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Evaluation of certain ureas and nitrosoureas of 2,3-dihydro-1,4-benzothiazines against Sarcoma-180 solid tumors *in vivo*

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Different derivatives of nitrosoureas were synthesized by a long sequence of reactions and were evaluated for their anticancer activities against female Swiss albino mice, 6–8 weeks old, weighing 18–24 g and bearing Sarcoma-180 (S-180) ascitic tumor. Experimental protocols include injecting a total of 2×10^5 S-180 viable ascitic cells s.c. on day 0 in the left flank of mice to obtain solid tumors, followed by the treatment of urea/nitrosourea derivatives on days 1, 5 and 9 at different dose levels. Of the 32 compounds tested, eight were found highly active against solid tumors with significant growth delay and tumor weight inhibition ratio. 5-Fluorouracil was injected s.c. as referent compound to positive control animals.

Key words: Alkylation, anti-cancer, 1,4-benzothiazine, nitrosourea, phenothiazine, Sarcoma-180.

Introduction

Derivatives of nitrosourea are known to be effective against a wide range of tumors including Hodgkin's disease, lung small cell carcinoma, lung non-small cell carcinoma, Burkitts lymphoma, diffuse histiocytic lymphoma, myeloma, prostate gland tumors, melanoma and renal cancers.[1] Nitrosoureas exhibit dual capacity while interacting with nucleic acid and proteins, by nucleic acid–protein cross-linking and alkylation of DNA.[2,3] The lipophilicity of nitrosoureas helps them to cross the blood–brain barrier, making them useful in the treatment of human gliomas with good response rates.[3,4] Despite excellent anti-cancer activity of nitrosources against a variety of neoplasms, they could not achieve widespread usage due to their substantial renal and bone marrow toxicity.[5] Another class of compounds which possess such anti-cancer property are the heterocyclic phenothiazines.[6–8] Since phenothiazines alone do not show significant anti-tumor activity, they cannot be used as potential drugs to treat cancer. However, some of their derivatives help indirectly to minimize cytotoxic effects caused by radiation and other carcinogens.[9] Additionally, the selective accumulation of phenothiazine derivatives in certain tissues can provide an effective treatment of such tumors.[9] In this group, their derivatives interact with DNA by producing complexes.[6,10] Biological activities of phenothiazines were ascribed to structural specificity due to a fold along the nitrogen–sulfur axis and a similar fold is present in the benzothiazines.[11,12] Keeping these properties in mind, a new series of bifunctional anti-cancer compounds containing nitrosourea and 1,4-benzothiazine nucleus were synthesized and tested against female Swiss albino mice bearing Sarcoma-180 (S-180) solid tumors.

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Materials and methods

Synthesis method

Title compounds have been synthesized by the sequence of reactions depicted in Figures 1–3.[13]

Chemicals

Newly synthesized compounds were identified by IR, mass spectroscopy, nuclear magnetic resonance and thin layer chromatography. They were dissolved in 95% ethanol (50 mg/ml) and diluted with physiological saline (0.9% NaCl in H_2O) just before use. All reagents used in anti-tumor assays were of analytical grade.

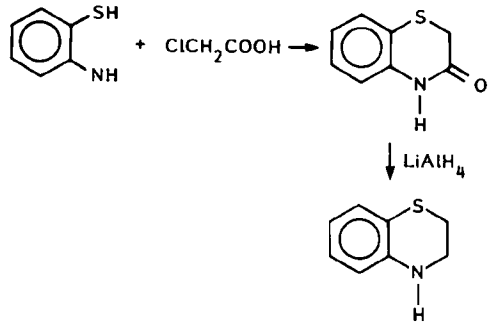
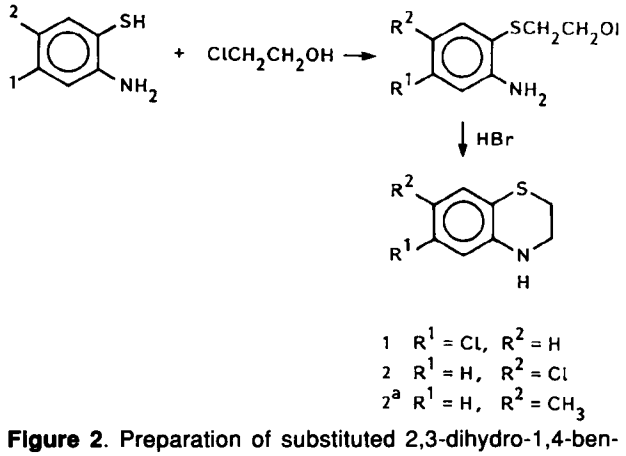
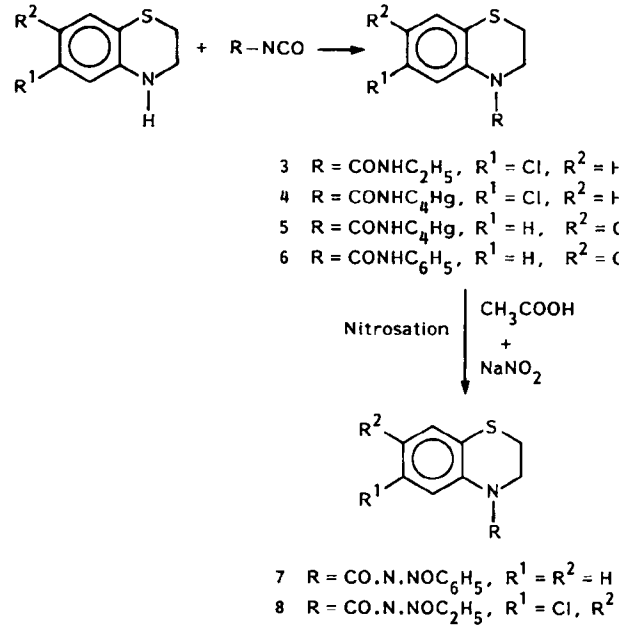


