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PRELIMINARY COMMUNICATIONS

5-(N-PHENYLCARBOXAMIDO)-2-THIOBARBITURIC ACID (NSC 336628), A NOVEL POTENTIAL ANTITUMOR AGENT

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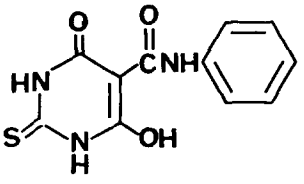
Antitumor activity is rarely observed among derivatives of barbituric acid. More than 700 barbiturates have been examined in the National Cancer Institute screening program, with none showing significant activity in murine test systems such as L1210 leukemia and B16 melanoma (1). We now wish to report, however, the exceptional antitumor activity of 5-(N-phenylcarboxamido)-2-thiobarbituric acid (NSC-336628) (1)[1], a compound synthesized in the research laboratories of Uniroyal Ltd., Guelph, Ontario, Canada and examined for antitumor activity by the National Cancer Institute. The synthesis of 1 and related compounds will be reported elsewhere.

The antitumor activity of 1 was discovered by pre-screening against the P388 murine leukemia, followed by testing in three additional murine tumors, utilizing previously described testing protocols (2-4) (Table 1). In the i.p. implanted P388 leukemia system, a maximum increased life span (ILS) of 101% was achieved following daily i.p. treatment with a

[1]Patent pending A.D. Brewer (Uniroyal Ltd.) and J.A. Minatelli (Uniroyal Chemicals).

50 mg/kg dose on days 1-5. In two additional experiments, maximum ILS values of 62 and 94% were obtained. Greater therapeutic efficacy was observed against the i.p. implanted L1210 leukemia. In repeated experiments, a 100 mg/kg dose of 1 administered i.p. on days 1-9 was curative (no gross evidence of tumor on day 30) in at least 50% of the mice, and increases in the median life span of the test groups were at least 200%. Activity (ILS ≥25%) was usually observed over a four-fold dosage range. Using the same treatment regimen, an optimal ILS of 93% was achieved in the i.p. implanted B16 melanoma model, with maximum values ranging from 67 to 85% in three other studies. As in the L1210 leukemia model, activity (ILS ≥25%) was noted over a four-fold dosage range. Intermittent treatment of the M5076 sarcoma with 1 produced maximum ILS values of 55% and 72%.



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NSC 336628

Table 1. Antitumor Activity of 1 in Four Murine Tumor Models

Tumor System	I.P. Treatment Schedule	FED[a]	Optimum Dose mg/kg	ILS %
i.p. P388 leukemia	QD, Days 1-5	30	50	101
i.p. L1210 leukemia	QD, Days 1-9	30	100	>237[b]
i.p. B16 melanoma	QD, Days 1-9	60	100	93
i.p. M5076 sarcoma	Q4D, Days 1-13	60	100	72

Experiments were conducted according to established NCI protocols (3). Groups of six CD_2F_1 (L1210 and P388) or ten $B_6C_3F_1$ (B16 and M5076) mice were inoculated with tumor on Day 0. Treatment with 1 suspended in saline plus Tween 80 was initiated 24 hours later according to the schedules listed in the table. Vehicle-treated control mice (30-40) were included in each experiment. The median survival times of the drug-treated mice were compared to those of the vehicle-treated controls and expressed as a percentage increase in life span (ILS). The data obtained with the most effective dose (optimum dose) are shown. [a] Final Evaluation Day. [b] 4/6 mice were alive with no gross evidence of tumor on the final evaluation day (day 30); thus the true median survival time and maximum ILS value were not reached.

