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Research Article

IN-VITRO AND *IN-VIVO* ANTITUMOR ACTIVITY OF 1, 3-DIACETOXY ACRIDONES AGAINST EHRLICH ASCITES CARCINOMA

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ABSTRACT

Several structural classes of compounds were discovered against tumor, but many of the existing antitumor agents exhibit severe side effects. Hence there is a need to identify a novel chemical entity having a broad range of therapeutic activity with fewer side effects. In this direction, several 1,3-diacetoxy acridones [1-15] were screened for their antitumor activity against Ehrlich Ascites Carcinoma (EAC) using *in-vitro* and *in-vivo* models. Compound 11 showed highly significant antitumor activity against EAC in comparison with vincristine as standard.

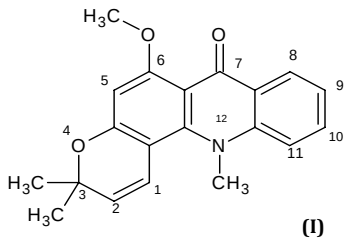
Keywords: 1, 3-Diacetoxy Acridone, Antitumor Activity, Ehrlich Ascites Carcinoma

INTRODUCTION

Cytotoxic drugs remain mainstay of cancer chemotherapy and are being administered with novel ways of therapy such as inhibitors of signals. It is therefore important to discover cytotoxic agents with spectra of activity and toxicity that differ from those current agents[1].

Acronycine, also known as acronine is an acridone alkaloid which was first isolated in 1948 from the bark of Australian tree Acronychia baurri [2]. In subsequent investigation it was found that acronycine possess anticancer activity against a white spectrum of experimental neoplasms in laboratory animals [3]. Dorr et al. [4] studied the *in vitro* cytotoxicity of acronycine in a variety of solid malignancies against human tumor cells isolated from patients. A structural activity relationship (SAR) in the acronycine group of compounds appears to be very inflexible considering that most of the acronycine derivatives tested against cancer, showed much lower activity than acronycine itself [5]. Several series of analogues, including cis- and trans-1,2-dihydroxy-1,2-dihydro acronycine diesters, exhibited marked antitumor properties with broad spectrum and increased potency when compared with acronycine. Among these derivatives diacetate was selected for further investigation, but its pre-clinical development was subsequently discontinued for toxicological reasons [6-8]. Nevertheless, its low water solubility and moderate potency have severely hampered its clinical trials, which have been given so far only poor results [9]. Consequently, the developments of structural analogues possessing a basic nitrogen atom able to give water soluble salts seem highly desirable. Schneider and Co-worker [10] attempted to synthesize structural analogues by modifying side chain in the 6-position on acronycine (I). However, the acronycine containing an oxy-dimethyl amino ethyl side chain in lieu of the methoxy group in the 6-position of acronycine

exhibited significant antitumor activity. Furthermore, inaddition to the compound, several acronycine analogues, in which the methoxy group at C-6 has been replaced by dialkyl amino alkyl side chain (including a dimethly amino ethyl analogue) have recently been tested in cytotoxicity. These modifications resulted in a significant increase of the cytotoxic activity towards L-1210 cells compared with acronycine [5].



(I)

We designed 1,3-diacetoxy N-substituted Acridones[11] by structural modification into the acronycine molecule for better antitumor activity. The fused planar tricyclic moiety contained in acronycine is necessary therefore, it was maintained therefore acetoxy group was introduced in the 6-position by replacing methoxy group. The second acetoxy group was introduced by replacing pyran ring. Further the length of the carbon chain at 12-position was increased to 3 and 4 and tertiary amine group at terminal end.

In this article, we have reported the antitumor evaluation of 1,3-diacetoxy Acridones[1-15] against Ehrlich Ascites Carcinoma. The antitumor activity was screened by determining various parameters like *in vitro* cytotoxicity, percentage increase in life span and percentage decrease in body weight [12,13].

EXPERIMENTAL

Animals

Swiss albino mice of female sex, weighing between 20-23 g, were used for the experiment. The animals were kept in plastic cages with free access to standard diet and water. The animals were maintained at a temperature of 23±2oC with a 12 h of light/dark cycle and relative humidity of 50±10%.

The EAC cells were collected, counted, and adjusted to 10^6 cells/mL with normal saline. The drug dilutions were made with phosphate buffer saline (PBS) and were further adjusted to concentrations ranging from 125-1000 µg/mL. The drug dilutions were then added to the EAC cells and incubated at 37oC for 3 h. At the end of 3 h, the cell viability was determined by tryphan blue exclusion method. Under identical conditions, standard antitumor agent vincristine was evaluated for its *in-vitro* antitumor activity. The percentage cytotoxicity was calculated using the formula, Percentage cytotoxicity = 100 – *Tc*-*Dc*/*Tc* × 100, where *Tc* = total EAC cells, and *Dc* = dead EAC cells. The IC_{50} was determined from concentration percentage cytotoxicity curve and recorded in Table I.

