SEARCH FOR NEW DRUGS

ANTITUMOR ACTIVITY OF ACTINOCIN-BASED LIPID INTERCALATORS

I. S. Golubeva,[1] I. Yu. Kubasova,[1] N. P. Yavorskaya,[1] E. N. Glibin,[2] and E. B. Popova[1]

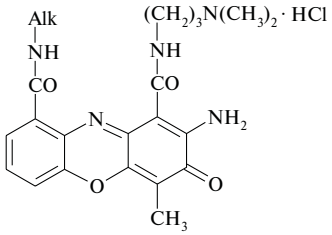
Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 34, No. 7, pp. 3 – 5, July, 2000.

Original article submitted November 30, 1999.

In the previous communication [1] we demonstrated that the class of actinocin derivatives includes compounds possessing antitumor activity, the effect of which in tests on animals bearing model tumors was comparable with that of actinomycin D. The results of that investigation showed that the antitumor activity in the series of compounds studied depends both on the ability to complex formation with DNA and on the presence of lipophilic-hydrophilic mobility in the molecules.

Earlier, Denny et al. [2] also concluded that the balance of lipophilic-hydrophilic properties is one of the factors determining the antitumor properties of acridine derivatives. Behr [3] synthesized an acridine-based compound, containing a cationoid center and an aliphatic radical. He demonstrated that the presence of these fragments in the molecule makes it capable of intercalating into DNA and modifying the DNA properties as a result of the aliphatic fragment "sticking" in the drug – DNA complex, for which reason the compound was classified as a lipid intercalator. Unfortunately, the biological properties of such compounds were never reported.

The presence of two nonequivalent carboxy groups in the actinomycin D molecule implies the possibility to introduce substituents of various types into amides of actinocin and its derivatives. For example, by introducing an alkyl radical into one amide group and a substituent with cationoid center into another amide group we may synthesize compounds with properties analogous to those of the lipid intercalator designed in [3]. We aimed at studying the effects of lipophilic fragments and cationoid groups (imparting hydrophilic properties to a molecule) in DNA complexes. For this purpose, we have synthesized a series of 6-demethylactinocin diamides (with 4-methylcinnabarinic acid) containing various alkyl substituents [butyl (I), cyclohexyl (II), dodecyl (III), and octadecyl (IV)] in the amide group of the quinoid ring. Differing in length, the substituents account for different lipophilicities of the synthesized compounds [4].



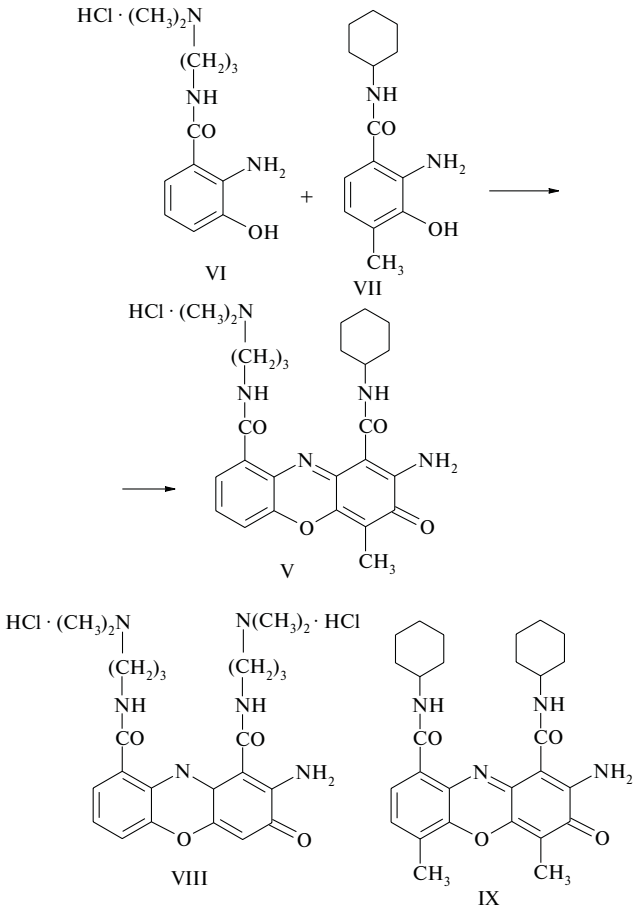
I – IV

Alk = *n*-C_4H_9 (I); *cyclo*-C_9H_{11} (II); *n*-$C_{12}H_{25}$ (III); *n*-$C_{18}H_{37}$ (IV).

