J. Pharm. Pharmac., 1969, **21**, 573–576 *Received February* 24, 1969

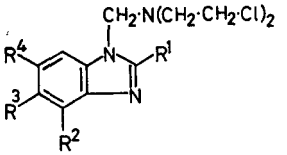
The effects of 1-[di(2-chloroethyl)aminomethyl]benzimidazole and related compounds on the growth of experimental tumours

V. V. SUBBA REDDY*, M. SIRSI*, G. R. REVANKAR† and S. SIDDAPPA†

* *Microbiology and Pharmacology Laboratory, Indian Institute of Science, Bangalore, India.*
† *Department of Chemistry, Karnatack University, Dharwar, India.*

The inhibitory activity of some benzimidazole Mannich-base nitrogen mustards on the growth of experimental tumours, viz. mouse fibrosarcoma in mice and Yoshida ascites sarcoma in rats has been examined. Amongst the compounds tested 5,6-dichloro-1-[di(2-chloroethyl)aminomethyl]benzimidazole and 1-[di(2-chloroethyl)-aminomethyl]-2-phenylbenzimidazole showed inhibitory effect on mouse fibrosarcoma; while 4-bromo-1-[di(2-chloroethyl)amino-methyl]benzimidazole, 4-chloro-1-[di(2-chloroethyl)aminomethyl]-benzimidazole and 1-[di(2-chloroethyl)aminomethyl]-5-methoxybenzimidazole were active against Yoshida ascites sarcoma.

The concept of incorporating an alkylating function e.g. the 2,2-dichloroethylamino-group, into a molecule known to play an important role in the biogenesis of a biologically active molecule, has received much attention (Benitez, Ross, & others, 1960). The phenylalanine mustards have been studied exhaustively in animals and in man as anticancer drugs (Greene, Baker & Greenberg, 1960). Their encouraging biological properties have created an increasing interest in the synthesis of allied hetero-aromatic compounds in a search for potential anticancer agents of high activity with minimum toxicity. In view of the biological importance of the benzimidazole moiety, a variety of benzimidazole mustards, i.e. 2-[di(2-chloroethyl)aminomethyl]benzimidazoles have been synthesized (Herschberg, Gellhorn & Gump, 1957, Gump & Nikawitz, 1959). Revankar & Siddappa (1967), employed a Mannich-type reaction, condensed benzimidazole and substituted benzimidazoles containing a labile N–H bond with formaldehyde and di(2-chloroethyl)amine to obtain a number of benzimidazole Mannich-base nitrogen mustards (I) as potential anticancer agents. Their inhibitory activities have been examined on experimental tumors in rats and mice and the results are here reported.



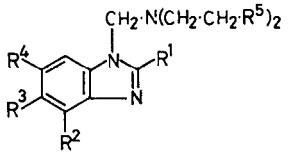
I

EXPERIMENTAL

Materials and methods

Mouse fibrosarcoma (MFS). This was induced by subcutaneous injection of 6,12-dimethylbenzo[1,2-*b*,4,5-*b'*]dithianaphthene in an inbred SWR mouse (Waravdekar & Ranadive, 1957), obtained from Indian Cancer Research Centre (ICRC), Bombay and has been maintained in the inbred strain of mice SWR/IISc.

Table 1. *Benzimidazole mustards tested*



Compound No.	R^1	R^2	R^3	R^4	R^5	Chemical name
A	H	Cl	H	H	OH	4-chloro-1-[di(2-hydroxyethyl)amino-methyl]benzimidazole
B	H	Br	H	H	OH	4-bromo-1-[di(2-hydroxyethyl)amino-methyl]benzimidazole
C	H	Br	H	H	Cl	4-bromo-1-[di(2-chloroethyl)aminomethyl] benzimidazole
D	H	Cl	H	H	Cl	4-chloro-1-[di(2-chloroethyl)aminomethyl] benzimidazole
E	H	H	H	H	Cl	1-[di(2-chloroethyl)aminomethyl]benzimid-azole
F	H	H	MeO	H	Cl	1-[di(2-chloroethyl)aminomethyl]-5-meth-oxybenzimidazole
G	H	H	Cl	Cl	Cl	5,6-dichloro-1-[di(2-chloroethyl)amino-methyl]benzimidazole
H	Ph	H	H	H	Cl	1-[di(2-chloroethyl)aminomethyl]-2-phenyl-benzimidazole
I						1-[di(2-chloroethyl)aminomethyl]-1*H*-naphth[1,2-*d*]imidazole

Yoshida ascites sarcoma. This was obtained from ICRC, Bombay and has been maintained in a closely inbred substrain of Wistar rats A/IISc.

Benzimidazole mustards. Chemical structures and names are in Table 1. Solutions were prepared by dispersing the compounds in 30% propylene glycol in saline, to give the required concentration in 0·2 ml of solution, i.e. the volume injected intraperitoneally each time.

Screening studies were made in rats weighing 120–150 g and mice 20–25 g of the respective strains. The animals were provided with dry diet (cracked wheat 60%, cracked Bengal gram 20%, fish meal 8%, shark liver oil 2%, peanut oil 5%, commercial casein 4% and common salt 1%) and water *ad libitum*. The weights and general behaviour of all animals were recorded regularly.

