be noted that compounds **23**, **26**, **29**, **30**, **37**, **46**, **49**, and **50** show moderate to good activity in the guinea pig lung screen and, in addition, compounds **26**, **29**, and **30** have moderate to good activity in the human lung screen.

For comparative purposes, the results obtained in our tests with ketotifen hydrogen fumarate, a recently introduced antiallergic compound,[18] are shown in Tables I–IV.

We conclude from this that the acylaminooxazoles described in this paper represent a new class of antiallergic compounds which warrant investigation under clinical conditions.

Table IV. Effect of 2-(Acylalkylamino)oxazoles on Bronchospasm in Concious Sensitized Guinea Pigs upon Antigen Challenge (Hexheimer)

no.	dose, mg/kg po	M^a	D^b	$M + D^c$	$\frac{M + D^d}{M}$
21	2 × 100	5.1	0.8	5.8	1.1
22	2 × 100	4.4	1.4	4.7	1.1
24	2 × 100	4.6	4.3	6.4	1.4
26	2 × 100	3.9	4.6	5.9	1.5
27	2 × 100	6.4	4.4	7.7	1.2
29	2 × 100	9.7	12.2	13.9	1.4
30	2 × 100	3.8	1.5	7.4	1.9
Ket[e]	1 × 2	3.7	4.2	7.2	1.9

[a] Mean collapse time after pretreatment with mepyramine/mean collapse time of control animals. [b] Mean collapse time after pretreatment with compound/mean collapse time of control animals. [c] Mean collapse time after pretreatment with compound and mepyramine/mean collapse time of control animals. [d] Ratio of *c*/*a*. [e] Ket, ketotifen.

Experimental Section

Melting points were taken on a Gallenkamp apparatus using capillaries and are uncorrected. All compounds were characterized by IR, UV, NMR, and elemental analyses (C, H, N), which were within ±0.4% of the theoretical values. Solvent extracts were dried using anhydrous $MgSO_4$ and evaporated under reduced pressure (water pump) using a Buchi Rotavap. A Buchi Kugelrohr air bath was used for distillation of products where noted.

***n*-Butylcyanamide (5, R^2 = Bu).** Cyanogen bromide (94.6 g, 0.89 mol) in dry ether (200 mL) was stirred with anhydrous sodium carbonate (200 g, 1.88 mol) and cooled at −20 to −10 °C during the addition of $BuNH_2$ (88 g, 0.88 mol) over 1 h. Stirring was continued for a further hour as the temperature rose to 0 °C. The mixture was then filtered and evaporated to leave a colorless oil, yield 84 g (96%).

A sample of the above (50 g) was distilled under reduced pressure to give a colorless mobile liquid, bp 100 °C (2 mmHg).

Since alkylcyanamides tend to polymerize but can be stabilized in the presence of cyanogen bromide, it was important that the above reaction was carried out with an excess of cyanogen bromide (e.g., up to 1.4%, w/w). In the presence of this stabilizing agent, the BuNHCN could be stored at room temperature with little or no decomposition.

When subsequent reactions were to be conducted in aqueous media, the cyanamide was prepared in a suitable water–miscible solvent (preferably THF) and the filtered solution used.

2-(Butylamino)-4-methyloxazole (6, R^2 = Bu; R^3 = Me; R^4 = H). (a) Base-Catalyzed Procedure. A mixture of BuNHCN (13 g, 0.13 mol) and anhydrous hydroxyacetone (9.7 g, 0.13 mol) in THF (25 mL) was stirred during the dropwise addition of 2 N NaOH solution (70 mL, 0.14 mol). The temperature rose spontaneously to 35 °C. After the addition was complete, stirring was continued at room temperature for a further 2 h.

Water (100 mL) was then added and the product extracted into ether. The extract was washed with saturated brine, dried, and evaporated to leave a pale yellow oil.

Distillation gave a colorless oil: 14.6 g (73%); bp 80 °C (0.5 mmHg). Anal. ($C_8H_4N_2O_2$) C, H, N.

