Biochemical Pharmacology, Vol. 33, No. 22, pp. 3681–3688, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00

EFFECTS OF 7*H*-PYRIDOCARBAZOLE MONO AND BIFUNCTIONAL DNA-INTERCALATORS ON CHINESE HAMSTER LUNG CELLS *IN VITRO*

JEAN-PIERRE BENDIRDJIAN,* CHARLOTTE DELAPORTE,* BERNARD P. ROQUES† and ALAIN JACQUEMIN-SABLON‡

*Unité de Biochimie et Enzymologie, Institut Gustave Roussy, 94800 Villejuif, France; and †Département de Chimie Organique, ERA 613 du CNRS, et U.266 de l'INSERM—U.E.R. des Sciences Pharmaceutiques et Biologiques, 4 Avenue de l'Observatoire, 75006 Paris, France

(*Received* 1 *March* 1984; *accepted* 6 *June* 1984)

Abstract—The effects of two 7*H*-pyridocarbazole dimers, PyDi1 and PyDi2, on Chinese hamster lung cells in culture *in vitro*, were compared to those of the corresponding monomers, PyMo1 and PyMo2, by measuring the rates of macromolecule syntheses, the growth kinetics of the drug-treated cells, and the cell cycle progression. The dimers, which are endowed with a very high DNA affinity, were about 10- and 40-fold more cytotoxic than the monomers from which they markedly differ in the following ways: in contrast to monomers, the dimers do not provoke the arrest of cell cycle progression in the G_2 + M phase; after a transitory exposure to either one of the dimers, the cell growth arrest was delayed for 6–8 generations.

Therefore, the 7*H*-pyridocarbazole dimers express their cytotoxicity through a mechanism of action different from that of their mono-intercalating counterparts. They might then constitute a new series of antitumour drugs.

Drugs, such as actinomycin D, anthracyclines and ellipticines, which bind to DNA through intercalation between adjacent base pairs, are among the most currently used antitumour agents. On *in vitro* cultured cells, these drugs are known to be active at several biochemical levels. Inhibition of DNA replication and transcription, arrest of cell cycle progression in the G_2 + M phase, and induction of DNA single and/or double strand breaks are common effects to most of them. Nevertheless, the precise event responsible for their cytotoxicity, as well as the mechanism of their selectivity toward tumour cells, are still unknown. Several reports have shown that, in the anthracycline and the ellipticine series which are amenable to multiple substitutions, a correlation was observed between the DNA binding affinity of the drugs and their cytotoxicity [1–3]. This indicates that, although this characteristic is not sufficient, a high DNA affinity is an important parameter for the biological activity of the DNA intercalating drugs.

In an effort to obtain new antitumoural agents along this line, various 7*H*-pyridocarbazole (a stereoisomer of ellipticine) dimers were prepared [4]. These molecules bis-intercalate into DNA, depending on the nature, the flexibility, and the ionization state of the linking chain. Several of them displayed a very high antitumour activity on the mouse L1210 leukemia [4, 5].

The purpose of this study was to compare the effects of two 7*H*-pyridocarbazole dimers on Chinese hamster lung cells in culture *in vitro* with those of the corresponding monomers. The results show that both monomers inhibited the macromolecule syntheses at doses very close to that required to inhibit the cell cloning efficiency. In addition they provoked the arrest of the cell cycle progression in the G_2 + M phase. The 7*H*-pyridocarbazole dimers were about 10- and 40-fold more cytotoxic than their mono-intercalating counterparts, from which they essentially differ in two ways: they do not provoke the accumulation of the cells in the G_2 + M phase of the cycle; after a transitory exposure to either one of them, the cells continued to grow for about 6–8 generations prior to irreversibly arresting their growth. Therefore it appears that the dimerization of the 7*H*-pyridocarbazole derivatives leads to compounds which exert their cytotoxic action through a mechanism of action markedly different from that of the monomers.

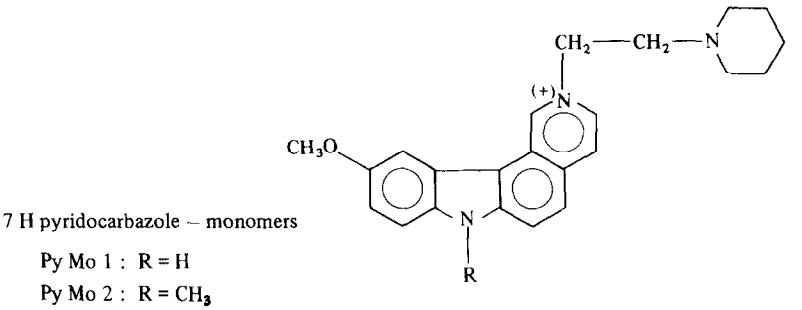
MATERIALS AND METHODS

Cells and culture medium. The Chinese hamster lung cells DC-3F have been previously described [6], as have the maintenance conditions of the monolayer cultures. Routinely, the cells were grown in Eagle's minimum essential medium (MEM), supplemented with 10% fetal calf serum (FCS), streptomycin (50 μg/ml) and penicillin (100 IU/ml).

Drugs. The structures of the drugs used in this work are shown in Fig. 1. The 7*H*-pyridocarbazole monomers (PyMo1 and PyMo2) and dimers (PyDi1, NSC 335153D and PyDi2, NSC 335154D) have been described by Pelaprat *et al.* [4, 7] and were kindly provided by the Laboratoire R. Bellon (Neuilly, France).

