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SHORT COMMUNICATION

Analogues of 4-(3,4-Dimethoxybenzyl)-2-imidazolidinone as Potent Inhibitors of Rat Erythrocyte Adenosine Cyclic 3′,5′-Phosphate Phosphodiesterase

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SUMMARY

A number of analogues of 4-(3,4-dimethoxybenzyl)-2-imidazolidinone (Ro 7-2956), a hypotensive and lipolytic agent, have been tested for their ability to inhibit rat erythrocyte phosphodiesterase (adenosine 3′,5′-cyclic phosphate, adenosine 5′-phosphate phosphohydrolase, EC 3.1.4.c). Elongation of the alkyl group in ether linkage with the oxygen at position 3 increased activity, whereas the reverse was true with the oxygen at position 4. The most potent compound, the 3-butoxy-4-methoxy derivative, was 5000 times more potent than theophylline. Both isomers derived from the asymmetrical carbon of the imidazolidinone ring were quite active, but opening of the ring markedly reduced activity, and addition of a carboxyl group yielded an almost inactive compound. This series introduces a new and potent group of inhibitors of phosphodiesterase which, in addition to their physiological effects, may be useful in probing the inhibitory site of the enzyme.

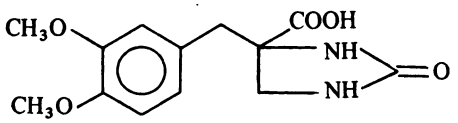
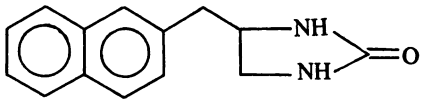
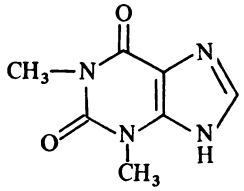
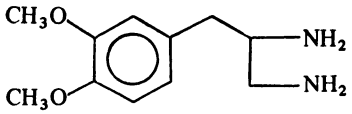
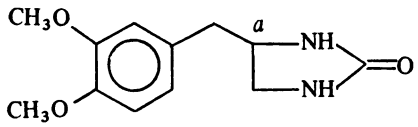
Evidence has been presented (1) that 4-(3,4-dimethoxybenzyl)-2-imidazolidinone (Ro 7-2956) is an agent which stimulates lipolysis in isolated fat cells, acts synergistically with adenosine cyclic 3′,5′-phosphate in this regard, and is somewhat more potent than theophylline as an inhibitor of fat cell lysate phosphodiesterase (adenosine 3′,5′-cyclic phosphate adenosine 5′-phosphate 3′-phosphohydrolase, EC 3.1.4.c). The agent was also found to produce positive inotropic and chronotropic effects along with peripheral vasodilation even in the presence of the *beta* receptor blocking agent propranolol (2).

This report describes the activity of several analogues of Ro 7-2956 which have been tested for inhibition of a phosphodiesterase present in a hemolysate of rat erythrocytes (3). It was found that increasing the length of the alkyl group attached to the oxygen at position 3 enhanced the activity whereas the reverse was true for the oxygen at position 4. The 3-butoxy derivative was found to be 5000 times more potent than theophylline.

Rat blood was collected and the erythrocytes were washed and hemolyzed as described previously (3). The activity of the phosphodiesterase was determined by measuring the disappearance of cyclic AMP-8-^{3}H and the appearance of 5′-AMP-^{3}H and adenosine-^{3}H as described previously (1). The cyclic AMP-8-^{3}H (1 μCi/79 pmoles) was diluted to give a specific activity of 1 μCi/2500 pmoles and a concentration of 5 μM per incubation mixture. All analyses were run in quadruplicate, and each incuba-

Table 1

Activity of a variety of compounds as inhibitors of rat erythrocyte phosphodiesterase

Compound	Structure	I_{50} μM
I		6800
II		1400
Theophylline		500
III		420
Ro 7-2956 *dl*-		12
IV	*d*-Isomer of Ro 7-2956	21.5
V	*l*-Isomer of Ro 7-2956	6.3

[a] Asymmetrical carbon.

tion contained approximately 1.5 mg of protein.

By plotting the logarithm of three concentrations of the drug which would inhibit the enzyme linearly from 20 to 80%, it was possible to determine graphically the concentration which inhibited the hydrolysis of cyclic AMP by 50% (I_{50}).

