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THE ANTIBACTERIAL ACTIVITY OF SOME FLUORINE-CONTAINING BENZIMIDAZOLES

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Abstract—A series of fluorine-containing benzimidazoles has been tested for antibacterial activity against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Klebsiella aerogenes* and *Escherichia coli*. The highest activities were shown by compounds which had a perfluoroalkyl group at position 2 and one or more electron attracting groups in the benzene ring. The most active compound of the series was 2, 4, 5-tristrifluoromethyl benzimidazole, which *in vitro* inhibited *Staph. aureus* at 0·97 μg/ml and *Strep. pyogenes* at 0·24 μg/ml. No antagonism was shown by vitamin B_{12} or purines against the activity of 4, 5-bistrifluoromethylbenzimidazole.

THE similarity in chemical structure of benzimidazoles to purines and the presence of 5, 6-dimethylbenzimidazole in vitamin B_{12} has led to the study of benzimidazoles as possible antimetabolites. Thus Woolley[1] found that the inhibitory action of benzimidazoles against *Saccharomyces cerevisiae* was antagonised by adenine and guanine. Several workers[2–4] have shown that certain benzimidazoles have antibacterial activity and that this activity is greatest with chloro and nitro derivatives. In addition it has been shown that certain chlor- and nitro-benzimidazoles are antimetabolites of vitamin B_{12}.[5] Since it appeared that the presence of electron attracting groups enhanced antibacterial activity in this series of compounds, it was of interest to study benzimidazoles which contain the highly electron-attracting fluoro and perfluoroalkyl groups.

EXPERIMENTAL

Benzimidazoles

These were synthesised as described elsewhere[6] unless otherwise indicated in the table. Non-fluorine-containing benzimidazoles are included for comparison.

Antibacterial activity

The following organisms were used: *Staphylococcus aureus* (NCTC 7447), *Streptococcus pyogenes* (NCTC, 8322), *Klebsiella aerogenes* (NCTC 8172), and *Escherichia coli* (NCTC 86). The compounds were dissolved in ethanol (10 mg/ml) and the solution diluted with sterile nutrient broth (Oxoid bacteriological peptone (1% w/v), Oxoid Lab Lemco (0·5% w/v), NaCl (0·5% w/v) 39 vol.) to give a solution which contained 250 μg/ml. Doubling dilutions were made from this in sterile nutrient broth. Many of the compounds were not soluble at 250 μg/ml so dilutions were made from an appropriate concentration at which they were soluble. After checking for sterility, the solutions (1 ml) were inoculated with a 3-mm loopful of a 1:1000 dilution of a 24-hr culture of the organism in nutrient broth and incubated at 37° for 24 hr. The minimum

inhibitory concentration was taken as that concentration at which no growth was visible to the naked eye. Suitable controls showed that the presence of the ethanol did not affect the growth of the organisms at the concentrations used. The results are shown in Table 1.

The effect of potential antagonists on the activity of 4, 5-*bistrifluoromethyl benzimidazole*

The effect of vitamin B_{12} on the activity of 4, 5-bistrifluoromethylbenzimidazole was studied with *Staph.aureus* (NCTC 7447) and *Sarcina lutea* (BUCD 23) grown in a medium containing Oxoid vitamin-free casein hydrolysate (1% w/v) and NaCl (0·25% w/v), and *Klebsiella aerogenes* (NCTC 8172) grown in a mineral–salt–glucose medium [$(NH_4)_2$ SO_4, (0·5%); $MgSO_4$, $7H_2O$ (0·1%); Na_2 H PO_4 12 H_2O (1·73%); KH_2PO_4 (2·7%); glucose (5%)] as the test organisms. The minimum inhibitory concentration of the compound (after 48 hr growth at 37°) was in all three cases, 31·2 μg/ml. Vitamin B_{12} (125 μg/ml) had no effect on this activity. Similarly, guanine, adenine and histidine, each at a concentration of 125 μg/ml had no antagonistic effect on the inhibitory activity of 4, 5-bistrifluoromethylbenzimidazole against *Sarcina lutea* nor did guanine or adenine mixed with the same concentration of vitamin B_{12}.

In-vivo *experiments*

These were carried out by Glaxo Laboratories Ltd. and showed that 2,4-bistrifluoromethyl benzimidazole, 2, 5-bistrifluoromethylbenzimidazole, and 2, 4, 5-tristrifluoromethylbenzimidazole were inactive against *Staphylococcus aureus* in mice. The latter two compounds, but not the first were toxic at the 1 mg/mouse (50 mg/kg) dose level. The acute intraperitoneal toxicity of 5-nitro-2-trifluoromethylbenzimidazole was found to be between 25 and 50 mg/kg.

RESULTS AND DISCUSSION

The fluorine-containing benzimidazoles which show activity (minimum inhibitory conc. 62·5 μg/ml) have certain common features. Each has the 2-position substituted with a perfluoroalkyl group and one or more electron attracting groups in the benzene ring. Thus these results are in accord with those of other workers[2–4] that the presence of electron attracting groups is necessary for high activity. 2-Hepta-fluoropropyl compounds have higher activity than the corresponding 2-trifluoromethyl compounds, except in the case of the 5-nitro derivatives, but they had lower water-solubility.

Benzimidazoles containing a water solubilising group (hydroxymethyl, carboxyl or amino) in addition to a perfluoroalkyl group showed only low activity.