All drugs were dissolved in water, and sterilized by filtration through 0.2 μm polycarbonate membrane (Nucleopore Corp., Peasanton, CA). Drug con-

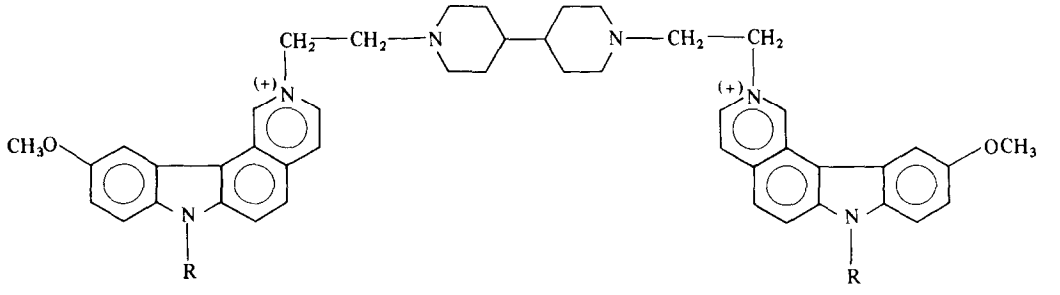
‡ To whom correspondance should be addressed at: Unité de Biochimie et Enzymologie, Institut Gustave Roussy, 94800 Villejuif, France.



7 H pyridocarbazole – monomers

Py Mo 1 : R = H

Py Mo 2 : R = CH_3



7H pyridocarbazole – dimers

Py Di 1 : R = H

Py Di 2 : R = CH_3

Fig. 1. Structures of 7*H*-pyridocarbazole monomers and dimers.

centrations, in the sterile solutions, were determined by absorption spectroscopy. These solutions were stored at 4°, in the dark. Immediately before use the solutions were serially diluted to the appropriate concentration in culture medium.

Drug exposure and cell survival. Throughout this study, the effects of the drugs were determined on exponentially growing cells.

For drug treatment, 16-mm wells of 24-well Costar dishes (Costar, Cambridge, MA; No. 3524) were seeded with 1×10^5 cells in 2 ml of MEM-FCS, and incubated at 37° in a humidified 5% CO_2 atmosphere, for about 20 hr. The medium was then replaced either with 1 ml of fresh medium (controls), or with 1 ml of medium containing the drug at the indicated concentrations. After 3 hr of incubation at 37°, the drug was washed off by rinsing the wells twice with 1 ml of MEM. Then 1 ml of MEM was poured into each well, and the cells were further incubated at 37° for 1 hr. This treatment was designed to allow the efflux of the exchangeable fraction of the drug retained within the cells. The 1 hr duration of this further incubation was determined on the basis of previous experiments with 6*H*-pyridocarbazole treated cells [8], showing a rapid efflux of these drugs reaching a plateau after about 30 min. After removal of the medium, the cells were washed twice again with 1 ml of MEM and trypsinized.

For the determination of the colony forming ability, about 200 cells were plated in triplicate in 35 mm diameter Petri dishes containing 1 ml of MEM-FCS and 1 ml of conditioned medium. The colonies were counted about 1 week later. In these conditions, the cloning efficiency of the controls was about 70%.

Incorporation of radioactive precursors into DNA, RNA and proteins. The cells were incubated with the drug as described above. After drug removal and washings, 1 ml of medium containing a DNA, RNA or protein labelled precursor was added into each well. After 1 hr of incubation at 37°, the wells were washed twice with 1 ml of MEM and, after trypsinization, were mixed with 1 ml of cold 10% (w/v) trichloracetic acid. After 30 min, the precipitate was collected on a 2.5 cm diameter glass microfibre filter (Whatman, GF/C) and washed 3 times with 1 ml of cold 5% trichloracetic acid, and twice with ethanol. After drying, the filters were transferred to scintillation vials with 6 ml of Permafluor III (Packard Instruments Co.), and the radioactivity was determined.

Flow cytometry. For analysis of DNA content by flow cytometry, the cells were fixed as described by Crissman *et al.* [9]. The fixed cells (1 to 2×10^6) were stored at 4° for 18 hr, and after rinsing twice with water, they were centrifuged at 700 *g* for 4 min and resuspended in a solution containing 1 mg of ribonuclease (Worthington) per ml of distilled water adjusted at pH 7.0. After incubation at 37° for 30 min, the cells were centrifuged, rinsed in distilled