(b) Acid-Catalyzed Procedure. Hydroxyacetone (2.25 g, 0.03 mol) in cold (10 °C) concentrated hydrochloric acid (2.75 mL) was treated with BuNHCN (3 g, 0.03 mol). After the addition (5 min), the cooling bath was removed and the temperature allowed to rise to 40 °C. One hour later, the solution was poured into cold 5 N NaOH (50 mL) and the product isolated by extraction into ether. The extract was washed with brine, dried, and evaporated to leave a yellow oil, 3.5 g. Distillation gave a colorless product, 2.6 g (56%), which was identical with the product obtained as above.

2-Methyl-*N*-butyl-*N*-(4-methyloxazol-2-yl)propanamide (26). A solution of 2-(butylamino)-4-methyloxazole (106.7 g, 0.69 mol) and triethylamine (110 g, 0.77 mol) in dry benzene (1500 mL) was stirred during the addition of 2-methylpropanoyl chloride (81.0 g, 0.76 mol). The mixture was stirred at room temperature for 15 h and then water (1 L) was added. After stirring the mixture

for a further 1 h, the organic phase was separated and the aqueous phase extracted twice with Et_2O. The combined extract was washed successively with 2 N HCl (2 × 500 mL), 10% sodium carbonate solution (2 × 500 mL), and saturated brine (2 × 500 mL). Evaporation of the dried ($MgSO_4$) organic phase gave an oil, which was distilled to give the product, 118 g (80%), bp 75–76 °C (0.15 mmHg), which existed as an oil at room temperature but crystallized on cooling to 0 °C. Anal. ($C_{12}H_{20}N_2O_2$) C, H, N.

2-Amino-4-methyloxazole (7, R^3 = Me; R^4 = H). NaOH (5 N, 125 mL) was added dropwise to a stirred solution of hydroxyacetone (74 g, 1 mol) and NH_2CN (42 g, 1 mol) in water (110 mL). The mixture rapidly became hot and was cooled to 20 °C, stirred at this temperature for 1 h and then extracted with ether (3 × 250 mL). The ether extract was washed with brine, dried, and evaporated, and the residue was distilled under reduced pressure to give the product as a colorless oil: 76.8 g (78%); bp 67–69 °C (0.5 mmHg); n_D^{23} 1.495. Anal. ($C_4H_6N_2O$) C, H, N.

2-Ethyl-*N*-(4-methyloxazol-2-yl)butanamide (3, R^2 = R^4 = H; R^3 = Me; R^1 = Et_2CH). A stirred solution of 2-amino-4-methyloxazole (8.8 g, 0.089 mol) and 2-ethylbutanoic anhydride (19.0 g, 0.089 mol) in toluene (50 mL) was heated under reflux for 2 h. The cooled solution was washed with sodium carbonate solution and then brine, dried, and evaporated. The solid residue was crystallized from EtOAc–petroleum ether (60–80 °C), giving white crystals: 10.1 g (58%); mp 106 °C. Anal. ($C_{10}H_{16}N_2O_2$) C, H, N.

2-Methyl-*N*-(5-methyloxazol-2-yl)propanamide (3, R^1 = $CHMe_2$; R^2 = R^3 = H; R^4 = Me). 2-Methylpropanoic anhydride (5.90 g, 0.0372 mol) was added to a solution of 2-amino-5-methyloxazole[17] (3.30 g, 0.0336 mol) in dry C_6H_6 (40 mL), and the mixture was heated under reflux for 3 h. Methanol (5 mL) and triethylamine (5 drops) were then added, and the mixture was heated for a further 30 min. The mixture was then cooled and washed with water (2 × 20 mL), 10% aqueous Na_2CO_3 (3 × 25 mL), and water (3 × 20 mL). The organic phase was dried and evaporated, and the residue was crystallized from hexane, giving colorless needles: 1.44 g (25.5%); mp 109–109.5 °C. Anal. ($C_8H_{12}N_2O_2$) C, H, N.