2-Difluoromethyl-4-trifluoromethylbenzimidazole is much less active than 2:4. bistrifluoromethylbenzimidazole and this suggests that a group at least as large or as electron attracting as a trifluoromethyl group substituted at position 2, is necessary for high activity. For 2-trifluoromethyl derivatives it appears that electron attracting substitutents at the 5-position confer the highest activities, and that the effectiveness is in the order $CF_3 > NO_2 \simeq Cl$. In the case of the 2-heptafluoropropyl derivatives the effectiveness of substituents at position 5 is in the order $Cl > CF_3 > NO_2$. It would have been of interest to determine the activity of compounds with a nitro group at position 2 but these could not be synthesized. The highest activity was

obtained with a derivative containing three electron attracting groups namely 2; 4, 5, tristrifluoromethyl-benzimidazole. However 4; 5, 6, 7 tetrafluoro-2-trifluoromethyl-benzimidazole was less active despite the presence of an electron attracting trifluoromethyl group and four electron attracting fluorine atoms.

TABLE 1. ANTIBACTERIAL ACTIVITY OF BENZIMIDAZOLES

Benzimidazole		Minimum inhibitory conc. (μg/ml) against			
		Staph. aureus	*Str. pyogenes*	*K.aerogenes*	*E.Coli*
Unsubstituted		*i*	*i*	*i*	*i*
5-Nitro-		*i*	*i*	*i*	*i*
5-Amino-		*i*	*i*	*i*	*i*
2-Hydroxymethyl-		*i*	*i*	*i*	*i*
5, 6-Dicarboxy-		*i*	*i*	*i*	*i*
2-Trifluoromethyl-	a	*i*	125	*i*	*i*
2-Pentafluoroethyl-		125	15·6	125	125
2. Heptafluoropropyl-		Solubility < 15·6, inactive at this conc.			
4-Trifluoromethyl-	a	*i*	250	*i*	250
5-Trifluoromethyl-	a	*i*	62·5	250	250
2-Methyl-4.trifluoromethyl-	a	*i*	125	*i*	*i*
2-Methyl-5-trifluoromethyl-		250	*i*	*i*	*i*
4-Methyl-2-trifluoromethyl-		250	*i*	250	250
2-Heptafluoropropyl-4-methyl-		Solubility < 15·6, inactive at this conc.			
2-Phenyl-4-trifluoromethyl-	a	250	31·2	*i*	250
5-Methoxy-2-trifluoromethyl-		*i*	250	250	250
2-Difluoromethyl-4-trifluoro-methyl-		125	125	250	*i*
2, 4-Bistrifluoromethyl-		62·5	15·6	62·5	62·5
2, 5-Bistrifluoromethyl-		10·0	3·9	15·6	15·6
2-Heptafluoropropyl-4-trifluoromethyl-		3·9	1·9	Solubility < 15·6, inactive at this conc.	
2-Heptafluoropropyl-5-trifluoromethyl		1·9	3·9	Solubility < 62·5, inactive at this conc.	
4, 5, 6, 7-Tetrafluoro-2-trifluoromethyl-	b	7·8	7·8	125	125
4, 5-Bistrifluoromethyl-	c	62·5	7·8	250	125
2-Methyl-4, 5-bistrifluoromethyl-	c	31·2	31·2	62·5	*i*
2, 4, 5-Tristrifluoromethyl-	c	0·97	0·24	31·2	62·5
4, 5-Dimethyl-2-trifluoromethyl-		250	*i*	250	250
5, 6-Dimethyl-2-trifluoromethyl-		125	125	250	*i*
4, 5-Benz-2-trifluoromethyl-		62·5	31·2	62·5	62·5
5-Nitro-2-trifluoromethyl-		15·6	7·8	31·2	31·2
2-Heptafluoropropyl-5-nitro-		31·2	31·2	*i*	*i*
5-Chloro-2-trifluoromethyl-		15·6	15·6	15·6	15·6
5-Chloro-2-Heptafluoropropyl-		1·95	1·95	31·2	15·6
2-Amino-4-trifluoromethyl-		250	*i*	*i*	*i*
2-Amino-5-trifluoromethyl-		125	62·5	125	125
2-Amino-4, 5, 6, 7-tetrafluoro-	b	250	250	250	125
2-(3H)-4-trifluoromethylbenzimidazalone		*i*	125	*i*	*i*
2-Hydroxymethyl-4-trifluoromethyl-		*i*	*i*	*i*	*i*
2-Hydroxymethyl-5-trifluoromethyl-		*i*	*i*	*i*	*i*
2-Carboxy-4-trifluoromethyl-		*i*	*i*	*i*	*i*
2-Carboxy-5-trifluoromethyl-		*i*	125	*i*	*i*
4-Carboxy-2-trifluoromethyl-		*i*	*i*	*i*	*i*

Compounds synthesised as given in Ref. 6, except a, synthesised as in Ref. 7; b, as in Ref. 8; c, as in Ref. 9. *i* = inactive at $\leqslant$ 250 μg/ml.

The mode of action of these compounds is as yet unknown. It was apparent however that the activity of 4, 5.bistrifluoromethyl benzimidazole was not due to it being an antimetabolite of vitamin B_{12}, guanine, adenine or histidine. There does seem to be some specificity in the action of the compounds as shown in most cases by a higher

activity against the two gram positive organisms. The lack of activity *in vivo* could be attributed to the low solubility of the most active compounds.

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