Biochemical Pharmacology, Vol. 28, pp. 1811-1815.
Pergamon Press Ltd. 1979. Printed in Great Britain.

PRELIMINARY COMMUNICATION

DNA BIFUNCTIONAL INTERCALATORS : ANTILEUKEMIC ACTIVITY OF NEW PYRIDOCARBAZOLE DIMERS.

Bernard P. Roques★, Didier Pelaprat★, Irène Le Guen★, Gisèle Porcher★, Charles Gosse☆ and Jean-Bernard Le Pecq☆.

★ U.E.R. des Sciences Pharmaceutiques et Biologiques, Département de Chimie Organique, E.R.A. 613 (CNRS), 4 avenue de l'Observatoire, 75006 Paris, France.

☆ Institut Gustave Roussy, Unité de Physicochimie Macromoléculaire, L.A. 147 (CNRS) and U 140 (INSERM), 16 bis avenue Paul Vaillant Couturier, 94800 Villejuif, France.

(*Received* 1 *March* 1979; *accepted* 19 *March* 1979)

Several antitumor drugs (actinomycin D (1,2), daunorubicin (2,3), adriamycin (4), ellipticine derivatives (5)) interact with DNA by intercalation between two adjacent base pairs. Although the relation between DNA intercalation and antitumor activity is not completely understood, the design of DNA intercalating compounds binding to DNA with high affinity appears to be an efficient approach for the search of new antitumor drugs, as discussed recently (6,7, 8).

In order to obtain compounds of high affinity for DNA, a number of bisintercalating drugs, mostly in the series of acridine, phenanthridine, quinoline or ellipticine have been synthesized in our laboratories (9,10,11,12,13) and in others (14,15,16,17,18,19). These studies have recently led to the discovery of compounds having a significant activity on P388 leukemia (17, 20).

In previous works (11,12,21), the conformation of several bifunctional intercalators was studied. It was observed that these molecules tend to be folded in solution because the two aromatic rings exhibit a great tendency to stack on each other. The result of such an autostacking is a large decrease of the basicity of the nitrogen on the aromatic ring (21) followed by a dramatic decrease of the DNA affinity near the physiological pH (21,22).

Consequently our approach was to synthesize new derivatives made up of potentially active intercalating moities having a quaternary ammonium group linked by more rigid chains in order to prevent as much as possible the folding of the dimer. Various 7H pyridocarbazoles (23) were linked with different cyclic aminoalkyl chains (24) allowing the study of the importance of the nature and the position of the connecting chain in the dimer. In this preliminary communication we report the antitumor activity of some of these dimers as an illustration of the potential interest of these compounds. A more detailed study concerning the synthesis, the

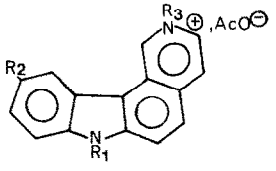
physicochemical properties, the DNA binding characteristics and the biological properties of the pyridocarbazole dimers will be reported later.

L 1210 murine leukemia was used for antitumor evaluations because of its good predicting value for human cancer (25). Determination of the antitumor activity was performed as reported earlier (26). 10^5 cells were intraperitoneally inoculated to female DBA/2 mice (15 animals per group). The compound under study was injected intraperitoneally 24 hours later. Deaths were recorded every day at the same hour. Animals which survived for more than 45 days were considered as cured. The mean survival time of treated animals (T) was compared to that of control animals (C). The increases in life span (ILS) were calculated as $\frac{T}{C} \times 100$. The acute toxicity of the compounds was determined by the usual procedure. The highest dose which could be administered without causing animal death (LD_o) was taken as unity, and dosages were expressed in fraction of LD_o to appreciate the chemotherapeutic indexes. The statistical significance of the results was determined using the test of Student. Unless otherwise stated all ILS values were statistically significative ($p < 0.05$).

The activities of the monomers and the corresponding dimers are reported in table 1 and 2, respectively.

Table 1.

