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Short report

Anticancer activities of 2,3-dihydro-1,4-benzothiazines, and of their 4-(*N*-alkyl amides) and 4-(*N*-alkyl *N*-nitrosoamides)

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Introduction

Phenothiazines possess a wide spectrum of biological activities and are well known for their central nervous system (CNS) activity. Their several derivatives are in clinical use.[1] Phenothiazines also exhibit significant anticancer activity[2–19] which has been assigned to their interaction with DNA via complexation.[20]

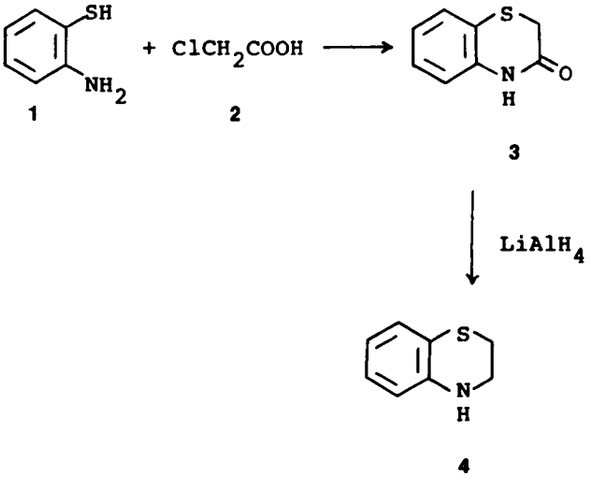
Biological activities of phenothiazines have been ascribed to structural specificity due to a fold along the nitrogen–sulfur axis. Such structural specificity of a fold along the nitrogen–sulfur axis is also present in 1,4-benzothiazines[21] and therefore, from a drug design point of view one can anticipate a pattern of anticancer activities similar to that of phenothiazines. 2,3-Dihydro-1,4-benzothiazines are less toxic and form an interesting class of heterocyclic drugs for anticancer activities.

Nitrosourea derivatives constitute an important class of anticancer agents and are clinically significant.[22–32] They interact with DNA by alkylation.[25,33] Their clinical use is limited, however, because of the cumulative and delayed side effects exerted by these compounds, bone marrow toxicity being dose-limiting. Therefore it is worthwhile to develop a series of nitrosoureas with minimum toxicity and side effects.

In 4-(*N*-alkyl *N*-nitrosoamides)-2,3-dihydro-1,4-benzothiazines, heterocyclic nitrogen with a side chain at the 4-position constitutes a *N*-nitroso-urea linkage, and as such these compounds contain both a 1,4-benzothiazine nucleus and a nitrosourea linkage. They will act with DNA by complexation as well as by alkylation and constitute a new class of bifunctional anticancer agents.

Synthesis methods

2,3-Dihydro-1,4-benzothiazine (Figure 1, compound **4**) was prepared by the condensation of 2-aminobenzenethiol with chloroacetic acid and subsequent reduction of lactum (Figure 1, compound **3**) obtained with lithium aluminium hydride.



1 2 3 4

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

7-Methyl-2,3-dihydro-1,4-benzothiazine (Figure 2, compound **8**) was prepared by the reaction of 5-methyl-2-amino-benzenethiol with chloroethanol and the subsequent dehydration of 2-amino-5-methylbenzenethioethanol (Figure 2, compound **7**) obtained with hydrobromic acid.

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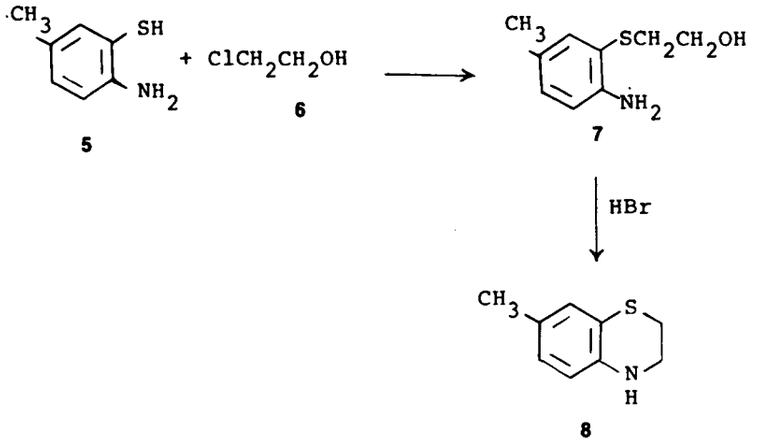


Figure 2. Preparation of 7-methyl-2,3-dihydro-1,4-benzothiazine.

2,3-Dihydro-1,4-benzothiazines (Figure 3, compounds **4** and **8**) were converted into 4-(*N*-alkyl amides) derivatives by reaction with alkylisocyanates. Amides obtained were converted into nitrosoamides (Figure 3).

Assay of antitumor activity

Antitumor activities of synthesized compounds (Figure 3, compounds **4** and **8–16**) were screened against sarcoma-180 tumor cells. Sarcoma-180 cells were transplanted intraperitoneally into female Swiss albino mice (2×10^5 cells/mouse), weighing 18–24 g, and this was considered as day 0. Six animals were used in each experimental and control group. The 10 compounds (100 mg/kg) solubilized in dimethyl sulfoxide (0.1 ml) were injected intraperitoneally into each animal of the experimental group on days 1, 5 and 9. Only one compound **15** was injected at three dose levels (50, 100 and 200 mg/kg). Survival times and animal weights