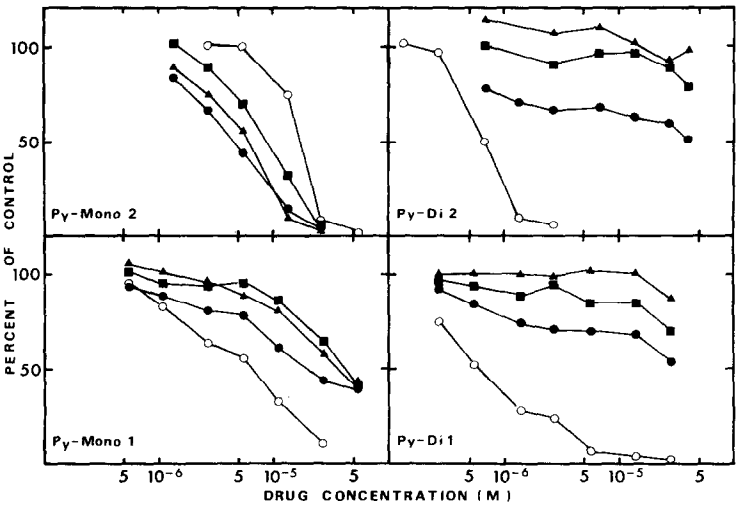


Fig. 2. Effects of 7*H*-pyridocarbazole monomers and dimers on cell survival and macromolecule syntheses as a function of drug concentration. After drug treatment, the cell cloning efficiency and macromolecule syntheses were measured as described in Materials and Methods. Drug-treated cells were incubated for 1 hr in medium containing 0.1 μCi/ml of [^{14}C]amino-acid mixture (54 mCi/mAtom) and either 1 μCi/ml of [6-^{3}H]thymidine (28 Ci/mmole) or 1 μCi/ml of [5,6-^{3}H] uridine (37 Ci/mmole). Each curve represents an average of 6 independent experiments: ○, cells survival; ●, uridine; ■, aminoacids; ▲, thymidine.

water, and resuspended in staining solution containing 50 μg/ml of ethidium bromide in 1.12% (w/v) aqueous sodium citrate. The stained cells were then analysed for DNA content using either a Cytofluorograf 4800A (Bio/Physics Systems, Inc.), coupled to a 2100 distribution analyser, or a FACS IV flow cytofluorometer (Becton-Dickinson). The data were analysed using the Dean and Jett model [10]. The Fortran program was kindly provided by Dr. M. Fox and adapted to Digital Minc 11/23.

RESULTS

Cell lethality and inhibition of macromolecule syntheses

Figure 2 shows the effects of the 7*H*-pyridocarbazole monomers and dimers on the cell survival and macromolecule syntheses. DC-3F cells in exponential phase of growth were incubated with increasing concentrations of the different drugs for 3 hr. After removal of the drug, the cells were tested for their ability to synthesize macromolecules by a 1 hr pulse labelling with the appropriate precursors, and the drug cytotoxicity was evaluated from the determination of their residual plating efficiency. In these conditions, DNA, RNA and protein syntheses were almost simultaneously inhibited by both monomers (PyMo1 and PyMo2). However, when one compares the doses yielding a 50% inhibition of these different syntheses, PyMo1 was about 4–5-fold less efficient than PyMo2. Decrease in the cell survival occurred at concentrations close to that corresponding to the inhibition of macromolecule syntheses, but, with regard to this parameter, PyMo1 was about 4–5-fold more active than PyMo2.

In similar experiments, the 7*H*-pyridocarbazole dimers (PyDi1 and PyDi2) displayed a markedly different behaviour. It first appeared that the colonies formed after treatment of the cells with either one of both dimers presented a peculiar aspect. Figure 3 (A–C) shows that treatment of the cells with increasing concentrations of PyMo1 led to a decreasing number of normal size colonies. Incubations of the cells with PyDi1 rather resulted in a reduction of the size of the colonies, the number of which remained about constant (D–F). The fraction of small colonies was increasing with PyDi concentration.

Treatment of the cells with PyMo2 and PyDi2 provoked the same effects as PyMo1 and PyDi1 respectively. Figure 2 shows that, after a 3 hr incubation, the cell plating efficiency, measured by scoring the number of residual control-like colonies, was 50% inhibited by PyDi1 or PyDi2 at a concentration of about 5×10^{-7} M. Therefore, PyDi1 and PyDi2 are about 10- and 40-fold more cytotoxic than PyMo1 and PyMo2 respectively. At concentrations ranging from 1×10^{-7} M to 1×10^{-6} M, corresponding to a marked cytotoxic effect, PyDi1 and PyDi2 had little influence on DNA, RNA and protein syntheses. RNA synthesis, which was the most sensitive to both drugs, was only 50% inhibited by one or the other at a concentration of 3×10^{-5} M.

The development of the small-size colonies, and the lack of inhibition of macromolecule synthesis suggested that, after removal of the drug, the dimers treated cells remained capable of dividing several times before the arrest of their growth. Therefore, the survival kinetics of the cells treated either with monomers or dimers were analysed in further detail.

Cell survival studies

Exponentially growing cells were incubated for 4.5 hr with either PyMo1 or PyDi1 at different concentrations. The drug was then removed and, after