The substances used in this study were adenosine cyclic 3′,5′-phosphate-8-^{3}H, Schwarz BioResearch; adenosine cyclic 3′,5′-monophosphate, adenosine 5′-phosphate, and adenosine, Sigma Chemical Company; theophylline, Hoffmann-La Roche, Inc.; and Ro 7-2956 and its analogues, which were synthesized by Dr. M. Hoffer, Hoffmann-La Roche, Inc.

The concentration of theophylline which was found to inhibit rat erythrocyte phosphodiesterase by 50% was 0.5 mM (Table 1). Ro 7-2956 was found to be about 42 times more potent than theophylline in

this system. The addition of a carboxyl group to the imidazolidinone ring, as in compound I, yielded a very weak inhibitor. Opening the imidazolidinone ring to give the diamine (III) reduced the activity, yielding a compound which was only a little more potent than theophylline. The replacement of the two methoxy groups by an aromatic ring (II) also reduced the activity to yield a compound which was about one-third as active as theophylline.

Ro 7-2956 is a racemic mixture of two enantiomers. Both isomers are active, and the *l*-form (V) is approximately 3.4 times more active than the *d*-form (IV).

Manipulation of the substituents on the aromatic ring yielded the results obtained in Table 2. With hydroxyl groups in place of the methoxy groups at positions 3 and 4 (VI) no activity could be detected. Methylation of the 3-hydroxyl group resulted in a compound (IX) which was almost 10 times more potent than theophylline. Subsequent methylation of the 4-hydroxyl groups (Ro 7-2956) increased the activity an additional 4-fold. If the 4-methoxy was replaced by an ethoxy group (X) the activity fell. Substitution of an ethanoloxy group (XI) for the 4-ethoxy increased the activity slightly. Substitution of an ethoxy for a methoxy group at position 3 (XIV), however, markedly enhanced the activity. The activity was increased further with an isopropoxy (XV) and even further with a butoxy group (XVI), for which the I_{50} was 0.1 μM.

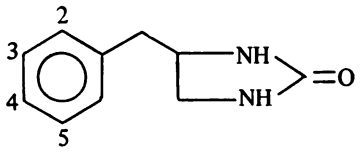
In agreement with the reduced activity seen with elongation of the alkyl chain attached to the oxygen at position 4, compound XIII, with an ethoxy group at position 4, had less activity than XIV, even though both had the ethoxy group at position 3. It was also observed that the presence of the ethanoloxy group at position 3 yielded a compound (XII) which was less potent than XIV but more potent than Ro 7-2956.

The addition of a third methoxy group at position 5 (VIII) markedly lowered activity. All activity was lost if the 4-methoxy group was transferred to position 2 (VII).

Linearity of hydrolysis was maintained over the 15-min period in the presence of 0.5 μM compound XVI and 50 μM Ro 7-2956. In addition, prior incubation of these in-

TABLE 2

Potency of various substituted 4-(benzyl)-2-imidazolidinones as inhibitors of rat erythrocyte phosphodiesterase



Compound	Substituent at position				I_{50}
	2	3	4	5	
					μM
VI	H	OH	OH	H	Inactive
VII	OCH_3	OCH_3	H	H	Inactive
VIII	H	OCH_3	OCH_3	OCH_3	3200
IX	H	OCH_3	OH	H	54
X	H	OCH_3	OC_2H_5	H	32
XI	H	OCH_3	OCH_2CH_2OH	H	24
Ro 7-2956	H	OCH_3	OCH_3	H	12
XII	H	OCH_2CH_2OH	OCH_3	H	9
XIII	H	OC_2H_5	OC_2H_5	H	2.3
XIV	H	OC_2H_5	OCH_3	H	0.7
XV	H	$OCH(CH_3)_2$	OCH_3	H	0.5
XVI	H	OC_4H_9	OCH_3	H	0.1

