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THE ANTITUMOUR ACTIVITY OF A SERIES OF BENZOTHIOPHENES

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Abstract—The monofunctional halogenoalkylamine and 5-HT antagonist 3-(N-β-chloroethyl-N-ethyl aminomethyl) benzothiophene HCl, was tested against experimental tumours. It proved to have high antitumour activity but was also very toxic. Only one compound, 5-bromo-3-(N-β-chloroethyl-N-ethyl aminomethyl) benzothiophene HCl, of a series of analogues had greater antitumour action combined with acceptable toxicity. The extent of the antitumour activity was surprising in view of the general acceptance that alkylating agents require at least two functional side chains for effective antitumour activity.

HALOGENOALKYLAMINES such as dibenamine and dibenzyline are related to the potent antitumour agents, the nitrogen mustards, in that they possess a β-chloroethylamine side chain. The benzothiophene halogenoalkylamine AGN 1319 (Table 1), which, like dibenamine and dibenzyline, has strong adrenolytic properties[1] and a β-chloroethylamine side chain, was also found to possess anti-5-hydroxytryptamine activity.[2] This activity together with the general resemblance to the nitrogen mustards suggested that this compound ought to be examined for antitumour action.

Preliminary screening showed that AGN 1319 possessed useful antitumour activity, but that this was associated with considerable toxicity. A series of related benzothiophenes was therefore made[3] and examined to see if the toxicity could be dissociated from the antitumour property.

MATERIALS AND METHODS

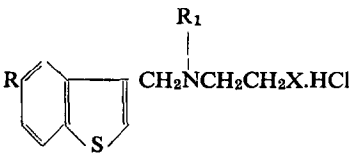
All compounds were tested against the Crocker sarcoma, (S180) and the mouse leukaemia L1210. Where the results warranted further testing, the effect on other experimental tumours was also investigated. In general conventional methods of testing were used[4] and the size of the groups was based on the statistical analyses and conclusions of Schneiderman.[5]

Mice of one sex, and weighing between 18 g and 22 g were used in each experiment. Solid tumours were implanted as fragments of tissue weighing about 30 mg; they were placed subcutaneously in the flank by means of a Bashford needle. For tests on ascitic tumours 0·2 ml ascitic fluid was inoculated intraperitoneally into each animal. The leukaemia L1210 was transplanted subcutaneously as a suspension of spleen cells from an animal carrying this leukaemia.

After implantation, animals were randomly distributed into groups of not less than 6. The first dose of the compound being tested was administered 24 hr later and successive doses were given as indicated in Table 2.

Compounds were normally given at maximum tolerated doses, these having previously been determined in acute toxicity tests. They were administered either as

TABLE 1. ACTIVITY OF SOME BENZOTHIOPHENES AGAINST S180 MOUSE TUMOUR



$\frac{T}{C} = \frac{\text{Mean weight of treated tumours}}{\text{Mean weight of untreated tumours}}$ For significance. $\frac{T}{C} \leqslant 0{\cdot}53$;

$\left(\frac{T}{C}\right)1 \times \left(\frac{T}{C}\right)2 \leqslant 0{\cdot}19$; $\left(\frac{T}{C}\right)1 \times \left(\frac{T}{C}\right)2 \times \left(\frac{T}{C}\right)3 \leqslant 0{\cdot}07$

where $\left(\frac{T}{C}\right)1$, $\left(\frac{T}{C}\right)2$, $\left(\frac{T}{C}\right)3$ are the $\left(\frac{T}{C}\right)$ values of three successive tests