Compounds, which had significant *in-vitro* antitumor activity 3, 5-15 were further selected for screening *in-vivo* antitumor activity. The animals were divided into various groups and each group consisting of eight animals. The EAC cells containing 10^6 cells/0.1 mL of PBS were injected into the peritoneal cavity of all the animals and treatment was started 24 h after inoculation of tumor cells, (once daily as a single dose) for 10 days. Group I served as control and received 0.3% CMC suspension. Group II served as standard and received vincristine (520 µg/kg body weight). Group III-XII served as test groups and received test compounds 3, 5-15 (11.89mg/kg body weight). The control, standard, and test compounds were administered intraperitoneally. Antitumor activity was screened by determining different parameters like body weight analysis, mean survival time and percentage increase in life span.

After tumor cell inoculation, all the mice were weighed daily up to 11 days. Those compounds that had significant *in-vitro* antitumor activity, opposed the average increase in the body weight of the carcinoma induced mice. Percentage decrease in the body weight was determined by using the formula, percentage decrease in body weight = (*Gc*-*Gt*)/*Gc* ×100, where *Gc* = gain in body weight of control group, and *Gt* = gain in body weight of treated group. Percentage decrease in body weight shown by the tested compounds is recorded in Table I.

The survival times of EAC bearing mice were noted. Percentage increase in life span was calculated by the formula, % ILS = (MST of treated group-MST of control group)/MST of control group ×100. % ILS shown by the tested compounds is recorded in Table I.

The cytotoxicity of fifteen compounds was examined on EAC cell lines by Tryphan blue exclusion method with several concentrations of acridones. The IC_{50} values of N^{10}- chloropropyl substituted and chlororbutyl 1,3-diacetoxy acridones derivatives against EAC cells, revealed that antitumor activity relatively increased as the chain length increased from 3 to 4 suggesting that hydrophobicity may plays an important role in biological activity. The increase of distance between ring nucleus and amino group increased the antitumor activity of these compounds. It is clear from the data, the comparison of the cytotoxicity of the butyl derivatives as shown that the cell killing potency following order, 11>15>14>10>12>13 and propyl derivatives 6>8>5>7>3>4. However, comparison of IC_{50} values within the series revealed that the butyl derivatives have higher potency than propyl derivatives.

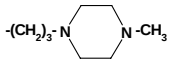
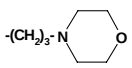
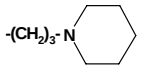
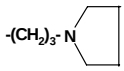
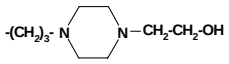
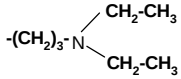
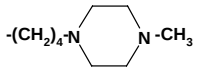
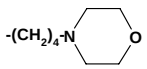
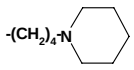
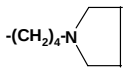
In vivo antitumor activity results showed that compounds 11(%ILS, 103.21), 14(%ILS, 92.94), 15(%ILS, 91.78), 12(%ILS, 62.12), 8(%ILS, 90.61) and 6(%ILS, 66.61) have increased the percentage increase in life span (%ILS) as compared to compounds 10(%ILS, 47.26), 5 (%ILS, 48.42), 9(%ILS, 15.27), 7(%ILS, 15.27), 3(%ILS, 14.17) and 13(%ILS, 13.01) (Table 1, Figure 1) .

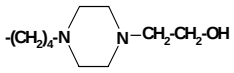
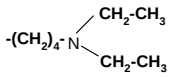
The *in vitro* and *in vivo* antitumor activity against EAC cell line showed that compound 11 exhibited highest antitumor activity among the series of acridones when compared to vincristine. Compounds 6, 12, 14 and 15 exhibited good *in vitro* and moderate antitumor activity. Compounds 5 and 10 exhibited moderate antitumor activity.

The new Acridones derivatives derived from acridone with tertiary amino group at the terminal end of the alkyl side chain had strong inhibiting activity against EAC cell lines. The tricyclic N^{10}-sustituted acridones with acetoxy groups at positions C-1 and C-3 and a secondary amine side chain containing a tertiary amine group at a distance of atleast three to four carbon atoms from the tricyclic ring may be responsible for marked antitumor activity.

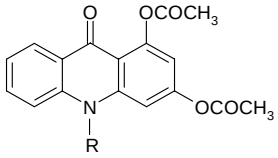
Therefore, it can be concluded that the structural features required with in the series to cause a maximum anticancer activity in EAC cells include hydrophobic substituted acridone ring with an alkyl side chain preferably four methylene units with substitution positively charged morpholine group. However, further pharmacological screening on different types of cell lines is required to select the potent molecule without severe side effects.

