Biochemical Pharmacology, Vol. 30, No. 16, pp.2381-2384, 1981.
Printed in Great Britain.

0006-2952/81/162381-04$02.00/0

PRELIMINARY COMMUNICATION

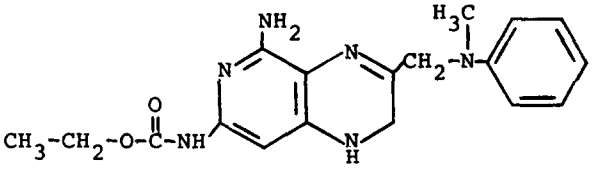
1-DEAZA-7,8-DIHYDROPTERIDINES, A NEW CLASS OF MITOTIC INHIBITORS WITH ANTICANCER ACTIVITY

Glynn P. Wheeler, Bonnie J. Bowdon, Jacqueline A. Werline, and Carroll Temple, Jr.

Kettering-Meyer Laboratory, Southern Research Institute
Birmingham, Alabama 35255

(Received 11 May 1981; accepted 21 May 1981)

As part of a continuing program for the synthesis and evaluation of potential folic acid antagonists [1,2] the compound having the structure shown below was synthesized.



Ethyl 5-amino-1,2-dihydro-3-[(N-methylanilino)methyl] pyrido-[3,4-b] pyrazin-7-ylcarbamate (NSC-181928)

It was submitted to the Division of Cancer Treatment, National Cancer Institute, which evaluated its anticancer activity against a spectrum of experimental tumors and provided us with the resulting data. We have examined these data, and our interpretation of them is given here. The compound was active against L1210 leukemia, P388 leukemia, and intraperitoneally implanted murine colon tumor #26, it had borderline activity against subcutaneously implanted murine colon tumor #38, $CD8F_1$ mammary tumor, and CX-1 colon xenograft, and it was inactive against Lewis lung carcinoma, LX-1 lung xenograft, MX-1 breast xenograft, and B-16 melanocarcinoma. It is significant that this agent was also active against P388/Meth, a line of murine leukemia P388 that is resistant to methotrexate.

In our initial studies with NSC 181928 we observed that its inhibition of the proliferation of cultured L1210 cells was not prevented by folinic acid, although in a parallel experiment folinic acid did prevent inhibition by methotrexate, and that doubling the normal quantities of vitamins and amino acids in the culture medium did not prevent the inhibition. In a subsequent experiment we observed that the agent caused an accumulation of cultured L1210 cells in mitosis. Therefore, tests were performed with other lines of cultured cells to determine the generality of the effect.

METHODS

The cell lines used in these experiments are routinely maintained in continuous culture in this laboratory. The L1210 cells are grown in Fischer's medium containing 10% horse serum, and the P388/0 and P388/VCR (resistant to vincristine) cells are grown in Dulbecco's medium containing 10% horse serum; all three of these lines are grown in stationary plastic culture flasks. The H. Ep. #2 cells and the murine colon tumor #26 cells are grown both in swirl cultures and as plastic-attached cultures in SRI-14 medium [3] containing 10% calf serum; in the plastic-attached cultures 25μg of calcium chloride per milliliter is

added to the medium to facilitiate adhesion of the cells to the surface of the plastic flask. The murine colon tumor #38 cells are grown similarly to the plastic-attached #26 cells. The drugs were added to the experimental cultures of cells that were growing at approximately exponential rates (24-hour-old cultures of L1210, P388/0, and P388/VCR and 48- or 72-hour-old cultures of the cells of the other lines), and the cells were harvested at selected times thereafter by taking aliquots of the cultures that were grown in suspension or swirl culture and by decanting the medium from the plastic-attached cultures and removing the cells from the surface by trypsinization. The cells were washed and used for the preparation of microscope slides as has been described [4]. Briefly, the cells are treated with cold citric acid and resuspended in ethanol-acetic acid. The suspension is spread on microscope slides, which are allowed to dry at room conditions. The slides are then immersed for 5 minutes in 1.0 N hydrochloric acid at 60 degrees, rinsed with water and absolute ethanol, and allowed to dry. After the slides are stained with toluidine blue in borax solution, 1000 cells per slide are counted with the aid of a microscope and classified as interphase or mitotic.

RESULTS AND COMMENTS

The experimental results are presented in Table 1. For all of the lines of cells tested, NSC 181928 caused arrest of the cells in mitosis. The effectiveness of this new agent was quantitatively similar to that of vincristine and vinblastine for P388/0, colon tumor #26 cells, and colon tumor #38 cells. Its activity against P388/VCR was similar to that of vinblastine and similar to its activity against P388/0. The data suggest that there is little cross-resistance of P388/VCR to NSC-181928 and to vinblastine, but there is some cross-resistance to colchicine. In several instances there is evidence that is suggestive that higher concentrations of the agents and longer periods of exposure of the cells to the agents caused lysis of the mitotic cells with a resulting lower fraction of the remaining cells in mitosis.

A number of other compounds structurally related to NSC 181928 have been synthesized and are currently being studied to establish structure-activity relationships. Other studies to compare compounds of this type with vincristine, vinblastine, and other mitotic inhibitors with respect to the effects upon the formation and functioning of microtubules will also be performed. Nevertheless, sufficient data are at hand to establish that compounds of this type prevent the progression of cells through mitosis.