Compound (AGN)	R	R_1	X	$\left(\frac{T}{C}\right)1$	$\left(\frac{T}{C}\right)1 \times \left(\frac{T}{C}\right)2$	$\left(\frac{T}{C}\right)1 \times \left(\frac{T}{C}\right)2 \times \left(\frac{T}{C}\right)3$
1319	H	C_2H_5	Cl	0·50	0·21	0·09
1318	H	C_2H_5	OH	1·3	—	—
1614	H	$(CH_3)_2CH$	Cl	1·5	—	—
1616	H	$(CH_3)_3C$	Cl	0·9	—	—
1618	H	$Ph.CH_2$	Cl	1·1	—	—
1314	Cl	C_2H_5	Cl	0·71	—	—
1312	Cl	C_2H_5	OH	0·94	—	—
1602	Cl	$(CH_3)_2CH$	Cl	0·57	—	—
1604	Cl	$(CH_3)_3C$	Cl	0·51	0·24	—
1606	Cl	$Ph.CH_2$	Cl	0·84	—	—
1414	Br	C_2H_5	Cl	0·43	0·14	0·06
1412	Br	C_2H_5	OH	0·72	—	—
1608	Br	$(CH_3)_2CH$	Cl	0·77	—	—
1610	Br	$(CH_3)_3C$	Cl	1·2	—	—
1612	Br	$Ph.CH_2$	Cl	1·4	—	—
1621	F	C_2H_5	Cl	0·36	0·20	0·13
1623	F	$(CH_3)_2CH$	Cl	1·1	—	—
1625	F	$(CH)_3C$	Cl	0·7	—	—
1627	F	$Ph.CH_2$	Cl	1·1	—	—
1431	CH_3	C_2H_5	Cl	0·51	0·39	—

freshly prepared aqueous solutions, or as suspensions in 0·5% carboxymethylcellulose in normal saline (C.M.C.), or in corn oil. Control animals received solvent or suspending agent only. Administration was normally by the intraperitoneal route unless otherwise indicated. Food and water were given *ad libitum* throughout the experiments.

Solid tumours were excized and weighed, usually on the 8th day following implantation. The weight of ascitic tumours was determined by weighing the animals before and after removal of ascitic fluid. Survival of leukaemic animals was noted by counting the survivors daily until all animals were dead.

TABLE 2. INHIBITION PRODUCED BY THE TWO MOST ACTIVE BENZOTHIOPHENES AGAINST S180 MOUSE TUMOUR

Number (AGN)	Dose mg/kg	Vehicle	Route	Dosed on days	No. survivors Test	No. survivors Control	% Animal weight change Test	% Animal weight change Control	$\frac{T}{C}$
1319	30	Aq. dist.	i.p.	1, 3, 5, 7	8/15	15/15	−8	+17	0·50
	30	Aq. dist.	i.p.	1, 3, 5, 7	15/15	15/15	0	+26	0·42
	30	Aq. dist.	i.p.	1, 3, 5, 7	10/14	14/14	−2	+16	0·46
1414	62·5	Aq. dist.	i.p.	1, 3, 5, 7	12/15	15/15	−5	+17	0·43
	62·5	Aq. dist.	i.p.	1, 3, 5, 7	14/15	14/14	+1	+24	0·34
	62·5	Aq. dist.	i.p.	1, 3, 5, 7	14/15	15/15	+2	+26	0·40
	62·5	Aq. dist.	i.p.	1, 3, 5, 7	8/9	10/10	−7	+22	0·21
	62·5	Aq. dist.	i.p.	1, 3, 5, 7, 9	8/8	9/9	+7	+43	0·69
	100 × 1 then 62·5 × 3	Aq. dist.	i.p.	1, 3, 5, 7	12/20	13/13	−15	+34	0·42
	90·0	Aq. dist.	i.p.	1, 3, 5, 7	7/13	15/15	−19	+10	0·44
	125·0	Aq. dist.	i.p.	1, 3, 5, 7	3/6	6/6	−5	+5	0·20
	62·5	Aq. dist.	S.C.	1, 3, 5, 7	15/15	15/15	−19	+10	0·57
	500	CMC	Oral	1, 2, 3, 4, 5, 7	7/9	9/9	−4	+12	0·42
	400	CMC	Oral	1, 2, 4, 7, 8	5/9	9/9	+19	+36	1·1
	40	Aq. dist.	i.v.	1, 3, 5, 7	10/10	9/9	+7	+3	0·9
	100	Aq. dist.	i.v.	1, 2, 3, 4, 5, 6, 7 continuous infusion	11/12	6/6	+11	+18·5	0·75
	30	Aq. dist.	i.v.	6, 7, 8, 9	15/15	15/15	—	—	0·64

In one experiment the effect of compound AGN 1414 (Table 2) on an established tumour was determined by starting administration 6 days after implantation. The effects of constant blood levels were also studied by a method of continuous infusion[6] into the tail veins of tumour-bearing mice over a period of 7 days.