2-Methyl-*N*-butyl-*N*-(5-methyloxazol-2-yl)propanamide (3, R^1 = Me_2CH; R^2 = Bu; R^3 = H; R^4 = Me). The above amide (2.1 g, 0.0124 mol) was dissolved in dry DMF (10 mL) and cooled to 5 °C, and NaH (50% oil dispersion, 1.4 g, 0.029 mol) was added portionwise with stirring. When addition of the NaH was complete, the temperature was allowed to rise to room temperature and BuI (5.0 g, 0.0271 mol) was added. The mixture was left overnight at room temperature, then the solvent was evaporated under reduced pressure, and the residue was dissolved in ether (50 mL), washed with water (3 × 25 mL), 10% Na_2CO_3 (3 × 25 mL), and water (25 mL), and dried. Removal of the solvent gave a clear oil, which was distilled in the Kügelrohr apparatus to give the product as a clear oil: 1.74 g (62%); bp 100 °C (0.1 mmHg). Anal. ($C_{12}H_{20}N_2O_2$) C, H, N.

2-(*tert*-Butylamino)-4-methyl-5-pentanoyloxazole (15). A solution of 2-(*tert*-butylamino)-4-methyloxazole (8.3 g, 0.054 mol) and pentanoic anhydride (14.0 g, 0.076 mol) in dry toluene (50 mL) was heated under reflux for 28 h. Methanol (20 mL) and triethylamine (1 mL) were added, and refluxing was continued for a further 1 h. Excess methanol was removed under vacuum, and the residual solution was washed with 2 N HCl solution and brine, dried, and evaporated to dryness. The residue (11.5 g) was distilled under vacuum, and the fraction (7.8 g) boiling at 120–130 °C (1.0 mmHg) crystallized from petroleum ether (bp 60–80 °C) to give the product as colorless prisms: 5.6 g (50%); mp 60 °C. Anal. ($C_{13}H_{22}N_2O_2$) C, H, N.

2-(Butylamino)-4-methyl-5-(phenylacetyl)oxazole (17). Phenylacetyl chloride (11.2 mL, 0.085 mol) was added dropwise to a stirred solution of 2-(butylamino)-4-methyloxazole (10.9 g, 0.071 mol) and triethylamine (11.8 mL, 0.085 mol) in dry benzene (100 mL), and the stirred mixture was heated under reflux for 5 h, cooled, and filtered. The filtrate was washed with sodium carbonate solution and water, dried, and evaporated to give a brown oil. Trituration of this oil with petroleum ether (bp 60–80 °C) deposited a solid (11.8 g), which was twice recrystallized from CCl_4–petroleum ether (bp 60–80 °C) to give needles: 4.4 g (23%); mp 81–82 °C. Anal. ($C_{16}H_{20}N_2O_2$) C, H, N.

2-(Phenylacetamido)-4-methyloxazole (3, R^1 = $PhCH_2$; R^2 = R^4 = H; R^3 = Me). Phenylacetyl chloride (29 mL, 0.22 mol) was added dropwise to a stirred solution of 2-amino-4-methyloxazole (20 g, 0.2 mol) and triethylamine (30 mL, 0.22 mol) in dry benzene (200 mL) at 5–10 °C, and the mixture was stirred for 2 h at room temperature. The pale solid was filtered off and dissolved in ethyl acetate (1.5 L) and washed with water (300 mL). The solvent layer was washed further with water (300 mL), dried, and evaporated, and the residue was recrystallized from ethyl acetate: 17.1 g (39.5%); mp 177 °C. Anal. ($C_{12}H_{12}N_2O_2$) C, H, N.

2-(*N*-Butylphenylacetamido)-4-methyloxazole (33). 1,5-Diazabicyclo[4.3.0]non-5-ene (19.5 mL, 0.165 mol) was added dropwise to a stirred suspension of the phenylacetamide (10.0 g, 0.046 mol) and butyl iodide (13 mL, 0.11 mol) in dry benzene (400 mL) at room temperature. The resulting solution was stirred for 16 h, washed with water, dilute HCl, Na_2CO_3 solution, and brine, dried, and evaporated. The residue was distilled under reduced pressure to give the product as a clear oil: 8.3 g (66%); bp 126–130 (0.2 mmHg). Anal. ($C_{16}H_{20}N_2O_2$) C, H, N.