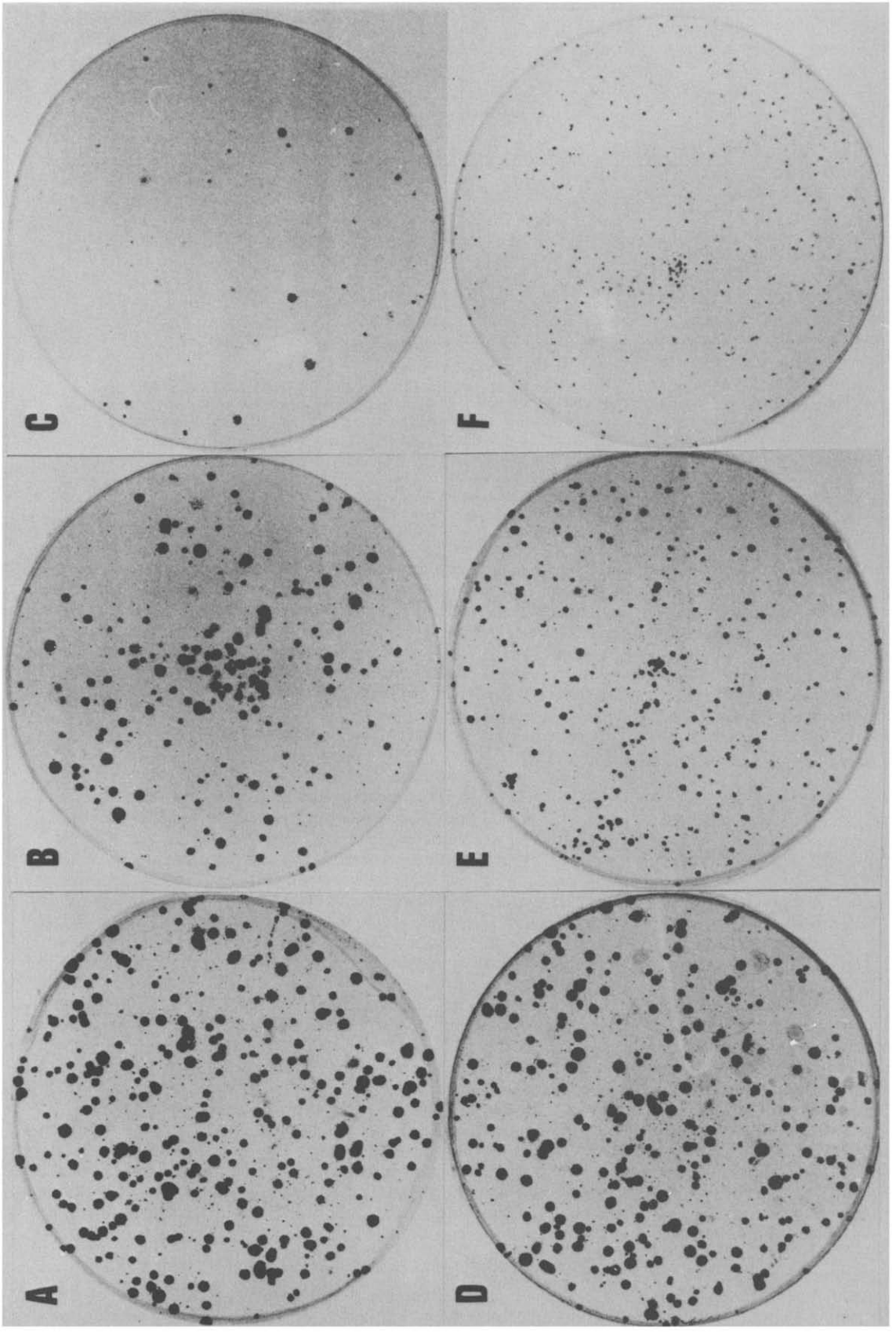


Fig. 3. Morphology of cell colonies after treatment with PyMo1 or PyDi1. Exponentially growing cells were treated for 3 hr either with PyMo1 at 2.6×10^{-6} M (B) and 2.8×10^{-5} M (C), or PyDi at 2.8×10^{-7} M (E) and 1.4×10^{-6} M (F). 300 untreated cells (A and D) or drug treated cells were then plated in 60 mm diameter Petri dishes and then incubated at 37° in 5% CO_2 atmosphere for 6 days. The clones were stained with crystal violet and photographed.

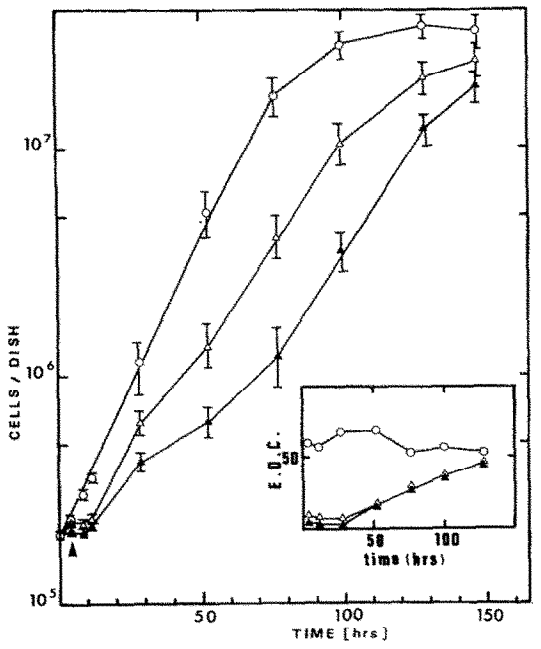


Fig. 4. Growths kinetics of PyMol treated cells. Twenty-four hours before drug treatment, 1×10^5 DC-3F cells were seeded in 100 mm diameter Petri dishes. At zero time, the cells were incubated with PyMol at 1.4×10^{-5} M (△) or 2.8×10^{-5} M (▲) for 4.5 hr (the arrow indicates the end of the treatment). The drug containing medium was then replaced by fresh medium which was renewed daily. At the indicated times, aliquot samples were taken for cell counting and cloning efficiency determination (insert to the figure). The figure represents an average of 5 independent experiments and the error bars correspond to the standard deviations on each point.

washing, the cells were incubated in fresh medium at 37° in a humidified 5% CO_2 atmosphere. At different times after the drug treatment, the cells were trypsinized and resuspended for counting and determination of the efficiency of cloning.

