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Antitumor Activity and Pharmacokinetics in Mice of 8-Carbamoyl-3-methylimidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one (CCRG 81045; M & B 39831), a Novel Drug with Potential as an Alternative to Dacarbazine[1]

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ABSTRACT

A number of 3-alkyl analogues of the experimental antitumor drug mitozolomide [8-carbamoyl-3-(2-chloroethyl)imidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one] have been screened against murine tumors *in vivo*. Only the compounds with a 3-methyl- or 3-bromoethyl group possessed significant antitumor activity against the TLX5 lymphoma. The 3-methyl analogue, 8-carbamoyl-3-methylimidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one (CCRG 81045), was investigated further and found to possess good activity, when administered i.p., against the L1210 and P388 leukemias, the M5076 reticulum cell sarcoma, B16 melanoma, and ADJ/PC6A plasmacytoma. The drug was also active when administered p.o. to mice bearing the L1210 leukemia. A daily for 5 days schedule of 100 mg/kg CCRG 81045 produced increases of survival time of treated animals compared to controls of 176 and >235% against the P388 and L1210 leukemias, respectively. In the female C57BL × DBA/2 F_1 mouse the 10% lethal dose was 125 mg/kg daily for 5 days. CCRG 81045 was found to undergo mild alkaline hydrolysis and ring fission to form the linear triazene 5-(3-methyltriazen-1-yl)imidazole-4-carboxamide, which is the putative metabolite formed upon metabolic activation of the antitumor drug dacarbazine [5-(3,3-dimethyltriazen-1-yl)imidazole-4-carboxamide]. The half-life of CCRG 81045 at 37°C in 0.2 M phosphate buffer (pH 7.4) was 1.24 h, whereas that of 5-(3-methyltriazen-1-yl)imidazole-4-carboxamide at 25°C was reported to be 8 min (F. H. Shealy and C. A. Krauth, J. Med. Chem., *9:* 34–37, 1966). The half-life of CCRG 81045 in human plasma *in vitro* at 37°C was 0.42 h. Pharmacokinetic experiments conducted in BALB/c mice produced plasma profiles of CCRG 81045, administered i.p. or p.o., which showed a rapid absorption phase, elimination half-lives of 1.13 h (i.p.) and 1.29 h (p.o.), and a bioavailability of 0.98.

INTRODUCTION

We have recently identified a series of imidazo[5,1-*d*]-1,2,3,5-tetrazine derivatives which have intriguing chemical (2), structural (3), and biological properties (2, 4–7). One of these compounds, mitozolomide (Fig. 1), displays potent antitumor activity against a broad spectrum of murine tumors (8) and human tumor xenografts (9) and a Phase I evaluation of the compound has recently been completed in the United Kingdom (10). The drug is currently in Phase II trials in Europe. Both chemical and biochemical evidence suggests that mitozolomide may act as a prodrug for the chloroethylating agent 5-[3-(2-chloroethyl)triazen-1-yl]-imidazole-4-carboxamide (Fig. 1) (2, 4–8).

In this paper, the first describing the activity of analogues of mitozolomide, we report on the chemical and antitumor properties of a series of 3-substituted derivatives in which the chloroethyl group of mitozolomide has been replaced by alkyl groupings, and in particular on the methyl analogue of mitozolomide, CCRG 81045[3] (Fig. 1). This compound was potentially the most interesting to us because, by analogy with mitozolomide, it should be capable of cleavage to form a linear triazene, MTIC (Fig. 1), the putative active metabolite generated by host metabolism of DTIC (dacarbazine) (11). Although DTIC was reported to have good activity against murine tumors *in vivo* (11) its activity in the clinic as a single agent was disappointing (12, 13), but it has shown useful activity in drug combination therapy (14, 15). It has been suggested that one possible reason for its limited activity in humans is that, following administration of DTIC, plasma levels of MTIC are much higher in rodents than in humans (16). Efficient metabolic *N*-demethylation of DTIC is considered to generate the unstable MTIC which must then circulate from the host liver or other sites of metabolic activation to the tumor. There is no evidence to suggest tumor activation of the drug. Thus, a prodrug form of MTIC, which does not depend on host metabolic activation to an unstable species but relies instead on chemical transformation and which has good pharmacodynamics, might present advantages compared with DTIC. The proposition that CCRG 81045 may possess the required characteristics is the subject of this paper, as well as a discussion of the structural features of 3-alkyl-8-carbamoylimidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-ones which are required for significant antitumor activity.