α-Cyclohexyl-2-(hydroxymethyl)-1,3-dithiane (9, R^4 = c-C_6H_{11}). A solution of BuLi (44 mL, 8% w/v in heptane) was added dropwise to a stirred solution of 1,3-dithiane (5 g, 0.056 mol) in dry THF (50 mL) maintained at −30 °C, and cyclohexane carboxaldehyde (4.7 g) in dry THF (10 mL) was added slowly. The temperature was allowed to rise to 20 °C over 2 h and then water was added, and the product was extracted into ether (3 × 100 ml) and dried. Removal of the solvent gave an oil which crystallized from ether–petroleum ether (bp 40–60 °C) to give prisms: 6.8 g (70%); mp 64–65 °C. Anal. ($C_{11}H_{20}S_2O$) C, H, S.

α-Cyclohexyl-2-(acetoxymethyl)-1,3-dithiane. The above compound (6.8 g) was converted into the acetoxy derivative using excess acetic anhydride in pyridine at ambient temperature for 16 h. Removal of the solvent and excess reagent in vacuo gave an oil which crystallized as white prisms from petroleum ether: bp 40–60 °C; 6.7 g (85%); mp 84–85 °C. Anal. ($C_{13}H_{22}S_2O_2$) C, H, S.

Cyclohexylacetoxyacetaldehyde (10, R^4 = c-C_6H_{12}). A solution of α-cyclohexyl-2-(acetoxymethyl)-1,3-dithiane (6.0 g, 0.022 mol) in MeCN (300 mL) was added to a mixture of $HgCl_2$ (19.2 g, 0.07 mol) and freshly prepared cadmium carbonate (11.6 g, 0.067 mol). Water (4 mL) was added and the mixture stirred at 50 °C under N_2 for 36 h. The solvent was removed in vacuo, and the residue was extracted with benzene (2 × 100 mL) and chloroform (100 mL). The organic extract was evaporated to dryness and the residue distilled in the Kugelrohr apparatus to give the product as a clear oil: 3.9 g (95%); bp 120 °C (air bath) (0.5 mmHg). Anal. ($C_{10}H_{16}O_3$) C, H, N.

Cyclohexyl Acetoxymethyl Ketone (13, R^3 = c-C_6H_{11}). Cyclohexylcarboxaldehyde (22.4 g, 0.2 mol) and propanedithiol (21.0 g 0.2 mol) in $CHCl_3$ (300 mL) were added slowly to a solution of $BF_3{\cdot}Et_2O$ (24 mL) and AcOH (48 mL) in $CHCl_3$ (80 mL), and the mixture was heated under reflux for 1 h. On cooling, the solution was washed with 2 N NaOH (4 × 100 mL) and water (2 × 100 mL) and dried. Removal of the solvent and distillation under reduced pressure gave 2-cyclohexyl-1,3-dithiane as a clear yellow oil, 25.1 g (65%), bp 100 °C (0.5 mmHg), which crystallized, mp 51–53 °C (11, R^3 = cyclohexyl).

The above dithiane (26.0 g, 0.13 mol) in dry THF (200 mL) was cooled to −30 °C, and a solution of BuLi in hexane (60 mL, 15%, w/w, solution) was added dropwise. Stirring and cooling at −30 °C were maintained for 2 h and then paraformaldehyde (4.0 g, 0.14 mol) was added portionwise. The temperature of the mixture was allowed to rise to 0 °C overnight and then water (100 mL) was added. The THF was removed under reduced pressure and the residue extracted with ether (2 × 100 mL). The ethereal extract was washed with brine (2 × 50 mL) and water (1 × 50 mL) and dried. Removal of the solvent gave 2-cyclohexyl-2-(hydroxymethyl)-1,3-dithiane as a colorless oil, 31 g, which was immediately converted to the acetoxy derivative using excess acetic anhydride and pyridine. Distillation gave the product as a clear oil: 18.8 g; bp 138–141 °C (1 mmHg).