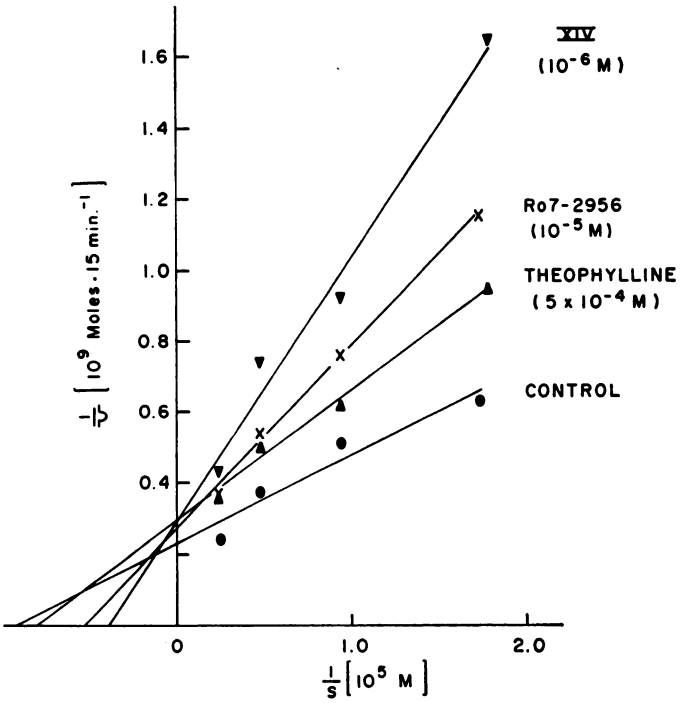


FIG. 1. *Inhibition of rat erythrocyte adenosine cyclic 3',5'-phosphate diesterase by theophylline, dl-4-(3,4-dimethoxybenzyl)-2-imidazolidinone (Ro 7-2956), and dl-4-(3-ethoxy-4-methoxybenzyl)-2-imidazolidinone (XIV)*

Lineweaver-Burk analyses of inhibitors of the rat erythrocyte phosphodiesterase. Each point represents the mean of quadruplicate determinations. The curves represent the best fit, obtained by the method of least squares.

hibitors with either substrate or enzyme failed to alter the extent of inhibition.

Lineweaver-Burk plots (Fig. 1) showed that the K_m for the substrate was 10.9 μM and the V_{max} was 4.3 nmole/15 min. The nature of the inhibition with theophylline, Ro 7-2956, and compound XIV was found to be of a mixed type. The absence of pure competitive kinetics was also evident from plots of $1/v$ with respect to inhibitor concentration.

Among the compounds which have been described as inhibitors of cyclic AMP phosphodiesterase, one can find the methylxanthines (4), purine and pyrimidine derivatives (5), benzothiadiazines (6), reserpine, and phenothiazines (7). The work presented here introduces another class of inhibitors which differ chemically from the compounds mentioned above. Inhibition of the enzyme cyclic AMP phosphodiesterase is one recognized mechanism for increasing the levels of cellular cyclic-AMP (8), and this could account for the cardiac stimulating (2) and lipolytic effects (1) of the parent compound, Ro 7-2956.

The structure-activity relationships which have been uncovered in these studies display a surprising specificity at the level of the methoxy groups attached to the aromatic ring. In essence, these studies demonstrate that elongation of the alkoxy group at position 3 (*meta*) of the aromatic ring increases the inhibition while elongation at position 4 (*para*) decreases the activity. In all probability, the *m*-alkoxy group projects in an axial plane opposite from that of the *p*-methoxy and imidazolidinone groups. While several models of drug-enzyme interaction could be constructed, the simplest view suggests that there exists a hydrophobic cavity on the surface of the enzyme

which has a high degree of acceptance for the *m*-butoxy group. The resultant hydrophobic binding would help to position the remainder of the molecule for increased inhibition. Elongation of the carbon chain at the *para* position provides a group which could also enter the postulated hydrophobic cavity but which positions the rest of the molecule for decreased inhibition. It is suggested that there exists a competition between the *p*- and *m*-alkoxy groups for the hydrophobic regions of the enzymes.

That the imidazolidinone group is also important for inhibition is evident from the observation that opening of the ring reduces potency appreciably whereas the addition of a carboxyl group eliminates activity completely. The slight spatial change in position of the imidazolidinone group which is seen in the *d*- and *l*-isomers of Ro 7-2956 has relatively little effect on the inhibitory potency of this compound. Both enantiomers are quite active, and the *l*-form is 3.4 times more active than the *d*-isomer.

It is apparent that the nature of the inhibition by two imidazolidinones and theophylline is rather complex, being of neither a purely competitive or a noncompetitive type. Competitive kinetics has been reported for methylxanthines with partially purified preparations from rat brain and beef heart (7, 9), and noncompetitive kinetics with preparations from dog heart and frog erythrocytes (10, 11). The nature of the inhibition therefore may depend on the relative purity and source of the enzyme. The presence of an activator (12) or multiple forms of the enzyme (13) could probably contribute to the nature of the enzyme kinetics that we observed.

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