MATERIALS AND METHODS

Drugs. DTIC (dacarbazine) was obtained from the National Cancer Institute by courtesy of Dr. V. L. Narayanan; all other drugs were synthesized and characterized in our laboratories or at May and Baker, Ltd., Dagenham, United Kingdom. The synthesis and characterization of further imidazo[5,1-*d*]-1,2,3,5-tetrazin-4-(3*H*)-ones and compounds modified in the imidazole ring have been described in detail elsewhere (1). The synthesis is suitable for the preparation of quantities of the drugs for clinical trials. Briefly, in this series, 5-diazoimidazole-4-carboxamide and the appropriate isocyanate in dichloromethane or ethyl acetate were stirred together at room temperature, in the dark, until reaction was complete. Details of reaction conditions and the physical properties of compounds are given in Table 1.

Decomposition Studies. Chemical decomposition of CCRG 81045 (0.2 g) to MTIC was accomplished in 5% aqueous sodium carbonate (10 ml) on incubation at 25°C for 0.5 h. The buff triazene (0.09 g) was collected. The solid MTIC, dried in a vacuum, decomposed with effer-

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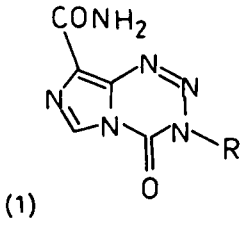
[1] Part 13 of the series "Antitumor Imidazotetrazines." Part 12 is Ref. 1. Supported by the Cancer Research Campaign, United Kingdom, Grant SP 1518, and in collaboration with May and Baker, Ltd., and Rhone Poulenc Santé.

[2] Recipients of postgraduate training awards from the Science and Engineering Research Council, United Kingdom.

[3] The abbreviations used are: CCRG 81045, 8-carbamoyl-3-methylimidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one, also known as temozolomide NSC 362856 and M & B 39831; MTIC, 5-(3-methyltriazen-1-yl)imidazole-4-carboxamide; DTIC (dacarbazine), 5-(3,3-dimethyltriazen-1-yl)imidazole-4-carboxamide; DMSO, dimethyl sulfoxide; AUC, area under plasma concentration *versus* time curve; s, singlet; brs, broad singlet.



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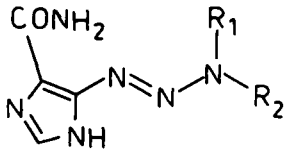

(2)

Fig. 1. Formulae of mitozolomide, CCRG 81045, and related structures. Compound 1a, R = CH_2CH_2Cl, mitozolomide; compound 1b, R = Me; CCRG 81045, M & B 39831 (see Table 1, other structures); compound 2, $R_1 = R_2 = Me$ (DTIC); R_1 = H, R_2 = Me (MTIC); R_1 = H, R_2 = CH_2CH_2Cl, (5-[3-(2-chloroethyl)triazen-1-yl]imidazole-4-carboxamide).

vescence at 178°C and was identical [λ_{max} (KBr) 3400 and 3250 (NH), and 1680 cm^{-1} (C=0); λ_{max} (ethanol) 230 and 317 nm; (DMSO-d^6) 3.0 (3H, s, CH_3), 7.6 (4H, brs, NH and NH_2) and 7.50 (1H, s, H-2)] to an authentic sample prepared by the reaction of 5-diazoimidazole-4-carboxamide with methylamine (17). Decomposition with time of CCRG 81045 in 0.2 M phosphate buffer and human plasma, pH 7.4, at 37°C, was monitored by high pressure liquid chromatography (see below).