The acetoxy compound 12 (R = cyclohexyl; 18.5 g, 0.068 mol) in MeCN (400 mL) and water (10 mL) was added to a stirred suspension of $HgCl_2$ (38.4 g, 0.14 mol) and freshly prepared $CdCO_3$ (23 g, 0.13 mol) in MeCN, and the mixture was stirred at 50 °C

for 36 h under nitrogen. The solvent was removed and the solid residue extracted with C_6H_6 (3 × 100 mL) and once with $CHCl_3$ (100 mL). Evaporation of the dried extract and distillation of the residue gave the product as a clear oil: 9.7 g (78%); bp 150 °C (air bath) (0.5 mmHg). The oil crystallized on standing to give prisms, mp 32–34 °C. Anal. ($C_{10}H_{16}O_3$) C, H, N.

Biological Methods. Cutaneous Vascular Permeability. Female Wistar rats were shaved, lightly anaesthetized, and injected intradermally in the abdominal skin with 0.05 mL of diluted rat antiovalbumin IgE serum. After 24 h, the rats were challenged intravenously with ovalbumin mixed with dye. Compounds (2 × 200 mg/kg) were administered at 2 h and again at 30 min before challenge. Forty-five minutes after challenge, the rats were killed, the ventral skins were removed, and the blue area on the inner surface was measured.[7]

The Guinea Pig Chopped-Lung Test. This test is based on the method of Mongar and Schild[8] and Brocklehurst.[9] Male albino guinea pigs (250–350 g) were sensitized with ovalbumin (100 mg sc + 100 mg ip) and, 3 weeks later, killed; their lungs were removed and perfused clear of blood with Tyrode's solution. The lungs were chopped into 0.5-mm cubes and divided into 100-mg aliquots. After washing and equilibration at 37 °C, the aliquots were challenged with antigen in the presence or absence of the test compound. One control group was not challenged and was used to assess spontaneous mediator release. Following incubation with rocking at 37 °C for 15 min, each supernatant was removed and bioassayed. Results are expressed as the percentage inhibition of mediator release. Each result is the mean of four samples and the usual variance is 5–10%.

Bioassay. Guinea pig ileum was suspended in Tyrode's solution at 37 °C. Contraction of the tissue was recorded with a Harvard isotonic transducer with a loading on the tissue of 1 g. Standard responses to histamine or partially purified SRS-AGP were obtained, and supernatants of the lung were assayed by interpolation.

Human Chopped-Lung Test (Passive Sensitization). Samples of apparently healthy human lung obtained at surgery for bronchial carcinoma were washed in Hank's solution and chopped into 0.5-mm cubes. The tissue was incubated for 18 h at 18 °C with atopic serum from patients allergic to cocksfoot pollen. The chopped tissue was washed and equilibriated in Tyrode's solution at 37 °C before being challenged with saline extract of the pollen in the presence or absence of the test compound. The design of the experiment and the assay procedures were as described for guinea pig chopped lung. The method is similar to that described by Sheard, Killingback, and Blair.[10]

Modified Herxheimer Test. The test is based on the method described by Herxheimer.[11] Male guinea pigs were sensitized with ovalbumin and then exposed to ovalbumin aerosol (1% w/v solution) in an observation chamber. The animals were kept in the exposure chamber until symptoms of respiratory distress, terminating in a characteristic convulsive cough, were observed. The time of exposure is the "collapse time". Exposure beyond this end point resulted in convulsions and death from severe bronchospasm. The "protection ratio" of a compound alone (D) was defined as: mean collapse time after pretreatment with compound/mean collapse time of control animals.

Histamine is known to play a significant part in the observed bronchospasm, and pretreatment with antihistamines such as mepyramine reveals that a nonhistamine component, probably SRS-A, is responsible for the remainder of the response. Accordingly, the response to each compound in the presence of mepyramine (0.5 mg/kg sc), given 0.5 h before challenge, was studied. The compound itself was either given orally 2 h before antigen challenge or in two doses 0.5 and 3 h before challenge.

The "protection ratio" of mepyramine alone (M) was defined as with compound alone, mepyramine-treated animals being substituted for compound-treated animals. The protection ratio ($M + D$) was defined as: mean collapse time after pretreatment with mepyramine + compound/mean collapse time of control animals.

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