Activity of monomeric 7H pyridocarbazoles on L 1210 mice leukemia.



Compound	R_1	R_2	R_3	LD_o (mg/kg)	Dose ip Fraction LD_o	Control[a,b] survival time (days)	Treated[a,b] survival time (days)	T/C %[c]
1	H	OCH_3	$-CH_3$	60	0.5	8.8±0.4	9.8±0.4	111
2	H	OCH_3	$-(CH_2)_2-N$ ◯	10	1	9.2±0.9	8.8±0.3	ns
3	CH_3	OCH_3	$-CH_3$	25	0.7	9.6±0.7	11.7±0.9	122
4	CH_3	OCH_3	$-(CH_2)_2-N$ ◯	5	0.5	8.4±0.9	8.4±0.4	ns
5	H	OH	$-CH_3$	25	1	9.6±0.7	9.3±0.5	ns

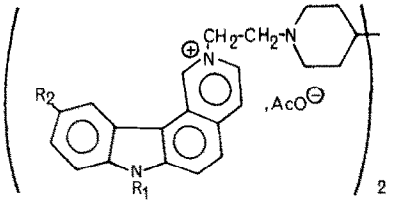
a : mean survival times are given with ± twice the standard error.

b : 10^5 cells are inoculated ip to female DBA/2 mice (15 animals per group). The product is injected ip 24 hours later and deaths are recorded every day at the same hour.

c : T/C % = $\frac{\text{treated survival time}}{\text{control survival time}} \times 100$. ns = statistically not significant.

Table 2.

Activity of 7H pyridocarbazole dimers on L 1210 mice leukemia.[a]



Compound	R_1	R_2	LD_o (mg/kg)	Dose Fraction LD_o	Control survival time (days)	Treated survival time (days)	T/C %
6	H	OCH_3	50	0.4	9.9±1.0	18.1±4.7	182
				0.25	8.6±0.7	18.6±4.7	216
				0.2	9.9±1.0	14.3±2.6	144
				0.1	9.9±1.0	13.6±2.9	137(1/15)[b]
				0.02	9.9±1.0	12.2±0.9	123
7	CH_3	OCH_3	25	0.8	8.3±0.4	12.9±1.9	155(4/15)[b]
				0.4	8.3±0.4	11.8±0.9	140(4/15)[b]
				0.05	8.3±0.4	11.9±1.2	143
8	H	OH	50	0.4	8.4±0.6	13.2±1.9	157
				0.2	8.4±0.6	12.9±1.5	153
				0.02	7.9±0.7	9.4±1.2	119

a : same conditions as in table 1.

b : 45 days survivors.

These results show clearly that in this series, a considerable increase in the antitumor activity is induced by the dimerization process. The DNA binding affinity of the dimers is als 100 to 1000 times higher than that of the monomers (results not shown).

While the monomers 1 to 5 demonstrate practically no valuable activity, the corresponding dimers exhibit T/C up to a value of 216. A substantial percentage of cured animals is obtained with compound 7. In addition, the therapeutic indexes of all dimers appear to be remarkably hig Compound 6 is still active at a dose 50 times smaller than the LD_o.

The dimerization of 7H pyridocarbazoles appears therefore to enhance considerably the ant tumor activity.

According to our primary assumption, the design of compounds with high affinity for DNA seems thus to increase the chances of getting more potent drugs. A more extensive study of a large variety of monomers and dimers with various experimental tumors is still necessary to ge a better undestanding of the mechanism of action of this type of agents.

Nevertheless these preliminary results emphasize the potential interest of bifunctional intercalators in the field of antitumoral drugs.

Acknowledgements.

This study was done under the auspice of EORTC (Screening and pharmacology group). The Technical assistance of J. Couprie and J. Morizet is gratefully acknowledged. This research was supported by grants of INSERM, DGRST, Université René Descartes (Paris V) and Université Pierre et Marie Curie (Paris VI).