Animals and Tumor Systems. BALB/c, DBA/2, C57BL × DBA/2 F_1 (hereafter called $BD2F_1$), and CBA/Ca mice weighing between 20 and 22g were obtained from Bantin and Kingman, Ltd. (Hull, United Kingdom). Murine tumors were used in protocols similar to those of the National Cancer Institute, using 5 or more mice per treatment group, and have been described previously by us in detail (8). Suspensions of the compounds in saline plus 10% Tween 80 or solutions in 10% DMSO-arachis oil were prepared immediately before use.

Toxicity Studies. Studies of the acute toxicity of CCRG 81045 were performed in male $BD2F_1$ mice by injection of five daily i.p. doses of the drug. Groups of five to ten mice were observed for mortality over a period of 30 days. Lethal dose values and their 95% confidence limits were calculated according to the method of Litchfield and Wilcoxon (18).

Pharmacokinetic Experiments. These were conducted in male 25-g BALB/c mice. CCRG 81045 was administered in a volume of 0.2 ml at 20 mg/kg i.p. or p.o. as a solution made by dissolving the drug in DMSO prior to dilution with sterile saline (final DMSO concentration, 10% v/v). Animals were dosed at zero time and blood samples, obtained by cardiac puncture from anesthetized mice, were taken at various time periods up to 8 h, added to 0.1 ml 3% trisodium citrate, and centrifuged immediately before storing at −20°C until analysis. The analysis of plasma samples for CCRG 81045 was similar to that described for mitozolomide (19, 20) and will be described in detail elsewhere.[4] Essentially, the internal standard 8-carbamoyl-3-ethylimidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one was added to acidified plasma and extracted with ethyl acetate. The residue, following evaporation, was redissolved in 5% acetic acid/methanol and analyzed by reverse phase high performance liquid chromatography with UV detection at 325 nm.

Kinetic Calculations. Values for the elimination rate constant (K_{el}) were estimated from the slope obtained from the linear regression analysis of the plots of the logarithm of plasma concentration *versus* time. AUC was obtained trapezoidally (using the trapezoidal rule) over 0–8 h. The p.o. bioavailability (F) was obtained by division of the p.o. AUC by the AUC obtained i.p.

[4] Manuscript in preparation.

RESULTS

The compounds synthesized in this series are described in Table 1. The antitumor activity of 3-alkyl-substituted 8-carbamoylimidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-ones, characterized in Table 1, was estimated in a primary screen *in vivo* using the TLX5 lymphoma, in a protocol which was identical to that which first identified the potent antitumor effect of mitozolomide (8) (Table 2, compound 1a). Mitozolomide has excellent activity against this tumor when administered as a single dose (percentage of treated *versus* control > 450%). A repeated dose schedule did not greatly improve its efficacy (8). In the series of 3-alkylimidazotetrazinones screened (Table 2) two had significant antitumor activity, compounds 1b and 1d. Compound 1b (CCRG 81045), although active when administered as a single dose, had an improved therapeutic effect when given daily for 5 days. In previous reports, the non-schedule dependence of the chloroethylnitrosoureas and the schedule dependence of the arylmethyltriazenes for activity against this tumor were noted (21, 22).

In the primary TLX5 screen, a single dose schedule was used to identify active drugs. The ethyl analogue (Table 1, compound 1c) was not active on this schedule or on repeated dosing, and on this precedent inactive drugs were not rescreened in a multiple schedule regimen. The activity of the bromoethyl analogue (Table 2, compound 1d) contrasted with that of the chloropropyl analogues (compounds 1g and 1h), which suggests that in those analogues capable of halogenoethylation of a biological target, the chain length of the alkyl group, *i.e.*, two carbons, is of importance. Such a relationship was previously observed in structure-activity studies of halogenoalkylnitrosoureas (23).