Figure 4 represents the increase in total cell numbers after treatment with PyMol at 1.4 and 2.8×10^{-5} M. After a 10 hr growth arrest, the cell growth resumed according to the following pattern: after a burst of division, lasting about 15 hr (about one generation time), the cell numbers slowly increased for the next 20 hr (low dose) or 40 hr (high dose); later, the cell numbers exponentially increased with an apparent doubling time of 16 hr (as compared to 12 hr for the control), and tended to the same plateau as the untreated cells. Comparable growth curves have been previously observed and analysed for irradiated cells [11, 12]. Such growth kinetics were interpreted as indicating that the drug-treated cell population is composed of two kinds of cells: surviving and non-surviving cells (defined as non-colony formers). The insert to Fig. 4 shows that, after Py-Mol treatment in the conditions of the experiment, the survival fractions were about 20% and 10% of the control respectively. These survival fractions slightly decreased during the first 30 hr after drug removal, and progressively increased after 50 hr to finally reach a control-like value. Thus, the initial burst of division indicates that, after the 10 hr division delay, the non-surviving cells are able to divide about once, and the exponential growth observed later corresponds to the growth of the surviving cells. Light and electron microscopy revealed that the cytotoxic effects of PyMol were also accompanied by cell morphological modifications, such as formation of giant cells and nucleoli fragmentation. These modifications developed during the first 48 hr after treatment and later disappeared.

Cells treated with PyDil elicited a markedly different pattern. Figure 5 shows that after the 4.5 hr drug treatment at 1.4×10^{-6} M the cell growth continued although with an average doubling time of about 18 hr as compared to 12 hr for the untreated cells. This residual growth was pursued for about 120 hr, which represents 7–8 generations. Then a plateau was settled at a cell density 8–10 times lower than that of the control. At lower PyDil doses, the doubling time of the treated cells tended to be closer to that of the untreated cells and the plateau which was only reached at higher cell densities was less clearly defined because of the growth of surviving cells. Doubling the dose used in the experiment described in Fig. 5 did not change significantly the shape of the curve, the drug effect being already maximum. The insert in Fig. 5 shows that the cells, which have been treated with PyDil at 1.4×10^{-6} M for 4.5 hr, have irreversibly lost their ability to form normal colonies. In fact, the size of the colonies decreased with the time of plating after drug treatment: later than 72 hr after treatment, the number

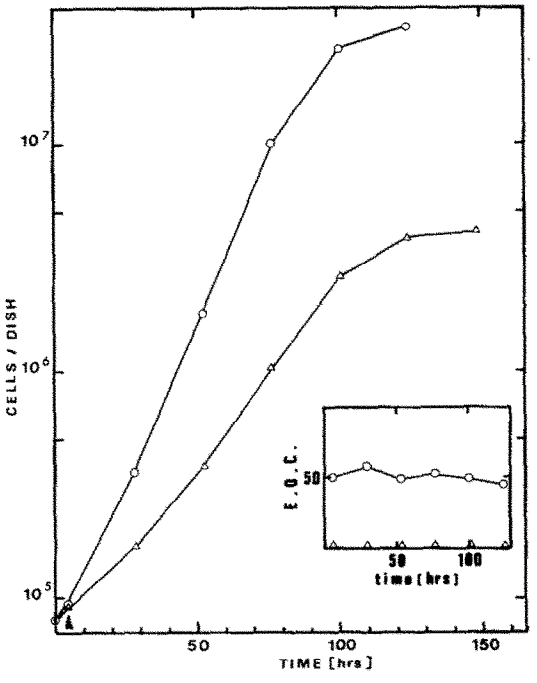


Fig. 5. Growth kinetics of PyDil treated cells. The cells were treated for 4.5 hr with PyDil at 1.4×10^{-6} M, and the experiment was carried out as described in legend to Fig. 4. The insert to the figure shows the cell cloning efficiency at the different times. As explained in the text, only normal size colonies were counted.

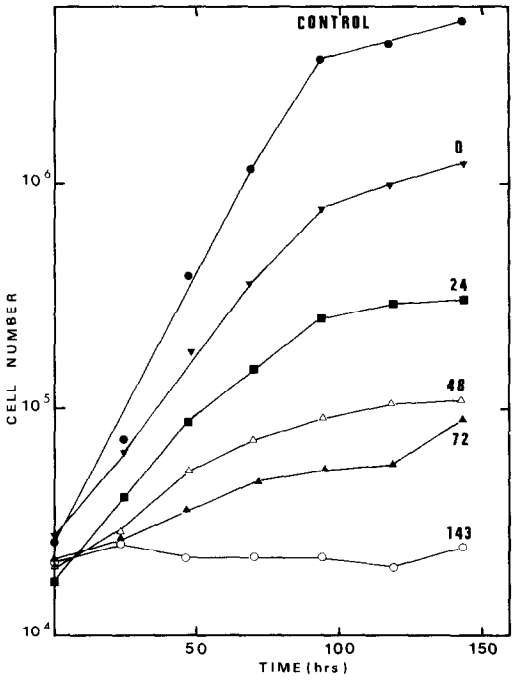


Fig. 6. Residual growth of PyDi1 treated cells. This figure represents the growth curves of the cells taken at different times after PyDi1 treatment in the experiment shown on Fig. 5. 2×10^4 cells from each aliquots were seeded in 60 mm diameter Petri dishes. Each point was carried out in duplicate. The number on each curve indicate the time after drug treatment at which the cells were plated.

of residual cell divisions was too small to form identifiable colonies. Similarly when the cells were seeded in conditions of mass culture (about 2×10^4 cells per 35 mm diameter Petri dishes) the rate and extent of residual growth was progressively decreasing when the time from drug treatment was increasing (Fig. 6). It is noteworthy that cells trypsinized at the plateau (143 hr after drug treatment) were able to re-attach and, although they did not divide, remained bound to the support for about 150 hr. Morphological modifications described in PyMo1 treated cells were not observed in PyDi1 treated cells which remained of normal size or became smaller at late times after drug treatment.