In order to study the dependence of the antitumor properties on the positions of the alkyl substituent and the radical with cationoid center, we have also synthesized compound V, in which the alkyl radical is situated (in contrast to compound II) in the quinoid ring. This compound, as well as compounds I – IV [4], was synthesized by mixed oxidative condensation of a 3-hydroxyanthranilic acid derivative (VI) and a 3-hydroxy-4-methylanthranylic acid derivative (VII). The latter compound was obtained by the catalytic hydrogenation of N-cyclohexyl-2-nitro-3-hydroxy-4-methylbenzamide hydrochloride [6].

The accompanying "symmetric" derivatives of cinnabarinic acid (VIII, cf. [5]) and actinocin (IX) were separated from the target compound V using the difference in their solubilities in water and organic solvents. The proposed structure of compound V was confirmed by the absence of a signal characteristic of protons of the 6-CH_3 group in the ^1H NMR spectrum (see data reported in [5] and in the experimental chemical part below) and by the presence of absorption characteristic of cinnabarinic acid and actinocin [5] in the electronic absorption spectrum. The corresponding characteristics were also obtained for the "symmetric" derivative IX.

[1] Blokhin Oncological Research Center, Russian Academy of Medical Sciences, Moscow, Russia.
[2] St. Petersburg State Technological Institute (Technical University), St. Petersburg, Russia.



Below we report on a comparative study of the antitumor properties of compounds I – V, the previously reported N,N′-di(3-dimethylaminopropyl)actinocyldiamide (compound X), and actinomycin D.

EXPERIMENTAL CHEMICAL PART

The UV spectra were recorded with a Specord UV-VIS spectrophotometer (Germany). The ^{1}H NMR spectra were measured on a Tesla BS-497 100-MHz spectrometer (Czech Republic) using freshly prepared solutions in trifluoroacetic acid with TMS internal standard. The melting temperatures were determined using an HMK heating table of the Koefler type. TLC analyses were conducted on Silufol UV-254 plates eluted in the systems chloroform – methanol – 25% aqueous ammonia, 8 : 2 : 0.2 (A), or 25%-aqueous-ammonia-saturated chloroform – methanol, 2 : 1 (B).

The data of elemental analyses (C, H, N) for compounds V and IX coincide with the results of analytical calculations. The physicochemical parameters of the synthesized compounds I – IV agree with the published data [4].