The antitumor activity of compound 1b, CCRG 81045, was evaluated further in other survival time models (Table 3). For comparison, the activity of DTIC is also shown. It should be noted that although the TLX5 lymphoma is used as a survival time model, it is transplanted s.c. and grows as a solid tumor before dissemination. Table 3 also shows the results of a test of CCRG 81045 against a line of the L1210 leukemia which was resistant to DTIC. Clearly, this tumor was also resistant to CCRG 81045. In addition, CCRG 81045 and DTIC were both shown to be active when administered p.o. to mice bearing the L1210 leukemia (Table 3). Each of the experiments which compares CCRG 81045 with DTIC was run simultaneously. Table 4 shows the activity of CCRG 81045 against five solid tumor models. Both the survival-time models and the solid tumor models show that CCRG 81045 has activity which is comparable to that of DTIC.

In acute toxicity tests, in which a daily for 5 days schedule was used in $BD2F_1$ mice, CCRG 81045 had a 10% lethal dose of 125 mg/kg (95% confidence limits, 119–132 mg/kg). This toxicity is marginally greater than the dose of 100 mg/kg found to be optimally effective against both the P388 and L1210 leukemias grown in the same strain of mouse.

CCRG 81045 had a half-life of 1.24 h at 37°C in phosphate buffer (0.2 M), pH 7.4, and 0.42 h in human plasma at pH 7.4 and 37°C. Under the defined chemical conditions of mild alkaline hydrolysis, it decomposed to form MTIC which was identified spectroscopically.

The plasma profiles obtained from the pharmacokinetic experiments are shown in Fig. 2. It can be seen that both i.p. and

Table 1 *Synthesis and physical characteristics of 3-alkylimidazotetrazines*

Compound substituent R (see Fig. 1)	Solvent used in synthesis[a]	Reaction duration (days)	Yield (%)	m.p. (°C) (with decomposition)	Formula[b]	UV λ_{max} (nm)	IR $\nu_{C=O}$ (cm^{-1})	^1H NMR[c] chemical shifts
1a. $(CH_2)_2Cl$	A	20	95	164–165	$C_7H_7ClN_6O$	325	1748 1673	4.05 (2H, t, CH_2CH_2Cl) 4.60 (2H, t, CH_2CH_2Cl) 7.70 (2H, brd, NH_2) 8.85 (1H, s, H-6)
1b. CH_3	B	21	98	210[d]	$C_6H_6N_6O_2$	327	1750 1680	3.90 (3H, s, CH_3) 7.75 (2H, brs, NH_2) 8.85 (1H, s, H-6)
1c. CH_2CH_3	A	15	79	172–173	$C_7H_8N_6O_2$		1740 1680	1.45 (3H, t, CH_3) 4.40 (2H, q, CH_2) 7.60 (1H, brs, NH) 7.65 (1H, brs, NH) 8.70 (1H, s, H-6)
1d. $(CH_2)_2Br$	C	2	56	156–157	$C_7H_7BrN_6O_2$		1750 1675	3.86 (2H, t, CH_2Br) 4.70 (2H, t, NCH_2) 7.67 (1H, brs, NH) 7.78 (1H, brs, NH) 8.85 (1H, s, H-6)
1e. $(CH_2)_2CH_3$	D	30	75	167	$C_8H_{10}N_6O_2$	328	1720 1690	0.95 (3H, t, CH_3) 1.80 (2H, m, $CH_2CH_2CH_3$) 4.25 (2H, t, $CH_2CH_2CH_3$) 7.75 (2H, brs, NH_2) 8.80 (1H, s, H-6)
1f. $(CH_2)_2OCH_3$	E	1	93	164–165	$C_8H_{10}N_6O_3$			
1g. $(CH_2)_3Cl$	A	3	56	153–154	$C_8H_9ClN_6O_2$		1735 1675	2.21 (2H, q, CH_2CH_2) 3.71 (2H, t, CH_2Cl) 4.40 (2H, t, NCH_2) 7.5–7.6 (2H, brs, NH_2) 8.68 (1H, s, H-6)
1h. $CH_2CHClCH_2Cl$	A	3	85	153–155	$C_8H_8Cl_2N_6O_2$		1740 1670	4.05 (2H, m, CH_2Cl) 4.60 (3H, brs, NCH_2CH) 7.60 (1H, brs, NH) 7.70 (1H, brs, NH) 8.75 (1H, s, H-6)
1i. $CH_2CH{=}CH_2$	C	18	100	149–150	$C_8H_8N_6O_2$		1730 1675	4.86 (2H, d, NCH_2) 5.1–5.50 (2H, m, $=CH_2$) 5.3–5.70 (1H, m, $=CH$) 7.55 (1H, brs, NH) 7.70 (1H, brs, NH) 8.72 (1H, s, H-6)
1j. $CH(CH_3)CH_2CH_3$	C	4	15	131–132	$C_9H_{12}N_6O_2$		1736 1690	
1k. $(CH_2)_5CH_3$	A	10	16	149–150	$C_{11}H_{16}N_6O_2$		1730 1695	0.9 (3H, t, CH_3) 1.1–1.60 (6H, m, $[CH_2]_3CH_3$) 1.8 (2H, q, NCH_2CH_2) 4.26 (2H, q, NCH_2) 7.6 (1H, brs, NH) 7.67 (1H, brs, NH) 8.72 (1H, s, H-6)
1l. CH_2Ph	C	18	74	182–183	$C_{12}H_{10}N_6O_2$		1736 1675	5.5 (2H, s, CH_2) 7.2–7.30 (5H, brs, ArH) 7.5–7.70 (2H, brs, NH_2) 8.75 (1H, s, H-6)