Treatment of the cells with PyDi2 in comparable conditions led to similar effects. The overall interpretation of these results is that, after a transitory exposure to 7*H*-pyridocarbazole dimers, the cells have acquired a limited life span of 7–8 generations, at the end of which they stop growing and do not recover.

Macromolecule syntheses in PyDi1-treated cells

In order to further analyse the physiological state of the cells after PyDi1 treatment, we determined the rates of macromolecule syntheses at different times after drug treatment. The cells were incubated for 4.5 hr with PyDi1 at 1.4×10^{-6} M and, every 24 hr after the treatment, were pulse-labelled for 1 hr with [^{3}H]-leucine and either [^{14}C]-thymidine or [^{14}C]-uridine. During the period of residual growth after drug treatment, the rates of incorporation of the different markers into acid-insoluble material remained about constant or slightly decreased. However, Table 1 shows that the PyDi-induced growth arrest was associated with a drastic and immediate decrease in the rates of DNA and protein syntheses which fell to about 1% or less of the initial values. Comparable decrease of the RNA synthesis was also observed, but with a 24 hr delay. In contrast, when the growth of the untreated cells was arrested at saturation density, macromolecule syntheses remained about constant (or even increase) for several days after reaching the plateau.

Table 1. Macromolecule syntheses after PyDi1 induced growth arrest

Time (hr)	% Incorporation					
	Tdr		Urd		Leu	
	C	T	C	T	C	T
0	100	100	100	100	100	100
24	97	0.3	66	65	185	0.7
48	94	0.4	82	0.5	260	1.0
72	29	0.4	107	0.8	180	1.3
96	10	0.2	132	1.3	135	1.4

This table shows the results of a typical experiment. The cells were first incubated for 4.5 hr with PyDi1 at 1.4×10^{-6} M. At different times after drug treatment, the cells were then pulse-labelled for 1 hr with radioactive precursors: ^{14}C-thymidine (0.1 μCi/ml; 52 mCi/mmole), ^{14}C-uridine (0.1 μCi/ml; 57.8 mCi/mmole) and ^{3}H-leucine (1 μCi/ml; 100 Ci/mmole). The precursor incorporation into acid-insoluble material was determined as described in Materials and Methods. Cell growth arrest occurred 96 hr after drug treatment (see Fig. 4): this time was taken as 0 time and the rates of macromolecule syntheses at the different times afterward are expressed as percent of their respective levels at 0 time (series T). Series C shows the rates of macromolecule syntheses in untreated cells arrested at saturation density.

Effects on cell cycle progression

When the cells were exposed for 24 hr to PyMo1 at the following concentrations, 1.2×10^{-6} M (46% survival), 6×10^{-6} M (18% survival) and 1.2×10^{-5} M ($\leq$1% survival), one could observe the progressive accumulation of the cells in the G_2 + M phase of the cycle. Such a G_2 block has already been reported for other DNA intercalating agents [13, 14]. In contrast, 24 hr treatment of the cells with PyDi1 at concentrations up to 3.5×10^{-6} M, which completely abolishes the efficiency of cloning, does not provoke significant modifications in the pattern of distribution of the cells between the different phases of the cycle (Fig. 7).

Figure 8 shows the evolution of the cell distribution in the cycle at different times after a 4.5 hr treatment either with PyMo1 at 2.8×10^{-5} M or with PyDi1 at 1.4×10^{-6} M. At the end of the treatment with PyMo1 (time 0 in series B), a relative increase of the proportion of cells in the G_2 + M phase was already observed. Four hours after treatment, the G_1 phase almost completely disappeared, whereas the cells were essentially in S and G_2 + M phases. At 24 hr after treatment, the profile began to reverse with the

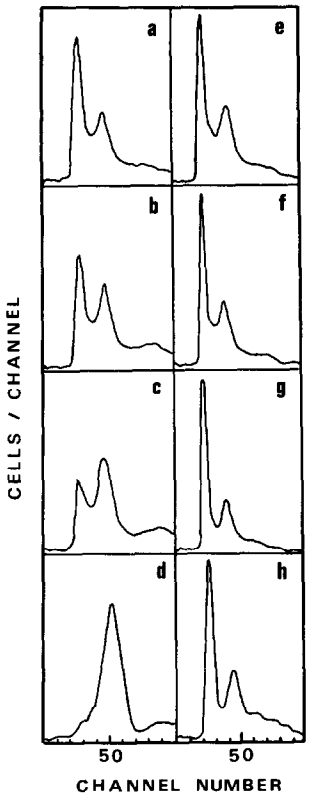


Fig. 7. Effect of PyMo1 and PyDi1 at different concentrations on the cell cycle progression. Exponentially growing cells were incubated for 24 hr either with PyMo1 at 1.2×10^{-6} M (b), 6×10^{-6} M (c), and 1.2×10^{-5} M (d), or with PyDi1 at 1.4×10^{-7} M (f), 1.4×10^{-6} M (g) and 3.5×10^{-6} M (h). After staining with ethidium bromide, as described in Materials and Methods, the cells were analysed for DNA content with a cytofluorograf 4800 A (Bio/Physics System, Inc.). Patterns a and e correspond to untreated cells.

reappearance of the G_1 peak. This reversion was pursued at 48 hr, and at 96 hr the pattern was that of a normally growing cell population.