Figure 1. Preparation of 2,3-dihydro-1,4-benzothiazine.



1 $R^1 = Cl$, $R^2 = H$
2 $R^1 = H$, $R^2 = Cl$
2[a] $R^1 = H$, $R^2 = CH_3$

Figure 2. Preparation of substituted 2,3-dihydro-1,4-benzothiazines.



3 $R = CONHC_2H_5$, $R^1 = Cl$, $R^2 = H$
4 $R = CONHC_4Hg$, $R^1 = Cl$, $R^2 = H$
5 $R = CONHC_4Hg$, $R^1 = H$, $R^2 = C$
6 $R = CONHC_6H_5$, $R^1 = H$, $R^2 = C$

Nitrosation: CH_3COOH + $NaNO_2$

7 $R = CO.N.NOC_6H_5$, $R^1 = R^2 = H$
8 $R = CO.N.NOC_2H_5$, $R^1 = Cl$, R^2

Figure 3. Preparation of 4-*N*-alkyl amide and their nitrosoamide derivatives from 2,3-dihydro-1,4-benzothiazines

Anti-tumour assay

Animals and tumor model. Close-bred female Swiss albino mice, 6–8 weeks of age weighing 18–24 g, were maintained under controlled temperature and humidity with sterile bedding. Food and water were served *ad libitum*. S-180 ascitic cells, originally procured from the Cancer Research Institute, Bombay, India, were maintained and propagated intraperitoneally (i.p.) by serial transplantation in adult female mice.

Experimental design. Solid tumors were developed by s.c. inoculation of 2×10^5 viable S-180 cells in the left flank of the hind limb in the female Swiss albino mice. Tumors that developed were measured from day 6 in three planes with a plastic Vernier caliper. Tumor weight (W) in mg was calculated using the following formula.[14]

$$\text{Tumor weight } (W) = \frac{l \times (W)^2}{2}$$

Where l is the length of the tumor in mm and W is the width of the tumor in mm.

Test compounds were diluted in 0.1 ml saline and injected s.c. at two different dose levels, i.e. 50 and 75 mg/kg body weight, at 24 h after inoculation of S-180 cells and further on days 5 and 9. Control mice were given the same volume of saline (0.1 ml) on days 1, 5 and 9. A positive control group was injected with referent compound, 5-fluorouracil, at 60 mg/kg body weight as per the above schedule, to assess the tumor curability of the test compounds.

Tumor response was assessed by taking tumor weight inhibition ratio (TWI%) and growth delay (GD) as quantitative end points. TWI expressed as a percentage is calculated as follows.

$$\frac{100(W_c - W_t)}{W_c} = 100\left(1 - \frac{W_t}{W_c}\right)$$

Where W_c is the mean tumor weight of the control group in g and W_t is the mean tumor weight of the test group in g.

Tumor GD is the difference in the number of days for the treated tumors (T_t) to reach 2000 mm^3 compared with the untreated control (T_c). Thus,

$$\text{GD} = T_t - T_c \text{ day}$$

Student's *t*-test was employed to analyze the results.

Results

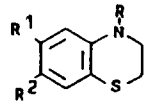
Results obtained from the administration of these compounds against solid tumor model are summarized in Table 1. All the compounds studied were found to be active at one dose level at least, causing significant GD and TWI compared with the control and positive control animals.

Discussion

A total of 32 compounds of urea and nitrosourea derivatives of 2,3-dihydro-1,4-benzothiazines was initially tested against an ascitic model of S-180 cells in female Swiss albino mice;[15] only the listed few were found to give encouraging results for further investigations. In the present experiments, only the latter compounds were tested using S-180 induced solid tumors.