[a] A, ethyl acetate; B, none; C, hexamethylenephosphoramide; D, dichloromethane; E, acetonitrile.
[b] All compounds analyzed for CHN (and Cl) and found to be ±0.4% of required composition.
[c] NMR, nuclear magnetic resonance; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; brs, broad singlet; brd, broad doublet.
[d] Polymorphic forms of CCRG 81045 were obtained depending on the precise reaction conditions; these forms differed in their appearances, melting points, and IR spectra (KBr discs) but had identical ^1H NMR solution spectra.

p.o. administration lead to rapid absorption, with a maximum plasma concentration achieved within 0.5 h. Although the maximum plasma concentration obtained from i.p. administration (25.84 mg/liter) was greater than that seen when CCRG 81045 was given p.o. (19.64 mg/liter) the p.o. bioavailability was 0.98. In common with our observations on the pharmacokinetics of mitozolomide in mice (20) the elimination half-life was slightly longer following p.o. administration compared to i.p. (1.29 h compared to 1.13 h). The use of pharmacokinetic data derived from i.p. administered drug is open to criticism with regard to the estimation of bioavailability. However, the purpose of this study was not only to demonstrate the equivalence of the two routes but also to draw attention to the activity of a triazene prodrug when given p.o. (Tables 3 and 4).

Table 2 *Activity of various 3-alkyl-8-carbamoylimidazo[5,1-d]-1,2,3,5-tetrazin-4(3H)-ones against the TLX5 lymphoma implanted s.c. in CBA/CA mice*

Compound (see Table 1)	3-Alkyl group R (see Fig. 1)	Schedule[a] [day(s) of injection(s)]	Optimum dose[b] (mg/kg/day)	Treated (T) death days		Control (C) death days		T/C[c] %
				Mean	Range	Mean	Range	
1a	(Mitozolomide) $(CH_2)_2Cl$	3	40	55	35–>60[d]	12.0	10–13	458[e]
		3–7	16	36.2	28–>60[d]	12.0	10–13	302[f]
1b	(CCRG 81045) CH_3	3	160	16	14–17	10.6	10–12	151
		3, 6, 9	80	19	18–20	12.3	11–14	154
		3–7	40	19.2	17–21	10.6	10–12	181
1c	CH_2CH_3	3	640	13.4	12–17	10.9	10–12	123
		3–7	80	13.8	13–15	12.4	12–13	111
1d	$(CH_2)_2Br$	3	160	16.2	14–18	11.8	10–13	137
1e	$(CH_2)_2CH_3$	3	320	11.2	10–13	10.9	10–12	103
1f	$(CH_2)_2OCH_3$	3	320	11.6	11–12	11.8	11–13	98
1g	$(CH_2)_3Cl$	3	320	12.8	12–13	11.8	11–13	108
1h	$CH_2CHClCH_2Cl$	3	320	12.2	11–13	11.8	11–13	103
1i	$CH_2CH{=}CH_2$	3	320	11.4	7–13	11.8	11–13	97
1j	$CH(CH_3)CH_2CH_3$	3	320	9.8	6–12	11.8	10–13	83
1k	$(CH_2)_3CH_3$	3	320	11.2	10–12	10.9	10–13	103