Importantly, the antitumor efficacy of 1 against the L1210 leukemia was retained when the tumor implant site was distant from the drug injection site (Table 2). Following 5 daily i.p. treatments with a 45 mg/kg dose of 1, 5 of 10 mice were still alive and apparently free of tumor 60 days after s.c. inoculation with L1210 ascites, and the median survival time of the 5 mice that died was increased 110%. Similarly, 4 of 10 mice receiving 9 daily i.p. injections of a 55.7 mg/kg dose of 1 were alive on day 60, but the survival of the 6 remaining mice was not increased over that of the controls. However, on the same schedule, the 37.3 mg/kg dose produced a 71% ILS and, in a separate experiment, 5 of 6 mice receiving a 100 mg/kg dose of 1 suspended in Tween 80 were alive and free of tumor on day 30. Oral treatment for 9 days was as effective as i.p. treatment for 5 days although a greater cumulative dose was required to achieve the same response (Table 2). Intravenous bolus administration of 1 for 5 days also produced an antitumor effect, but was less efficacious than either i.p. or oral treatment.

Table 2. Effect of Route of Administration of 1 on its Activity Against the S.C. Implanted L1210 Leukemia[a]

Route of Administration	Treatment Schedule	Opt. Dose mg/kg	ILS[b] %	Cures[c] /Total
i.p.	QD, Days 1-5	45	110	5/10
i.p.	QD, Days 1-9	55.7	- [d]	4/10
p.o.	QD, Days 1-9	124	100	4/10
i.v.	QD, Days 1-5	30.1	45	0/10

[a] L1210 cells (10^5) were implanted s.c. in groups of ten CD_2F_1 mice (60 mice in water-treated control group) 24 h before initiation of therapy. A solution of the N-methylglucamine salt of 1 was administered either i.p. or orally on days 1-9 or i.p. or i.v. on days 1-5. [b] Increased life span based on median survival time of dying animals only. Median survival time of control animals was 10.5 days. [c] Number of day 60 survivors without gross evidence of tumor. [d] No increase in survival of dying mice.

In contrast to its effects in the i.p. and s.c. implanted L1210 leukemia models, 1 demonstrated only marginal activity (maximum ILS values of 28 and 34%, data not shown) against the i.c. implanted tumor following daily i.p. administration for 9 days. The lack of strong i.c. effects, indicating that the compound does not penetrate the CNS, can be explained on structural grounds. Even though 1 is a barbiturate, the absence of 5,5-disubstitution permits a very acidic pKa of 4.0 and renders 1 highly ionized at physiological pH (5).

Based on these results, we believe that 1 is a structurally novel anticancer lead of exceptional promise. At the present time, we are pursuing it as a high priority candidate for clinical trials and also investigating the mechanism of its anticancer action.

In summary, 5-(N-phenylcarboxamido)-2-thiobarbituric acid, in contrast to other barbiturates, has shown outstanding antitumor activity against the murine L1210 leukemia as well as good activity against the B16 melanoma and M5076 sarcoma. The compound is being pursued as a high priority candidate for clinical trials.

REFERENCES

1. J.S. Driscoll, N.R. Melnick, F.R. Quinn, N. Lomax, J.P. Davignon, R. Ing, B.J. Abbott, G. Congleton, and L. Dudeck. Cancer Treat. Rep. 62, 45 (1978).

2. V.L. Narayanan, in "Structure-activity relationship of antitumor agents" (Eds. D.N. Reinhoudt, T.A. Conners, H.M. Pinedo, and K.W. Van de Poll) p.5, Martinus Nijhoff Publishers Hague, Boston and London (1983).

3. NIH Publication No. 84-2635, "In Vivo Cancer Models, 1976-1982", Superintendent of Documents, U. S. Government Printing Office, Washington, D.C. 20402 (1984).

4. J.M. Venditti, R.A. Wesley, and J. Plowman. In "Advances in Pharmacology and Chemotherapy" (Eds. S. Garattini, A. Goldin, F. Hawkins) Vol. 20, p.1 Academic Press: New York (1984).

5. A. Albert. "Selective Toxicity", 6th Ed, p.47, Chapman and Hall Ltd., London (1979).