Tumours used were (with strain of mouse carrying them in parentheses):

a. The Crocker sarcoma, S180 (Swiss)
b. Adenocarcinoma Ca755 (C57B1)
c. Lymphoid leukaemia L1210 (BDF_1)
d. Ehrlich ascites carcinoma (Swiss)
e. Sarcoma (ascitic form) BP8/Pl (C_3H/He)

A few tests were also made with AGN 1414 only against:

f. The Walker 256 carcinosarcoma maintained in rats.

These tumours are maintained by weekly aseptic transplantation.

Animals were weighed daily and signs of toxicity such as general appearance, weight loss, diarrhoea, and change of behaviour were noted. The number of survivors was also recorded daily.

Results for solid and ascitic mouse tumours are expressed as T/C, where T is the arithmetical mean of the tumour weights of treated animals, and C is the arithmetical mean of the tumour weights of control animals. In the case of the leukaemia, the results are expressed as mean survival time of test group divided by mean survival time of control group. Mean survival times are calculated according to the formula adopted by the Cancer Chemotherapy National Service Center for this tumour.[4]

Peripheral blood obtained from the tail veins of mice treated with 62·5 mg/kg AGN 1414 for 8 days was examined for abnormalities. Blood films were stained by conventional methods. Counts of red and white cells were also made.

In one experiment the effect of 5-HT 250 mg/kg (as creatinine sulphate) alone and in combination with AGN 1414 was investigated.

RESULTS

Preliminary results obtained with the first compound to be tested, compound AGN 1319, indicated that it might have promising tumour inhibitory properties. The toxicity of this compound was however, considerable. Examination of analogues of AGN 1319 showed that many of them inhibited the growth of S180 to some extent, but compound AGN 1414 showed the greatest inhibitory activity combined with the least toxicity. More exhaustive testing of this compound against S180 and other tumours was therefore undertaken.

When tested at maximum tolerated doses against S180, AGN 1414 consistently gave T/C values lower than the limit below which compounds are worthy of closer investigation[4, 7] (Table 1). Compounds giving a T/C above this limit are of little further interest. AGN 1414 did not cause any regression of well established S180 tumours although it slowed down further growth. It produced an almost total inhibition of the Ehrlich ascites carcinoma and showed appreciable activity against the BP8/Pl sarcoma. It was however ineffective against the Ca755, the leukaemia L1210 and the Walker 256. (Table 3.)

None of the analogues of AGN 1414 achieved a comparable activity against the S180, combined with an acceptable level of toxicity (defined as less than 35 per cent

TABLE 3. A. AGN 1414 AGAINST MOUSE TUMOURS. B. AGN 1414 AGAINST WALKER 256

Tumour	Dose mg/kg	Vehicle	Route	Dosed on days	No. survivors Test	No. survivors Control	% Animal weight change Test	% Animal weight change Control	% Inhibition
CA755	25	Aq. dist.	i.p.	1, 3, 5, 7, 9	9/10	9/10	−1	+14	0
CA755	30	Aq. dist.	i.p.	1, 2, 3, 4, 5 6, 7, 8, 9, 10	10/10	10/10	−10	+7	10
L1210	50	Aq. dist.	i.p.	1, 2, 4, 5, 6	—	—	—	—	0
Ehrlich	62·5	Aq. dist.	i.p.	1, 3, 5, 7	13/13	10/10	+0	+6	99
ascites	62·5	Aq. dist.	i.p.	1, 3, 5, 7	5/7	6/7	−7	+45	92
BP8/P1	62·5	Aq. dist.	i.p.	1, 3, 5, 7	15/15	10/10	−11	−10	58
BP8/P1	62·5	Aq. dist.	i.p.	1, 3, 5, 7	6/10	10/10	−3	+24	83

Test	Dose mg/kg	Vehicle	Admin.	No. survivors Test	No. survivors Control	T/C
1	50	Corn	Once	3/3		0·6
	100	oil	only	3/3	4/4	0·5
	200		i.p.	3/3		0·3
	300			3/3		0·5
2	100	Corn	Once	4/4		1·15
	200	oil	only	0/4	10/10	—
	300		i.p.	0/4		
3	100	Corn	Once	6/6		0·93
	200	oil	only	3/6	7/7	0·9
			i.p.			—

deaths in treated groups) and they were therefore not tested further. None of them was active against the L1210 leukaemia.