[a] TLX5 cells (2×10^5) injected s.c. inguinally on day 0 and survival times recorded.
[b] Greatest T/C% without mortality observed before that of the controls.
[c] T/C, treated *versus* controls.
[d] Survival >60 days considered to be "cures" and included in T/C%.
[e] 4/5 cures.
[f] 2/5 cures.

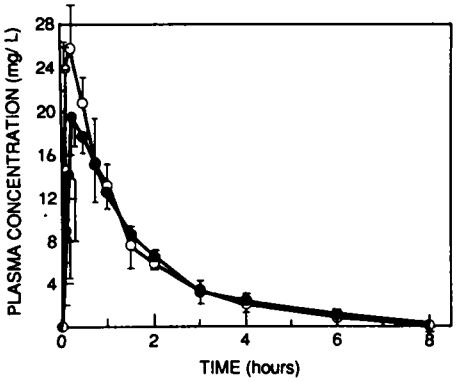

Fig. 2. Mean plasma concentrations of CCRG 81045 with time after administration of 20 mg/kg to 25-g male BALB/c mice. ○, i.p.; ●, p.o. *Bars*, +(i.p.)/−(p.o.) 1 SD of the mean value for each time point ($n = 5$).

DISCUSSION

Since the discovery of the potent and broad-spectrum activity of the imidazotetrazinone mitozolomide against murine tumor models (8) a large number of analogues have been prepared by us in an attempt to describe structure-activity relationships. The TLX5 lymphoma has been used as a primary screen for this purpose in the search for second generation agents of potential clinical value. The present series of 3-alkyl-substituted 8-carbamoylimidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-ones contains two compounds which show significant antitumor activity, the 3-methyl analogue CCRG 81045 (Fig. 1; Table 1, compound 1b) and the 3-bromoethyl analogue (Table 1, compound 1d). Neither compound demonstrated the curative action of mitozolomide against a number of murine tumors (8). The greater potency of mitozolomide suggests that, to be most effective, a chloroethyl group in position 3 of these imidazotetrazinones is optimal, although the bromoethyl analogue also has activity (Table 2). Chemical (2) and mechanistic (4, 5) studies suggest mitozolomide to be a prodrug of a chloroethylating agent which has the potential to cross-link important targets in the cell, such as DNA. However, the activity of the 3-methyl analogue CCRG 81045 questions the hypothesis that only those analogues capable of producing cross-links are active not only because of the good antitumor activity of CCRG 81045 *per se* but also because of our previous observations of cross-resistance between the chloroethylating agents, like mitozolomide and the nitrosoureas, and the aryldimethyltriazenes (8, 24), an observation which suggests some commonality of mechanism of action. Such a hypothesis has been given further support in a recent report which shows that the monomethyltriazene MTIC, for which CCRG 81045 is a progenitor, like alkylnitrosoureas is more toxic to cells which lack the guanine-O^6-methyltransferase enzyme (*Mer*$^-$ cells) yet does not cross-link DNA (25). The authors of this paper question whether cross-linking is an important facet of drug-induced cell death induced by triazenes (and thus possibly these imidazotetrazinones) and halogenoalkylnitrosoureas.

Among the 3-alkyl-substituted compounds in Table 2 only CCRG 81045, with an *N*-methyl group, possessed significant activity; substitution with an ethyl group (compound 1c) or higher alkyl groupings (compounds 1e–1k) brought about a substantial fall of activity. This is a result reminiscent of the structure-activity relationships among alkylaryltriazenes, alkylarylhydrazines, and alkylmelamines and as yet remains an unexplained phenomenon. The same structure-activity relationship established here using the TLX5 lymphoma was also observed when the L1210 leukemia was used (data not shown).

