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Synthesis and *in vivo* antitumor activity of new heterocyclic derivatives of the 1,3,4-thiadiazolium-2-aminide class

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Four new mesoionic compounds derivates of 4-phenyl-5-(4-X-cinnamoyl)-1,3,4-thiadiazolium-2-phenylamine chlorides were synthesized and their antitumor activities against Ehrlich carcinoma and Sarcoma 180 (S180) were evaluated. In the schedule assayed, the derivatives where X = OH and X = NO_2 injected i.p. in mice at a total dose level of 10 and 30 mg/kg respectively caused a significant inhibition of ascitic S180 growth, and at a dose of 25 mg/kg inhibited the growth of Ehrlich carcinoma. The derivatives where X = H and X = OCH_3 did not show activity. There are no significant changes of hematopoietic parameters of the derivatives in this treatment. These data suggest that the presence of more polar substituents, NO_2 and OH, strongly increases the antitumor activity of this class of compounds.

Key words: Antitumor activity, mesoionic compounds, synthesis.

Introduction

In previous works we reported the antitumor activity of 1,3,4-thiadiazolium-5-thiolates and a 1,2,3-oxadiazolium-5-olate compound of the mesoionic class.[1–3] These interesting compounds have well separated regions of positive and negative charges[4] associated with the polyheteroatomic system enabling strong interactions with biomolecules such as DNA and proteins. A further advantage of this class of mesoionic compound is that they can be prepared with relative readiness in a pure state and they also have high stabilities.

The present work reports the synthesis of new salt derivatives of 1,3,4-thiadiazolium-2-aminide of the mesoionic class and their antitumor activity against ascitic Sarcoma 180 (S180) and Ehrlich carcinoma. The hematological values were also determined.

Materials and methods

Chemicals and reagents

Dimethyl Sulfoxide (DMSO) was purchased from Aldrich (Milwaukee, WI). All other reagents were of analytical grade.

Synthesis of mesoionic compounds

4-Phenyl-5-(4-X-cinnamoyl)-1,3,4-thiadiazolium-2-phenylamine chlorides were synthesized by the coupling of the corresponding freshly prepared *p*-substituted cinnamoyl chlorides[5,6] (10 mmol) with 1,4-diphenyl-thiosemicarbazide (10 mmol) in dry 1,4-dioxane (20 ml) at room temperature. After 24–48 h standing, the products were separated by vaccum filtration, washed with dry 1,4-dioxane and recrystallized from ethanol:dichloromethane (1:1 v/v), where: X = H, yield 75%, m.p. 192°C; X = OCH_3, yield 70%, m.p. 260°C; X = OH, yield 65%, m.p. 205°C; X = NO_2, yield 78%, m.p. 198°C. The mesoionic compounds thus prepared had their structures confirmed by ^{1}H-NMR, ^{13}C-NMR and mass spectrometry.

Animals and tumor cells

C_3H or C57 B1/10 mice of either sex weighing 18–22 g were housed under standard laboratory conditions. S180 and Ehrlich carcinoma were maintained by sequential transplants i.p. in appropriate hosts.

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Evaluation of antitumor activity of ascitic tumors

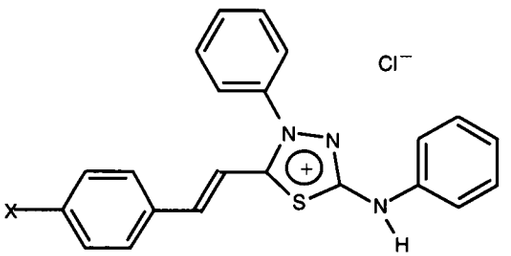
Tumor cells (5×10^5) were inoculated i.p. into C_3H mice. Treatment i.p. with the drugs dissolved in 80% DMSO in saline (v/v) was initiated 1 day after tumor inoculation, and was continued on days 1, 2 and 6. The control animals received 80% DMSO in saline at the same schedule. A total of 15 animals were used per group. The maximum tolerated dose was first chosen to test the effect. The antitumor effects of the drugs were determined by recording dead and surviving mice daily for 60 days. The efficiency of the ascitic tumor treatment was determined by the increase in the survival time of treated mice (T) as compared to that of the control group (C) using the expression:[7] %T/C = median survival time of treated animals (days)/median survival time of control animals (days).

Hematological data were recorded 1 day after injection of the drugs in normal SW mice.

Results

Synthesis

In this work we synthesized four new derivatives of the 1,3,4-thiadiazolium-2-aminide mesoionic class. These compounds were made from the corresponding *p*-substituted benzoyl chlorides and 1,4-diphenylthiosemicarbazide, and afforded the salt derivates 4-phenyl-5-(4-X-cinnamoyl)-1,3,4-thiadiazolium-2-phenylamine chlorides, where X = H, OCH_3, OH and NO_2 (Figure 1), with good yields and high purity confirmed by thin layer chromatography. The structures were confirmed by ^1H- and ^{13}C-NMR, spectroscopy and mass spectrometry. No alteration in drug structure in DMSO solution was observed by ^1H-NMR spectroscopy and the cytotoxic activity was maintained during at least 1 month of storage at −20°C.