The efficacy of the extract in controlling the growth of tumours was estimated by calculating the T/C values. These were calculated as follows: For solid tumours excised after two weeks the T/C value is the ratio of the mean tumour weight of treated animals divided by the mean tumour weight of control animals. T/C value for ascites tumour is the ratio (expressed as %) of the mean survival time of the treated group divided by the mean survival time of the control group.

T/C values of 0·5 or below in solid tumours and above 200 in ascites tumours were considered to be effective.

Design of experiments

Mouse fibrosarcoma. Tumour implantation was by aseptic subcutaneous injection of 0·1 ml of tumour homogenate (1:2 w/v in saline) at the axillary region. Animals were either injected with 0·2 ml of 30% propylene glycol in saline intraperitoneally or given five daily successive intraperitoneal doses of the compound 24 h after transplantation.

Yoshida ascites sarcoma. The rats received 10 million cells of actively growing tumour intraperitoneally and were either administered 30% propylene glycol in saline 0·2 ml intraperitoneally (20 animals) or in groups of 10 animals, treated in the same way as the mice.

RESULTS

Mouse fibrosarcoma. Doses and T/C values are shown in Table 2. Two of the animals receiving compound H had died by the end of the experiment. During treatment weights of the mice treated with compound G decreased but not significantly.

Yoshida ascites sarcoma. The survival period of rats treated with compounds C, D and F significantly enhanced (Table 3) with T/C values: in 534, 441 and 309 respectively. Compound H was inactive against this tumour.

Table 2. *Effect of intraperitoneal injections of benzimidazole mustards on mouse fibrosarcoma*

Compound	Dose (mg/kg)	Total dose (mg/kg)	Survivors	Weight* difference (g)	Tumour† weight (g)	T/C	Inhibition‡ (%)
Control	—	—	30/30	—	1·67 ± 0·15	—	—
A	8	40	10/10	+ 2·6	1·21 ± 0·23	0·70	30
B	8	40	10/10	— 1·2	1·85 ± 0·25	1·11	nil
C	8	40	10/10	— 0·7	0·97 ± 0·23	0·58	42
D	8	40	10/10	+ 0·4	0·99 ± 0·25	0·59	41
E	8	40	10/10	— 1·1	1·98 ± 0·63	1·19	nil
F	8	40	10/10	+ 0·2	1·26 ± 0·21	0·75	25
G	8	40	10/10	— 2·1	0·57 ± 0·22	0·34	66
H	8	32	8/10	— 3·8	0·65 ± 0·11	0·39	61
I	8	32	10/10	+ 0·4	1·25 ± 0·18	0·75	25

* The average animal weight change of treated hosts minus the average animal weight change of control hosts.

† Mean ± s.e.

‡ $\frac{\text{Control tumour weight} - \text{treated tumour weight}}{\text{Control tumour weight}} \times 100.$

Table 3. *Effect of intraperitoneal injections of benzimidazole mustards on Yoshida ascites sarcoma*

Compound	Dose (mg/kg)	Survival* period (days)	T/C
Control	—	8·2 ± 0·26	—
C	8	43·8 ± 12·39	534
D	8	36·2 ± 10·16	441
F	8	25·4 ± 10·86	309
H	8	7·8 ± 0·12	96

* Mean ± s.e.

DISCUSSION

In tests with mouse fibrosarcoma, compounds A, B and E were inactive. Introduction of a methoxy-group on the benzenoid ring in compound E gave a molecule (compound F) with slightly but not significantly increased activity compared to the parent molecule. Introduction of a chloro- or bromo-atom on the other hand resulted in molecules (compounds C and D) more active than compound F, while compound G, with two chloro-atoms on the benzenoid ring of compound E, was active. Similar activity was found in compound H, which is a 2-phenyl-substituted derivative of compound E.

The compounds C, D and F, though inactive with mouse fibrosarcoma, were active against Yoshida ascites sarcoma but the reverse applied to compound H.

Acknowledgements

One of the authors (V.V.S.R.) wishes to thank the authorities of the Council of Scientific and Industrial Research, India, for the award of a fellowship.

REFERENCES

BENITEZ, A., ROSS, L. O., GOODMAN, L. & BAKER, B. R. (1960). *J. Am. chem. Soc.*, **82**, 4585–4591.
GREENE, M. O., BAKER, B. R., & GREENBERG, J. (1960). *Cancer Res.*, **20**, 1160–1165.
GUMP, W. S. & NIKAWITZ, E. J. (1959). *J. org. Chem.*, **24**, 712–714.
HERSCHBERG, E., GELLHORN, A. & GUMP, W. S. (1957). *Cancer Res.*, **17**, 904.
REVANKAR, G. R. & SIDDAPPA, S. (1967). *Mh. Chem.*, **98**, 169–175.
WARAVDEKAR, S. S. & RANADIVE, K. J. (1957). *J. Nat. Cancer Inst.*, **18**, 555–567.