The activity of CCRG 81045 in the TLX5 test system prompted us to investigate the drug further, not least because studies of the chemistry of this class of agent (2) suggested that it was capable of base-catalyzed ring opening to form the linear triazene MTIC for which the clinically used drug DTIC is also a progenitor. Mild alkaline hydrolysis confirmed this (see "Results"). The activity of CCRG 81045 against a number of murine tumor survival time models was compared directly with that of DTIC, where it showed comparable activity (Table 3). CCRG 81045 was cross-resistant with DTIC in an L1210 leukemia with induced resistance to DTIC.

These comparisons of activity of CCRG 81045 with DTIC are important in considering the new drug as a potential alternative to DTIC. As mentioned in the introduction, DTIC may have limited clinical activity, particularly as a single agent, because of the reduced capacity of humans to metabolically activate the inert prodrug, to the putative active species MTIC,

Table 3 *Activity of CCRG 81045 and DTIC against murine tumor survival time models*

Drug	Tumor	Inoculum		Treatment		Dose (mg/kg/day)	Mean death day	Range	T/C[a] × 100%
		Route	No. of cells	Route	Days of injection				
CCRG 81045	TLX5 lymphoma	s.c.	2×10^5	i.p.	3–7	0	11.2	10–12	100
						10	14.8	13–16	132
						20	16.0	15–17	143
						40	17.2	17–18	154
						80	17.2	17–18	154
						160	13.5	12–15	120
						320	9.4	8–10	84
DTIC	TLX5	s.c.	2×10^5	i.p.	3–7	0	11.2	11–12	100
						10	13.0	12–14	114
						20	15.5	15–17	138
						40	16.4	15–18	146
						80	16.6	15–17	148
						160	16.8	13–20	150
						320	9.8	6–16	88
CCRG 81045	P388 leukemia	i.p.	1×10^6	i.p.	1–5	0	9.2	8–10	100
						25	10.8	8–13	117
						50	10.4	9–13	113
						100	16.2	15–17	176
						200	10.2	9–12	111
						300	6.6	5–7	72
DTIC	P388	i.p.	1×10^6	i.p.	1–5	0	9.2	8–10	100
						25	11.0	10–12	120
						50	11.2	11–12	122
						100	15.3	14–17	166
						200	7.8	2–13	85
						300	8.6	2–16	93
CCRG 81045	L1210 leukemia	i.p.	10^5	i.p.	1–5	0	8.4	8–9	100
						25	12.4	11–13	148
						50	14.4	12–15	171
						100	27.8	18–>60	>235[b]
						200	8.0	8	95
						300	5.8	5–6	69
DTIC	L1210	i.p.	10^5	i.p.	1–5	0	8.4	8–9	100
						25	11.0	9–12	131
						50	12.2	10–15	145
						100	13.2	10–16	157
						200	13.4	2–19	160
						300	11.6	2–16	138
CCRG 81045	L1210	i.p.	10^5	p.o.	1–5	0	8.4	8–9	100
						25	11.8	11–13	140
						50	13.4	13–14	160
						100	14.6	14–15	174
						200	10.8	8–13	137
						300	7.8	7–8	93
DTIC	L1210	i.p.	10^5	p.o.	1–5	0	8.4	8–9	100
						25	11.8	11–13	140
						50	11.6	10–14	138
						100	12.2	12–13	145
						200	13.6	13–14	162
						300	13.8	13–15	164
CCRG 81045	L1210-DTIC[c, d]	i.p.	10^5	p.o.	1–4	0	12.7	10–13	100
						25	12.7	11–14	100
						50	12.6	12–14	99
						100	10.7	9–12	84

[a] T/C, treated *versus* control.
[b] Long term survivor not included (1 of 5 survived >60 days).
[c] Resistant to DTIC (C. Fizames and F. Lavelle, unpublished).
[d] This experiment performed by Fizames and Lavelle at Vitry sur Seine and the drug administered p.o. according to previously described protocol (8).

via hepatic oxidative *N*-demethylation, whereas in the mouse this is apparently an efficient process (16). CCRG 81045 is capable of producing MTIC via a chemical decomposition, thus bypassing the species difference that may exist in the formation of MTIC from DTIC (Fig. 3).