The route of administration of compounds was found to be important, intraperitoneal being the most effective. AGN 1414 administered orally was only moderately active, and even less so when given subcutaneously or intravenously. By slow intravenous infusion over a period of 7 days, mice tolerated 100 mg/day without any toxic signs, but at the same time there was no evidence of tumour inhibition.

At maximum tolerated doses, toxic side effects observed with all active compounds were "writhing" immediately after injection, and loss of weight. Diarrhoea was observed in some cases.

Alteration in molecular structure (Table 1) produced profound changes in activity and toxicity. Substitution of a chlorine atom in the 5-position of the benzene ring reduced the toxicity slightly, whilst the 5-bromo and 5-fluoro derivatives were appreciably less toxic. The 5-chloro and the 5-fluoro compounds, together with the 5-methyl derivative, which was also highly toxic, were too inactive to merit further study. The only compound to show greater activity than AGN 1319 was the 5-bromo derivative, AGN 1414.

Replacement of the terminal chlorine atom in the alkylating side chain by a hydroxyl group reduced antitumour activity to insignificant levels even in the case of the 5-bromo substituted derivative (AGN 1412). Substitution of alkyl groups other than ethyl at the nitrogen atom reduced both antitumour activity and toxicity.

5-HT when given together with AGN 1414 did not influence either the toxicity or the antitumour activity of this compound.

Examination of blood films made from the peripheral blood of animals treated with 62·5 mg/kg AGN 1414 for 8 days revealed no abnormalities and blood counts showed no significant diminution of either red or white cell components.

DISCUSSION

The potency of the antitumour action of AGN 1319 and AGN 1414 was surprising since it is generally accepted that for effective antitumour action nitrogen mustard compounds must have at least two reactive side chains,[8] though there have been occasional reports of monofunctional nitrogen mustards with antitumour activities.[9] It is possible therefore that either the benzothiophene part of the molecule alone or the combination of the benzothiophene nucleus with the single β-halogenoalkylamine side chain was responsible for the activity of this class of compound. At first sight the activity might be supposed to be due to a "mustard-like" action. In favour of this is the fact that substituting a terminal hydroxyl radical for a chlorine in the side chain abolishes the activity. Against this, however, are two other findings.

1. Nitrogen mustards usually produce a profound and characteristic depressant effect on the haemopoietic tissues, but when mice were treated with maximum tolerated doses of AGN 1414 for 8 days no evidence of such pathological change was found.

2. Most nitrogen mustards possessing antitumour activity inhibit the growth of the Walker tumour, but AGN 1414, the most active of the benzothiophene mustards examined, showed only very poor activity against this tumour. The balance of argument seems therefore to be against the proposition that the antitumour activity of these compounds is by a "mustard-like" action.

In attempting to gain some insight into the mechanism of antitumour action of the benzothiophenes it seems pertinent to ask whether their other pharmacological actions, the adrenolytic, or, in particular, their anti-5HT activity is connected with their antitumour action.

It has been suggested[10] that anti-5HT compounds provide protection against the toxic effects of nitrogen mustards. If this were so then the anti-5HT effect of AGN 1414 might reduce the toxicity, but not the activity of the mustard part of the molecule sufficiently to permit a more selective antitumour action.

It has also been claimed[11] that 5-HT increases the toxicity and antitumour properties of Sarcolysin. In the present experiments, however, 5-HT did not influence the growth of the S180, either alone or in combination with AGN 1414.

It is therefore difficult to account satisfactorily for the antitumour properties of this group of compounds; though perhaps the most probable explanation lies in the combination of properties which the AGN 1414 molecule possesses. Many of the analogues of AGN 1414 had some antitumour activity, but the extent of this activity was insufficient to justify further investigation.

Inhibition of the S180 tumour, in excess of the criterion given in Table 1 provides a reasonably useful experimental basis for predicting whether or not a compound may be a potentially clinically useful antitumour agent. Despite the failure of others to obtain anything more than symptomatic improvement in carcinoid tumours with anti-5HT substances[12] it seems desirable to test AGN 1414 clinically in this condition since its anti-5HT activity combined with its antitumour properties might reinforce one another sufficiently to give a clinically useful result.

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