In addition to the agents mentioned above, other agents that cause arrest of cells in mitosis include griseofulvin [5], podophyllotoxin [5], trifluralin [5], aurantia [5], benzimidazol-2-ylcarbamates [6,7,8,9,10], 5-halopyrimidin-2-ones [11,12,13,14,15], and ansamitocins [16]. A number of these agents have anticancer activity.

It would be worthwhile to compare in a single laboratory representative compounds from the various classes mentioned above to determine if some of them might be more advantageous than others for use as therapeutic agents.

In summary, experiments with ethyl 5-amino-1,2-dihydro-3-[(N-methylanilino)methyl] pyrido[3,4-b] pyrazin-7-ylcarbamate (NSC-181928), a 1-deaza-7,8-dihydropteridine, have shown that this compound causes cultured cells of several lines to accumulate in mitosis. The agent is similar in activity to vincristine and vinblastine, and it is active against a line of P388 murine leukemia cells that is resistant to vincristine.

ACKNOWLEDGMENT

The work reported here was supported by Grant 1-R01-CA25311-01A1 from the National Cancer Institute, NIH.

Table 1

Effects of NSC-181928, Vincristine, Vinblastine, and Colchicine upon the Arrest of Several Lines of Cultured cells in Mitosis

Cell Line	Agent	Concentration (μM)	Time (hr)	Percent in Mitosis
L1210	NSC-181928	0.30	24	80
P388/0	NSC-181928	0.03	12	33
		0.30	12	46
P388/0	Vincristine	0.03	12	55
		0.30	12	40
P388/0	Vinblastine	0.03	12	53
		0.30	12	33
P388/0	Colchicine	0.03	12	27
		0.30	12	33
P388/VCR	NSC-181928	0.03	12	64
		0.30	12	58
P388/VCR	Vincristine	0.03	12	10
		0.30	12	55
P388/VCR	Vinblastine	0.03	12	69
		0.30	12	52
P388/VCR	Colchicine	0.03	12	3
		0.30	12	21
H.Ep. #2 (Swirl)	NSC-181928	0.30	24	48
H.Ep.#2 (Plastic-attached)	NSC-181928	0.30	24	87
Colon tumor #26 (Plastic-attached)	NSC-181928	0.30	14	42
			24	12
Colon tumor #26 (Plastic-attached)	Vincristine	0.30	14	36
			24	9
Colon tumor #26 (Plastic-attached)	Vinblastine	0.30	14	42
			24	12
Colon tumor #38 (Plastic-attached)	NSC-181928	0.30	14	47
			24	58
Colon tumor #38 (Plastic-attached)	Vincristine	0.30	14	47
			24	53
Colon tumor #38 (Plastic-attached)	Vinblastine	0.30	14	52
			24	61

Note: In each experiment listed in this table the percentage of the cells of a control culture that were in mitosis was determined, and the values were found to fall within the range 2-6%.

REFERENCES

1. R. D. Elliot, C. Temple, Jr., and J. A. Montgomery, J. Org. Chem. 33, 533 (1968).
2. C. Temple, Jr., R. D. Elliot, J. D. Rose, C. L. Kussner, and J. A. Montgomery, Abstracts of the 28th Southeast Region Meeting of the American Chemical Society, Gatlinburg, Tennessee, Oct. 27-29, Abstract No. 425 (1976).
3. G. G. Kelley, M. H. Vail, D. J. Adamson, and E. A. Palmer, Am. J. Hygiene 73, 231 (1961).
4. G. P. Wheeler, B. J. Bowdon, L. J. Wilkoff, and E. A. Dulmadge, Proc. Soc. Exp. Biol. Med. 126, 903 (1967).
5. G. Deysson, in Microtubules and Microtubule Inhibitors (Eds. M. Borgers and M. DeBrabander), p. 427. North-Holland Publishing Company, Amsterdam (1975).
6. G. Atassi, C. Schaus, and H. J. Tognon, Europ. J. Cancer 11, 609 (1975).
7. J. Hoebeke, G. Van Nijen, and M. DeBrabander, Biochem. Biophys. Res. Commun. 69, 319 (1976).
8. M. J. DeBrabander, R. M. L. Van de Veire, F. E. M. Aerts, M. Borgers, and P. A. Janssen, Cancer Res. 36, 905 (1976).
9. L. C. Davidse, and W. Flach, J. Cell Biol. 72, 174 (1977).
10. J. P. Laclette, G. Guerra, and C. Zetina, Biochem. Biophys. Res. Commun. 92, 417 (1980).
11. R. Oftebro, Ø. Grimmer, T. B. Øyen, and S. G. Laland, Biochem. Pharmacol. 21, 2451 (1972).
12. E. Wibe, R. Oftebro, T. Christensen, S. G. Laland, E. O. Pettersen, and T. Lindmo, Cancer Res. 38, 560 (1978).
13. E. Wibe, and R. Oftebro, Brit. J. Cancer 40, 222 (1979).
14 E. Wibe, R. Oftebro, S. G. Laland, E. P. Pettersen, and T. Lindmo, Brit. J. Cancer 39, 391 (1979).
15. E. Wibe, Cancer Res. 40, 2069 (1980).
16. K. Ootsu, Y. Kozai, M. Takeuchi, S. Ikeyama, K. Igarashi, K. Tsukamoto, Y. Sugino, T. Tashiro, S. Tsukagoshi, and Y. Sakurai, Cancer Res. 40, 1707 (1980).