Table 1: *In vitro* and *in vivo* antitumor activity of compounds 1-15

Compounds	R	IC_{50} (µg)	In vivo anticancer activity			
			Gain in body weight	Percentage Decrease in body weight	MST$^{\Upsilon}$ ± S.E.$^{\spadesuit}$	% ILS
Control	--	–	5.46 ± 0.19	–	14.6 ± 0.21	–
1	- H	>1000	ND$^{\blacklozenge}$	ND	ND	ND
2	$-CH_2-CH_2-CH_2-Cl$	>1000	ND	ND	ND	ND
3		859.5	4.53 ± 0.82	17.63	16.67 ± 0.61	14.17*
4		>1000	ND	ND	ND	ND
5		515.5	4.63 ± 0.22	15.81	21.67 ± 0.21	48.42***
6		235.5	2.25 ± 0.25	59	24.33 ± 0.71	66.64***
7		620.0	4.78 ± 0.77	13	16.83 ± 0.74	15.27*
8		260.2	1.21 ± 0.12	78	27.83 ± 0.70	90.61***
9	$-CH_2-CH_2-CH_2-CH_2-Cl$	700.0	4.61 ± 0.74	16.18	16.83 ± 0.65	15.27*
10		299.9	4.30 ± 0.21	21.8	21.50 ± 0.34	47.26***
11		205.0	1.0 ± 0.05	81.18	29.67 ± 0.21	103.21***
12		430.2	2.36 ± 0.15	57	23.67 ± 0.49	62.12***
13		619.5	5.11 ± 1.0	7.09	16.50 ± 0.56	13.01*

14		290.3	1.25 ± 0.11	77.27	28.17 ± 0.60	92.94***
15		255.8	1.18 ± 0.13	78.54	28.0 ± 0.63	91.78***
Vincristine	---	179.9	0.9 ± 0.1183	83.51	32 ± 0.25	119.17***

[Y] Mean survival time; [♣] Standard error; [♦] Not determined; * P <0.05; *** P <0.0001.



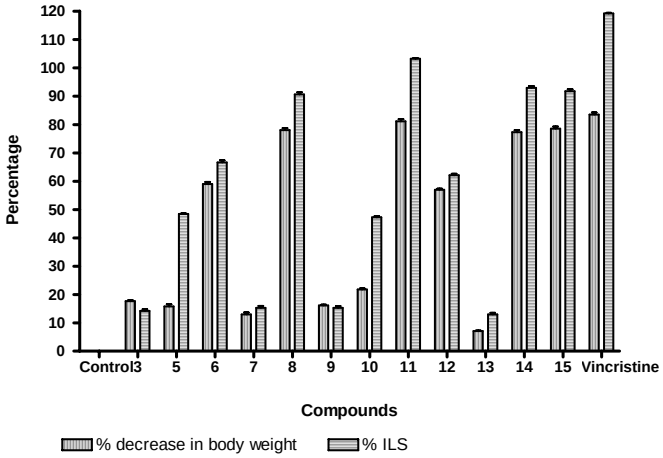


Fig. 1: *In vivo* antitumor activity of compound 3, 5-15 and the standard Vincristine

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REFERENCES

1. Nicolas, G,; Laurence, K.B.; Florencemeyer, L.; Virginie, Malivet, Christin, Sylvie, M.; Michel, K.; Bruno, P.; Ghanem, A.; John, H.; Alain, P.;*Clinical Cancer Res*., 2001, 7, 2573-2583.
2. Hughes, G.K.; Lahey, F.N.; Price, J.R.; Webb, L.J.; *Nature*, 1948,164, 223-224.
3. Svoboda, G.H.; Poore, G.A.; Simpsom, P.J.; Border, G.; *British J. Pharm. Sci.,* 1966, 55, 758-768.
4. Dorr, R.T.; Liddil, J.D.; Von Hoft, D.D.; Soble, M.;Osborne, C.K.; *Cancer Res.,* 1989, 49, 340-344.
5. James, P.D.; Werner, J.G.; Theodor, G.D.; Cornelis, J.V.S.; *Bio. Org. Med. Chem*., 2005, 13, 689-698.
6. Elomori, A.; Michel, S.; Skaltsounis, A.L.; Tillequin, F.; Koch, M.; Pierre, A.; Guilbaud, N.; Leonce, S.; Kraus, B.L.; Rolland, Y.; Atassi, G.; *J Med Chem.,* 1996, 39, 4762-4766.
7. Magiatis, P.; Mitaku, S.; Skaltsounis, A.L.; Tillequin, F.; Koch, M.; Pierre, A.; Atassi, G.; *J. Nat. Prod*., 1998, 61, 982-986.
8. Coster, N.; Michel, S.; Tillequin, F.; Koch, M.; Pierre, A.; Atassi, G.; *J. Nat. Prod*., 1999, 62, 490-492.
9. Scarff, J.H.; Beaumont, A.R.; Gowther, D.; *Can. Treat. Res.,* 1983, 67, 93-94.
10. Schneider, J.; Evans, E.I.; Grunberg, E.; Fryer, R.I.; *J.Med Chem.,* 1972, 15, 226-270.
11. Sathish, N.K.; Rajendra Prasad, V.V.S.; Raghavendra, N.M.; Shanta Kumar, S.M.; Mayur, Y.C.; *Sci. Pharma.,* 2009, 77, 19-22.
12. Khanam, J.A.; Bag, S.P.; Sur, B.; Sur, P.; *Ind. J. Pharmacol.,* 1997, 29, 157- 161.
13. Raghavendra, N.M.; Thampi, P.; Gurubasavarajaswamy, P.M.; Sriram, D.; *Archiv Der Pharm.,* 2007, 12, 635-64.