1-(N-Cyclohexylcarbamoyl)-9-(3-dimethylaminopropyl)carbamoyl-2-amino-4-methyl-3H-3-oxophenoxazine-hydrochloride (V). A mixture of 0.84 g of N-cyclohexyl-2-nitro-3-hydroxy-4-methylbenzamide [6] and 1.18 g of N-(3-dimethylaminopropyl)-2-nitro-3-hydroxybenzamide hydrochloride [5] in 30 ml of methanol was reduced by hydrogen at room temperature and atmospheric pressure in the presence of 0.2 g of 10% Pd on charcoal. The formation of aminophenols VI and VII was monitored by TLC in systems A and B. Upon completion of the reduction process, the catalyst was separated by filtration and the filtrate was mixed by stirring at room temperature with 0.972 g of *p*-benzoquinone in 10 ml of methanol. The mixture was stirred at room temperature for 2 h and allowed to stand without stirring for 15 h. The precipitate (0.38 g), separated by filtration after the mixture standing and identified by TLC in systems A and B as compound IX, was washed with ethanol and ether. The filtrate was evaporated to half volume, after which an additional amout of precipitated compound IX was obtained by filtration. The residual filtrate was diluted with 150 ml of absolute ether and allowed to stand for 16 h without stirring. The precipitate was separated by filtration and dissolved in 50 ml of water. The aqueous solution was filtered through a thin charcoal layer, saturated with NaCl to a concentration of 100 – 110 g/liter, and extracted with chloroform to obtain compound V (the aqueous layer analyzed by chromatography showed the presence of compound VIII). The chloroform extract containing compound V was doubly washed with a 100 – 110 g/liter aqueous NaCl solution, evaporated in vacuum to a residual volume of 10 – 15 ml, diluted with 100 – 150 ml of anhydrous ether, and filtered to obtain 0.389 g of compound V (chromatographically homogeneous in solvent systems A and B) in the form of finely crystalline powder; m.p., > 230°C; ^{1}H NMR spectrum (δ, ppm): 7.56 (m, 3H, H_{arom}), 2.85 (s, 6H, $N^+(CH_3)_2$), 2.12 (s, 3H, 4-CH_3), plus several multiplets in the region of 1 – 3 ppm attributed to methylene and methine protons of the propyl and cyclohexyl radicals; UV spectrum in EtOH, λ_{max} (log ε): 239 (4.53), 445 (4.42); $C_{26}H_{33}N_5O_4 \cdot HCl$.

1,9-Di(cyclohexylcarbamoyl)-2-amino-4,6-dimethyl-3H-3-oxophenoxazine (IX). N-cyclohexyl-2-nitro-3-hydroxy-4-methylbenzamide (0.28 g) [6] in 10 ml of methanol was hydrogenated at room temperature and atmospheric pressure in the presence of palladium black. The reduction process completion was monitored by hydrogen absorption and by TLC in solvent systems A and B. Upon completion of the reduction process, the catalyst was separated by filtration and the filtrate was mixed by stirring at room temperature with 0.162 g of *p*-benzoquinone in 3 ml of methanol. The mixture was allowed to stand at room temperature for 18 h and filtered to separate the precipitate of compound IX (0.23 g); m.p., > 230°C (chloroform – benzene); ^{1}H NMR spectrum (δ, ppm): 7.38 (m, 1H, 8-H), 7.18 (1H, J 8 Hz, 7-H), 3.86 (m, 2H, CH), 2.46 (s, 3H, 6-CH_3), 2.17 (s, 3H, 4-CH_3), 1.93 m, 1.69 m, 1.25 m (20H, CH_2); UV spectrum in EtOH, λ_{max} (log ε), nm: 245 (4.58), 445 (4.45); $C_{28}H_{34}N_4O_4$.

EXPERIMENTAL BIOLOGICAL PART

The antitumor activity was studied using a method developed at the Blokhin Oncological Research Center [7]. The experiments were performed on first-generation BDF_1 hy-

TABLE 1. Antitumor Activity of Lipid Intercalators I – V, Actinomycin D, and Compound X in Mice with Ca-755 Model Adenocarcinoma

Compound	Single dose,** mg/kg	TGI, % days after tumor inoculation: 7	10 – 11	13 – 14	17 – 18	SLI, %
I	120	64*	49	26	25	–
II	120	84*	88*	30	24	38
III	30	98*	99*	85*	42	39
IV	80	46	14	0	9	–
V	120	83*	93*	74*	65*	–
X	30	89*	75*	56*	23	21
Actinomycin D	0.04	96*	93*	81*	52*	36

* Reliable difference from control ($p < 0.05$).
** Treatment schedule: single daily injection over a period of 5 days.