Whether MTIC is the active antitumor species formed from DTIC remains to be established and some other pathways may be involved. We have previously questioned the role of monomethyl triazenes in the cytotoxic action of dimethyltriazenes (26, 27) and this question was raised again in a recent study of pyrazolotetrazines, analogous to the tetrazines reported here; surprisingly, the methyl congener was, unlike CCRG 81045, without antitumor activity (28).

Whatever the mechanism of action of CCRG 81045, it is a compound which demonstrates good antitumor activity and an adequate pharmacokinetic profile, particularly with regard to its bioavailability. For these reasons it will shortly enter a Phase I trial in the United Kingdom.

Table 4 *Activity of CCRG 81045 against murine solid tumor models*

Tumor	Schedule (days of injections)	Dose (mg/kg/day)	Wt (g)[a]	% of deaths[b]	Mean tumor volume (cm^3)	T/C%[c]
M5076 sarcoma[d]	1–17	0	+4.8	0	2.6 ± 0.7[e]	100
		2.5	+4.8	0	1.5 ± 0.3	56
		5	+4.3	0	0.7 ± 0.5	27
		10	+4.4	0	0.2 ± 0.1	8
		20	+4.5	0	0	0
		40	+3.2	0	0	0
		80	−4.7	80	0	0
ADJ/PC6A plasmacytoma[d]	14	0	+1.5	0	5.6	100
		10	+1.2	0	1.2	21
		20	+0.3	0	0.6	10
		40	+0.0	0	0	0
		80	+0.5	0	0	0
		160	−1.1	0	0	0
B16[d, f] melanoma	9	0	−0.4	30	1.4 ± 0.8	100
		50	−0.6	30	0.8 ± 0.8	57
		100	−0.6	0	0.6 ± 0.4	42
		200	−0.9	0	0.5 ± 0.4	36
C26[d, f]	1–4	0	−2.6	0	1.5 ± 0.45	100
		25	−1.3	0	1.4 ± 0.3	93
		50	−1.7	0	1.0 ± 0.5	66
Lewis lung[d]	7	0	0.7	0	5.6 ± 1.9	100
		50	1.0	0	4.9 ± 1.7	87
		100	−0.7	0	5.2 ± 1.3	93
		200	−1.5	0	4.1 ± 1.4	73

[a] Weight = change in mean weight between days 1 and 17 for M5076 sarcoma, days 14 and 19 for ADJ/PC6A plasmacytoma, days 1 and 14 for B16 and C26, and days 7 and 11 for Lewis lung.
[b] Measured on: day 24 (M5076 and ADJ/PC6A); day 22 (B16); day 15 (C26); day 22 (Lewis lung).
[c] T/C, treated *versus* control.
[d] Protocols as in Ref. 8.
[e] Mean ± SD.
[f] B16, C26, and Lewis lung: schedule per p.o. route.

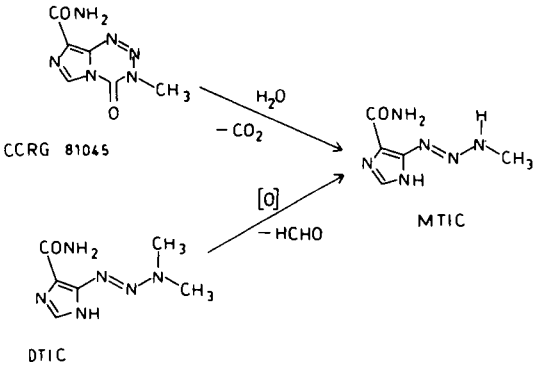

Fig. 3. Formation of MTIC from CCRG 81045 and DTIC.

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