Figure 8 (series C) shows that PyDi1 treated cells did not accumulate in the $G_2 + M$ phase. On the contrary, at late times after drug treatment, when the cell growth was arrested, the cells rather accumulated in G_1.

DISCUSSION

A possible rational approach in the search for new DNA-intercalating antitumour compounds consists in designing molecules having DNA binding affinities as high as possible [2, 4]. This rationale led Pelaprat *et al.* [4] to synthesize 7*H*-pyridocarbazole dimers which *in vitro*, in conditions where they behave as bifunctional intercalators, display apparent DNA binding constants ranging from 10^8 to 10^9 M^{-1}. These values are to be compared with 10^4 to 10^6 M^{-1} for the 7*H*-pyridocarbazole monomers [7]. It was also shown that in this series of considerable increase in the antitumour activity was induced by the dimerization process [4, 5]. A major problem was then to analyze whether monomers and dimers in this series would have the same mechanism of action, and thus would only differ by their DNA affinities, or whether the dimer would constitute a new series of antitumour drugs exhibiting a peculiar mechanism of action.

In this study, we have compared the cytotoxicity of two 7*H*-pyridocarbazole dimers (PyDi1 and PyDi2) with that of the corresponding monomers (PyMo1 and PyMo2) on a Chinese hamster lung cell line. The following differences were observed:

(i) Comparison of the doses inhibiting 50% of the cell cloning efficiency showed that the dimers are about 10- and 40-fold more cytotoxic than their respective mono-intercalating counterparts. The monomer concentrations inhibiting the macromolecule syntheses were close to that inhibiting the cell cloning efficiency. The shift of PyMo2 dose–response survival curve toward higher doses than that inhibiting DNA, RNA and protein syntheses (Fig. 2) indicates a partial reversibility of its effects, which is not observed with PyMo1. With regard to the dimers, a clear dissociation was established between the concentrations required to inhibit the macromolecule syntheses and that able to abolish the cell cloning efficiency. In both cases, a 50% inhibition of the cell viability was observed at a dose about 50- to 60-fold lower than that which inhibits the RNA synthesis 50%. Therefore, the lethal event triggered by the 7*H*-pyridocarbazole dimers was not associated with an immediate arrest of the macromolecule syntheses.

(ii) The dimers are characterized by a delayed cytotoxicity. After treatment with PyDi1 at 1.4×10^{-6} M for 4.5 hr, the cells continue to grow for 7–8 generations. They then stop dividing and do not recover. They remained attached to the support for several weeks without resuming their growth. This limited growth capacity of the drug-treated cells accounts for the development of the small abortive colonies.

(iii) The dimers do not provoke the arrest of cell cycle progression in the $G_2 + M$ phase of the cell cycle, as it is observed with the monomers as well as many other types of antitumour agents [13, 14].

The delayed cytotoxicity observed with the 7*H*-pyridocarbazole dimers, which has also been analysed in L1210 cells (C. Esnault, B. P. Roques, A. Jacquemin-Sablon and J. B. Le Pecq, to be published) can be compared with similar effects previously reported after X-ray irradiation of cultured cells [11]. For example, Chinese hamster cells committed to death after irradiation at 0.2 krad remained able to divide up to 5 times. However, the average number of post-irradiation divisions is decreasing with the dose [11]. In contrast, after treatment with the dimers, the number of residual divisions remains about constant (6–8 generations), whatever the drug concentration. Besides, X-irradiation provokes a G2 block [15] and the formation of giant cells [11]: none of these effects was observed in dimer-treated cells.

Therefore the 7*H*-pyridocarbazole dimers appear to kill the cells through a mechanism of action which is different from that of the corresponding mono-intercalating drugs. Although this mechanism is not yet understood, these molecules might constitute a new class of antitumour drugs.