TABLE 2. Antitumor Activity of Actinocin Derivatives

Compound	Single dose,** mg/kg	TGI, % (24 h after last drug injection) Ca-755	LLC	B-16	RShM-5	SLI, % P-388
I	120	53 – 64	20	48 – 53	83*	n/a***
II	120	84*	34 – 41	–	79 – 93*	n/a
III	30	96 – 98*	76 – 84*	66*	82*	n/a
IV	80	46	–	21	–	n/a
V	120	83*	–	–	–	n/a
X	30	68 – 89*	40 – 54	65 – 75*	64 – 75*	13 – 20
Actinomycin D	0.04	81 – 98*	63 – 64*	68 – 75*	75 – 77*	38 – 81

* Reliable difference from control ($p < 0.05$).
** Treatment schedule: single daily injection over a period of 5 days.
*** n/a = no antitumor activity.

brid mice inoculated with mammary adenocarcinoma Ca-755, which is the most sensitive model for both actinomycin D (XI) and actinocin derivatives [1].

The spectrum of antitumor action was determined on a series of transferred tumors, including lymphocyte leukemia P-388, Lewis lung carcinoma (LLC), melanoma B-16, and cervical carcinoma RShM-5. The lymphocyte leukemia P-388 was inoculated by intraperitoneally injecting 10^6 tumor cells with a 0.5 ml volume of a type 199 nutrient medium. The solid tumors were subcutaneously inoculated in the right axillary region (0.5 ml of a 10% tumor cell suspension in the same medium). The treatment was started 24 after intraperitoneal injections and 48 h after subcutaneous inoculation of the solid tumors. The drug solutions were prepared *ex tempore* and intraperitoneally injected in a range of doses.

The drug efficacy was evaluated by calculating the percentage tumor growth inhibition (TGI) for 7, 10 – 11, 13 – 14, and 16 – 17 days after the tumor transfer and by determining the survival lifetime increase (SLI) for the test mice with leukemia relative to the untreated control. Compounds inhibiting tumor growth by not less than 50% and/or increasing the lifetime by more than 25% were classified as possessing antitumor activity.

The toxicity of the tested compounds was judged by the number of lost animals, the macroscopic morphology pattern of the internal organs, and the gain in total body and spleen weights.

RESULTS AND DISCUSSION

As seen from the data presented in Table 1, all the studied compounds (except for derivative IV) injected in tolerable doses suppressed the growth of adenocarcinoma Ca-755. The maximum antitumor action (in some experiments, comparable with or even exceeding that of actinomycin D) was observed for derivative III (with dodecyl radical in position 9 of the amide group).

In the tests on solid tumors, the maximum effect was also produced by compound III (Table 2), the TGI values of which for Ca-755, LLC, RShM-5, and B-16 were comparable with that of actinomycin D. Compound II (with cyclohexyl radical in position 9 of the amide group) produced the most pronounced effect with respect to the model cervical carcinoma RShM-5.

None of the compounds tested, as well as the previously studied actinocin derivatives [1], was effective in the treatment of lymphocyte leukemia P-388; compounds I and II produced no protective effect in mice inoculated with Lewis lung carcinoma (Table 2).

Thus, the antitumor activity of the drugs studied is related to a certain extent to the hydrophilic-lipophilic properties of these compounds.

ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research (project No. 99–03–33–044) and by the INTAS Foundation (grant No. 97–31753).

REFERENCES

1. N. P. Yavorskaya, I. S. Golubeva, I. Yu. Kubasova, and E. N. Glibin, *Khim.-Farm. Zh.*, **30**(12), 22 – 26 (1996).
2. W. A. Denny, B. F. Cain, G. J. Atwell, et al., *J. Med. Chem.*, **25**(3), 276 – 315 (1982).
3. J. P. Behr, *Tetrahedron Lett.*, **48**, 5861 – 5864 (1986).
4. E. N. Glibin and E. B. Popova, *Zh. Org. Khim.*, **35**(3), 408 – 411 (1999).
5. Z. I. Korshunova, E. B. Popova, E. N. Glibin, and O. F. Ginzburg, *Zh. Org. Khim.*, **27**(2), 369 – 376 (1991).
6. E. N. Glibin, B. V. Tsukerman, S. S. Tsymbalova, and O. F. Ginzburg, *Zh. Org. Khim.*, **13**(2), 462 – 463 (1977).
7. Z. P. Sof'ina, *Chemotherapy of Tumor Disorders* [in Russian], Meditsina, Moscow (1984), pp. 11 – 42.