REFERENCES

1. W. Muller and D.M. Crothers, J. Mol. Biol., 35, 251 (1968).
2. M.J. Waring, J. Mol. Biol., 54, 247 (1970).
3. E. Calendi, A. Dimarco, M. Reggiani, B. Scarpinato and L. Valentini, Biochim. Biophys. Acta, 103, 25 (1965).
4. F. Dall'Acqua, M. Terbojevich, S. Marciani, D. Vedaldi and G. Rodighiero, Farmaco, Ed. Sci., 29(9), 682 (1974).
5. J.B. Le Pecq, M. Le Bret, C. Gosse, C. Paoletti, O. Chalvet and Nguyen-Dat-Xuong, Molecular and Quantum Pharmacology, vol.7, 515 (1974).
6. J.B. Le Pecq, C. Gosse, Nguyen-Dat-Xuong, C. Gosse and C. Paoletti, C.R. Acad. Sci. Paris, 281, Serie D, 1365 (1975).
7. J.B. Le Pecq, Nguyen-Dat-Xuong, C. Gosse and C. Paoletti, Proc. Natl. Acad. Sci. USA, 71 (12), 5078 (1974).
8. A. Di Marco and F. Arcamone, Arzneim Forsch. (Drug Res.) 25 (3), 369 (1975).
9. J.B. Le Pecq, M. Le Bret, J. Barbet and B.P. Roques, Proc. Natl. Acad. Sci. USA, 72 (8), 2915 (1975).
10. J. Barbet, B.P. Roques and J.B. Le Pecq, C.R. Acad. Sci. Paris, 281, serie D, 851 (1975).
11. B. Gaugain, J. Barbet, R. Oberlin, B.P. Roques and J.B. Le Pecq, Biochemistry, 17, 5071 (1978)
12. B. Gaugain, J. Barbet, N. Capelle, B.P. Roques and J.B. Le Pecq, Biochemistry, 17, 5078 (1978).
13. A. Delbarre, B.P. Roques and J.B. Le Pecq, C.R. Acad. Sci. Paris, 284, serie D, 81 (1977).
14. V.F. Marquez, J.W. Cranston, R.W. Ruddon and J.H. Burckhalter, J. Med. Chem., 17 (8), 856 (1974).
15. E.S. Canellakis, R.M. Fico, A.H. Sarris and Y.H. Shaw, Biochem. Pharm., 25, 231 (1976).
16. B.F. Cain, B.C. Baguley and W.A. Denny, J. Med. Chem., 21 (7), 658 (1978).
17. Birancha S. Sinha, R.M. Philen and R. Sato, J. Med. Chem., 20 (11), 1528 (1977).
18. P.B. Dervan and M.M. Becker, J. Amer. Chem. Soc., 100, 1968 (1978).
19. K.F. Kuhlmann, N.J. Charbeneau and C.W. Mosher, Nucleic Acid Research, 5 (7), 2629 (1978).
20. Teh Kuei Chen, R.M. Fico and E.S. Canellakis, J. Med. Chem., 21, 868 (1978).
21. J. Barbet, B.P. Roques, S. Combrisson and J.B. Le Pecq, Biochemistry, 15, 2642 (1976).
22. A. Delbarre, B.P. Roques, J.B. Le Pecq, J.Y. Lallemand and Nguyen-Dat-Xuong, Biophys. Chem., 4, 275 (1976).

23. D. Pelaprat, R. Oberlin, B.P. Roques and J.B. Le Pecq, C.R. Acad. Sci. Paris, 283, série D 1109 (1976).

24. B.P. Roques, J.B. Le Pecq, D. Pelaprat and I. Le Guen, Brevet ANVAR n° 78.23.801 (1978).

25. C.G. Zubrod, Proc. Natl. Acad. Sci. USA, 69, 1042 (1972).

26. J.B. Le Pecq, C. Gosse, Nguyen-Dat-Xuong, S. Cros, C. Paoletti, Cancer Research, 36, 3067 (1976).