Compounds 1 and 2, the two derivatives of 1,4-benzothiazine, exhibited marked potency. Although compound 1 could not cause regression of solid tumors significantly, compound 2 was found to be highly effective at both the dose levels. On the other hand, urea derivatives were found to be active at both test doses, i.e. 50 and 75 mg/kg, with the latter dose level delivering a better response. Benzothiazinyl ureas possess a fold along the nitrogen–sulfur axis for complexation with nucleic acid and protein,[6–8] and this structural specificity may explain their efficacy. Compounds 3–6 belong to this category. Compound 5 exhibited higher GD and TWI values at both dose levels. At the dose level 50 mg/kg, GD was 6.67 ± 1.52 days and TWI percentage was 65.87 ($p < 0.02$), while at 75 mg/kg dose level the values recorded were 11.95 ± 1.14 days and 75.72 ($p < 0.001$), respectively. The same observation holds true for compounds 3 and 4, whereas compound 6 was ineffective at the

Table 1. Anti-tumor activities of urea/nitrosourea and benzothiazinyl derivatives against female Swiss albino mice bearing S-180 ascitic cells (solid tumor model)



Compound	Structure of compound	Dose: 50 mg/kg × 3		Dose: 75 mg/kg × 3	
		GD (days)	TWI (%)	GD (days)	TWI (%)
1.	R = H R^1 = Cl, R^2 = H	+5.79 ± 1.00	64.74 ($p < 0.02$)	+5.26 ± 1.08	51.26 ($p < 0.05$)
2.	R = H R^1 = H, R^2 = Cl	+8.85 ± 1.51	72.47 ($p < 0.001$)	+6.92 ± 1.25	75.76 ($p < 0.001$)
3.	R = $CONHC_2H_5$ R^1 = Cl, R^2 = H	+5.66 ± 0.16	59.67 ($p < 0.02$)	+6.9 ± 0.61	68.88 ($p < 0.001$)
4.	R = $CONHC_4H_9$ R^1 = Cl, R^2 = H	+5.05 ± 1.15	61.56 ($p < 0.02$)	+6.12 ± 1.59	74.61 ($p < 0.001$)
5.	R = $CONHC_4H_9$ R^1 = H, R^2 = Cl	+6.67 ± 1.52	65.87 ($p < 0.02$)	+11.95 ± 1.19	75.72 ($p < 0.001$)
6.	R = $CONHC_6H_5$ R^1 = H, R^2 = CH_3	+2.17 ± 0.54	40.80 ($p < 0.10$)	+6.00 ± 3.33	62.11 ($p < 0.02$)
7.	R = CO–N(C_6H_5)–NO R^1 = R^2 = H	+4.57 ± 0.85	64.21 ($p < 0.02$)	+6.21 ± 0.81	74.88 ($p < 0.001$)
8.[a]	R = CO–N(C_2H_5)–NO R^1 = Cl, R^2 = H	+4.13 ± 1.08	55.94 ($p < 0.02$)	+9.84 ± 5.41	71.98 ($p < 0.001$)
9.[b]	5-FU	+5.76 ± 0.68	57.14 ($p < 0.02$)		

[a]Administered at 10 and 40 mg/kg body weight on days 1, 5 and 9.
[b]Administered at 60 mg/kg body weight on days 1, 5 and 9.
Figures in the parenthesis indicates the *p* value from Student's *t*-test.

50 mg/kg dose level, although at the higher dose level it was found to be significantly active.

In earlier experiments, it was observed that nitrosoureas were equally active at considerably low dose levels[13,15] and the same activity of nitrosoureas was observed in the present study as well. A probable reason could be their *N*-nitroso moiety[1] and a fold along the nitrogen–sulfur axis in the benzene ring,[12] which is considered to be the main factor responsible for imparting biological activities in BCNU and phenothiazine, respectively. The same *N*-nitroso moiety present in compound 8 could be responsible for its activity at the low dose level, i.e. 10 and 40 mg/kg, and exhibiting GDs of 4.13 ± 1.08 and 9.84 ± 5.41 days and TWI ratios of 55.94 ($p < 0.02$) and 71.98 ($p < 0.001$), respectively. However, when it was tested at the dose levels of 50 and 75 mg/kg, the compound turned out to be toxic to animals. Compound 7, which is also a nitrosourea derivative was found to be active at higher doses than compound 8 (i.e. 50 mg/kg). However, it also became toxic to animals at higher dose (i.e. 75 mg/kg). The reason could be their said dual capacity to interact with nucleic acid and protein via alkylation and complexation. Unfortunately, increasing the dose did not correspondingly increase its efficacy. Compound 8 was even found moderately effective when injected at the dose level of 10 mg/kg body weight. These doses are considered low in comparison with the doses for urea derivatives.

It is interesting to note that chlorine at the 6-position in the benzene ring of the test compound (e.g. compounds 1, 3, 4 and 8) was more potent than when it was at the 7- position (e.g. compounds 2 and 5), a configurational relationship that could be ascribed to their tumor curative property. 5-Fluorouracil was used as per the above schedule[14] to evaluate the efficacy of test compounds.

Conclusion

In the present investigation these compounds yielded better response with respect to GD and TWI ratio than currently used 5-fluorouracil, which yielded 5.76 ± 0.68 and 57.14 ($p < 0.02$), respectively.

Acknowledgments

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