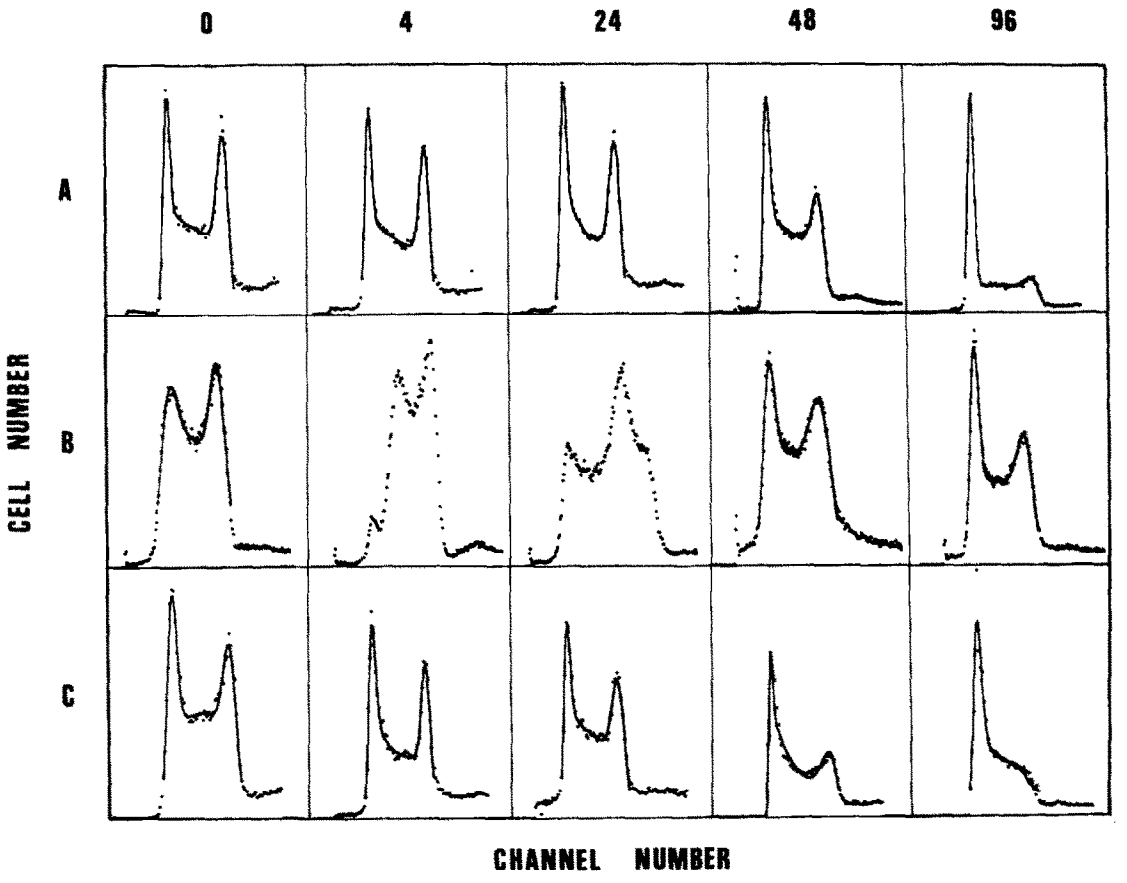


Fig. 8. Patterns of cell cycle progression after treatment with PyMol or PyDil. The cells were treated for 4 hr either with PyMol at 2.8×10^{-5} M or with PyDil at 1.4×10^{-6} M. At different times after the drug treatment (indicated by the numbers at the top of the figure), the cells were stained with ethidium bromide as described in Materials and Methods, and analysed for DNA content with a FACS IV flow cytofluorometer (Becton-Dickinson). The dots correspond to the experimental values. The continuous line is the best fit according to the Dean and Jett model.

Acknowledgements—This work was supported by the Centre National de la Recherche Scientifique (L.A. 147), the Institut National de la Santé et de la Recherche Médicale (U. 140), and the Commissariat à l'Energie Atomique. One of us (J-P. B.) was recipient of a C.N.R.S.–SANOFI fellowship. We are grateful to Dr. G. Muzard for chemical control of the different drugs and to Dr. J. B. Le Pecq for helpful discusssions.

REFERENCES

1. A. Di Marco, A. M. Casazza, T. Dasdia, A. Necco, G. Pratesi, P. Rivolta, A. Velcich and F. Zunino, *Chem. biol. Interact.* **19**, 291 (1977).
2. J. B. Le Pecq, N. Dat Xuong, C. Gosse and C. Paoletti, *Proc. natn. Acad. Sci. U.S.A.* **71**, 5078 (1974).
3. C. Paoletti, S. Gros, N. Dat Xuong, P. Lecointe and A. Moisand, *Chem. biol. Interact.* **25**, 45 (1978).
4. D. Pelaprat, A. Delbarre, I. Le Guen, B. P. Roques and J. B. Le Pecq, *J. med. Chem.* **23**, 1336 (1980).
5. B. P. Roques, D. Pelaprat, I. Le Guen, G. Porcher, C. Gosse and J. B. Le Pecq, *Biochem. Pharmac.* **28**, 1811 (1979).
6. B. Salles, J. Y. Charcosset and A. Jacquemin-Sablon, *Cancer Treat. Rep.* **66**, 327 (1982).
7. D. Pelaprat, R. Oberlin, I. Le Guen, B. P. Roques and J. B. Le Pecq, *J. med. Chem.* **23**, 1330 (1980).
8. J. Y. Charcosset, B. Salles and A. Jacquemin-Sablon, *Biochem. Pharmac.* **32**, 1037 (1983).
9. H. A. Crissman, R. J. Kissane, P. L. Wanek, M. S. Oka and J. A. Steinkamp, in *Third International Symposium on Detection and Prevention of Cancer* (Ed. H. E. Neiburg), p. 79. Dekker, New York (1977).
10. P. N. Dean and J. H. Jett, *J. Cell Biol.* **60**, 523 (1974).
11. M. M. Elkind and G. F. Whitmore, in *The Radiobiology of Cultured Mammalian Cells*, p. 303. Gordon & Breach, New York (1967).
12. Y. Okumura and Y. Uchiyama, *Int. J. radiat. Biol.* **26**, 321 (1974).
13. R. A. Tobey, M. S. Oka and H. A. Crissman, in *Flow Cytometry and Sorting* (Eds M. R. Melamed, P. F. Mullaney and M. L. Mendelsohn), p. 573. Wiley, New York (1979).
14. F. Traganos, L. Staiano-Coico, Z. Darzynkiewicz and M. R. Melamed, *Cancer Res.* **40**, 2390 (1980).
15. W. C. Dewey and D. P. Highfield, *Radiat. Res.* **65**, 511 (1976).