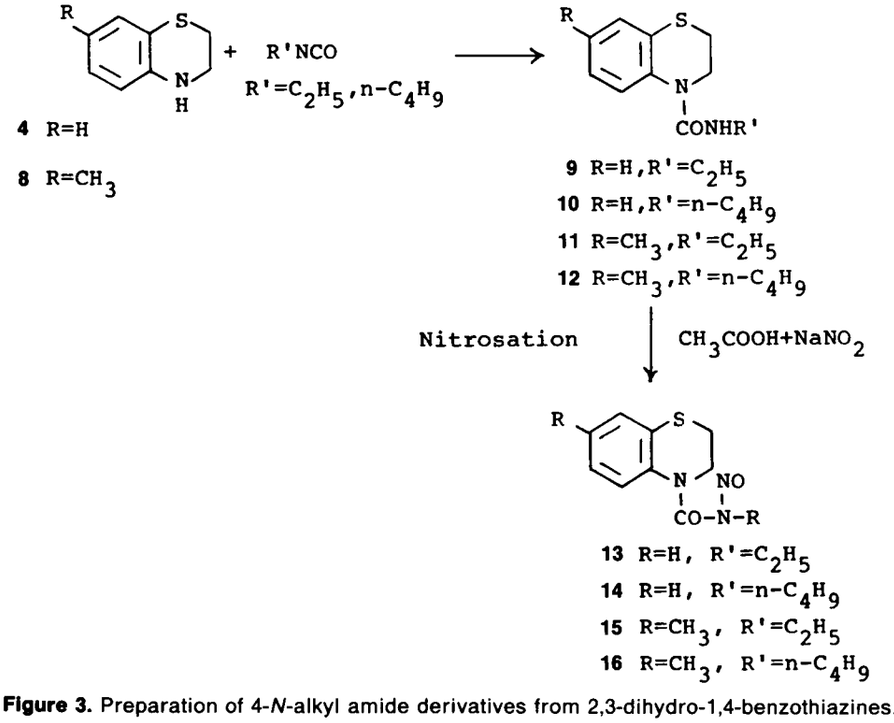
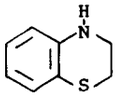
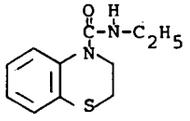
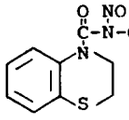
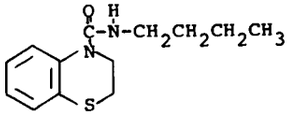
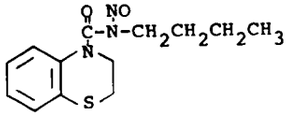
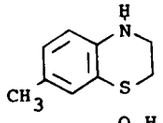
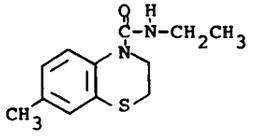
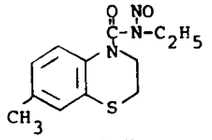
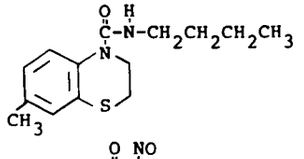
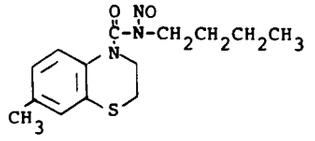


Figure 3. Preparation of 4-*N*-alkyl amide derivatives from 2,3-dihydro-1,4-benzothiazines.

Table 1. Antitumor activities of 2,3-dihydro-1,4-benzothiazines and of their 4-(*N*-alkyl amides) and 4-(*N*-alkyl *N*-nitrosamides) against sarcoma-180 cells transplanted intraperitoneally into female Swiss albino mice.

Complex	T/C at dose (mg/kg)[a]				Change in average body weight (g)[b]
	50	60	100	200	
4 	—	—	103.60	—	+7.64
9 	—	—	130.52[(1)d,e]	—	+8.8
13 	—	—	140.2[(2)]	—	+6.67
10 	—	—	136.6[(1)]	—	+5.60
14 	—	—	100.00	—	+5.85
8 	—	—	120.7	—	+2.34
11 	—	—	117.13	—	+4.75
15 	120.79	—	—	—	+4.00
	—	—	162.88	—	+6.00
	—	—	—	60.39[d]	+5.00
12 	—	—	107.97	—	+9.00
16 	—	—	137.26	—	+11.75
Positive control[c] (5-fluorouracil)	—	140.7	—	—	+8.00

[a] Administered on day 1, 5 and 9 (drug treatment began 24 h of inoculation of the tumor cells).
[b] Average weight change from onset to termination of drug treatment.
[c] Positive control compound (5-fluorouracil) was tested at the dose level of 60 mg/kg body weight as suggested in NCI Protocols.[34]
[d] Numbers in bracket indicate the number of mice which survived for more than 30 days.
[e] Underlining indicates positive effects (T/C ≥ 125).

were recorded. T/C was calculated from survival times. T/C and weight increase are recorded in Table 1. T/C is the ratio (express as a percent) of the mean survival time of the treated group divided by the mean survival time of the control group and is expressed as

$$T/C = \frac{\text{mean survival time of treated group}}{\text{mean survival time of control group}} \times 100$$

Discussion

All of the 10 compounds tested against sarcoma-180 at the dose level of 100 mg/kg possess $T/C > 85$ and are non-toxic according to NCI protocols.[34] Five compounds (**9**, **10**, **12**, **13** and **15**) possess $T/C > 125$ and can be considered to possess antitumor activity. Compound **15** (8-methyl-2,3-dihydro-4-*N*-ethyl *N*-nitrosoamide) has $T/C \approx 162.9$, which is much higher than that of the positive control (5-fluorouracil, $T/C = 140.7$) under identical experimental conditions and it possesses good antitumor activity. It has been tested at three dose levels (50, 100 and 200 mg/kg) and the optimum dose was found to be 100 mg/kg.

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