X = H(MI-A)

OCH_3 (MI-C)

NO_2 (MI-D)

OH (MI-J)

Figure 1. Chemical structure of the derivatives of 4-phenyl-5-(4-X-cinnamoyl)-1,3,4-thiadiazolium-2-phenylamine chlorides.

In vivo activity of mesoionic compounds

The *in vivo* antitumor activity of the four compounds was examined against S180 (Table 1). The tumor cells were inoculated i.p., drugs were injected by the same route until the maximum tolerated dose and the survival times were compared. The derivatives where X = H and X = OCH_3 did not show any appreciable antitumor activity at doses of 20 and 30 mg/kg.

In contrast, the derivatives where X = OH (30, 46 and 60 mg/kg) and X = NO_2 (10 and 30 mg/kg) showed marked antitumor activity against S180. We then assayed MI-D and MI-J at the dose of 25 mg/kg on days 1 and 5 against Ehrlich carcinoma with results comparable with that for S180.

Hematological toxicity

The hematological values of non-tumor bearing mice treated with saline, 80% DMSO v/v in saline (controls) or with drugs MI-D and MI-J (total dose 15 mg/kg) as shown in Table 2 were assayed 1 day after the last dose. No significant changes were seen in this schedule of treatment for the control and drug-treated animals in comparison with the normal values for hematological data obtained from the literature.[8] The platelets showed no alteration in morphology or coloring.

Discussion

Currently, we have an active program involving synthetic, mechanistic and biological activity studies of various classes of mesoionic compounds.[1–4,9,10] In this study, we synthesized four new 4-phenyl-5-(4-X-cinnamoyl)-1,3,4-thiadiazolium-2-phenylamine chlorides, where X = H, OCH_3, OH and NO_2.

A pilot study with the four derivatives was undertaken to utilize the maximum tolerated doses in SW

Table 1. Effect of mesoionic compounds against ascitic S180 and Ehrlich carcinoma

Tumor[a]	Compound	X	Schedule[b] (days)	Total dose (mg/kg)	%T/C[c]
S180	MI-A	H	1 and 6	20	103
				30	100
	MI-C	OCH_3	1 and 6	20	100
				30	115
	MI-D	NO_2	1 and 6	10	208
				30	160
	MI-J	OH	1, 2 and 6	30	155
				46	170
				60	170
			1 and 5	30	130
Ehrlich carcinoma	MI-A	H	1 and 6	30	100
	MI-C	OCH_3	1 and 6	30	100
	MI-D	NO_2	1 and 5	25	176
	MI-J	OH	1 and 5	25	135

[a]Tumor cells (5×10^5) were implanted i.p. into C_3H or C57 BL/10 mice on day 0.
[b]Drugs (treated group) or 80% DMSO in saline (control group) were administered i.p.
[c]%T/C $\geq$ 125 indicated significant activity.

Table 2. Effect of MI-D and MI-J treatment on mouse hematological values

Haematological values	Saline	DMSO	MI-D	MI-J
Haematocrit (%)	50.7 (±2.1)	49.7 (±1.2)	51.5 (±0.5)	50.0 (±1.4)
Leucocytes ($\times 10^3/mm^3$)	10 (±5.8)	8 (±1.9)	9 (±0)	9.5 (±5.0)
Basophils (%)	0	0	0	0
Neutrophils (%)	14.0 (±4.1)	11.5 (±7.5)	30.3 (±5.2)	17.5 (±12.0)
Lymphocytes (%)	78.5 (±5.1)	75.0 (±16.1)	61.0 (±4.1)	76.0 (±9.0)
Monocytes (%)	6.0 (±3.0)	12.1 (±0.8)	7.3 (±4.4)	5.0 (±2.0)
Eosinophyles (%)	1.5 (±0.9)	1.4 (±0.8)	1.4 (±1.0)	1.0 (±1.0)
Metamielocytes (%)	0	0	0	0
Mielocytes (%)	0	0	0	0

The hematological values are means (± SD) of three mice per group.

mice by the i.p. route in the treatment schedule used in this work.

As shown in Table 1, compound MI-A (X = H), i.e. without substituent, showed no significant antitumor activity in the schedule assayed.

The replacement of H (MI-A) by more hydrophilic moieties, OH (MI-J) and NO_2 (MI-D), results in an increase of the antitumor activity. This suggests that the charge of mesoionic heterocycles,[4,9,10] which becomes more delocalized with polar substituents, makes strong electrostatic interactions with biomolecules such as DNA and proteins possible.[11–13]

When we replaced the OH (MI-J) by a more lipophilic group OCH_3 (MI-C), the inhibitory effect against S180 of this derivative was decreased, corroborating the hypothesis that a more polar group contributes appreciably to antitumor activity by electrostatic interactions. These observations indicate that the nature of the substituent needs to be considered when trying to establish drug effects in biological activity.

No hematological toxicity was seen at a concentration of 15 mg/kg per dose since MI-D and MI-J did not significantly change haematocrit and WBC values in comparison with the control animals (Table 2).

Conclusion

The 1,3,4-thiadiazolium-2-aminide derivatives with NO_2 and OH substituents in the cinnamoyl moiety showed significant *in vivo* antitumor activity against

S180 and Ehrlich carcinoma. It is worthwhile to assay the four compounds in other treatment schedules, other murine tumors and other derivatives in an attempt to obtain relevant anti-tumor drugs.

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