pellet was resuspended in 1 ml of PBS-heparin. The total number of leukocytes was measured using a Coulter Counter. The number of PMNLs recovered from each pouch was then calculated.
Treatment was given 1 h before injection of the carrageenan into the pouch. Pouches were washed and 4 h later, PMNLs infiltration was measured as described above. Mean leukocyte numbers per ml of exsudate for each compound were compared to control values obtained from a similar group of animals receiving vehicle alone. The degree of anti-inflammatory response, produced in the air-pouch cavity was assessed by measuring the total cell number of the exudate. All the compounds tested produced a dose-dependent reduction in carrageenan-induced leucocyte migration *in vivo*. The dose of 10 mg/ml carrageenan caused a time-dependent PMNLs infiltration into the pouch with a maximal rate of influx between 2 and 4 h. Maximum cell numbers were detected in the cavity 4 h after irritation injection. The results are summarized in the Table.
The degree of inflammatory response was also assessed by the hind-paw edema test in mice. No local irritation and gastrointestinal side effects were observed.

2.4. Gastrointestinal ulceration studies

Mice were fasted for 24 h (with water ad libitum). Compounds were suspended in a methyl cellulose vehicle and administered orally by gavage in a volume of 0.5 ml/100 g of body weight. The animals were sacrificed after 4 h, and the stomachs were examined for lesions under a dissecting microscope [9].

2.5. Statistical analysis

Data were statistically evaluated by analysis of variance and Man-Whitney U.p value of less than 0.05 was considered to be significant.

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Syntheses and antitumor activity of 4-{*N′*-[*N*-(2-chloroethyl)-*N*-nitrosocarbamoyl]hydrazono}-2,2,6,6-tetramethylpiperidine-1-oxyl

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Chloroethylnitrosoureas are an important class of alkylating antitumor agents with a broad range of activity in experimental systems. Some of them, CCNU, BCNU and MeCCNU have been applied for treatment of human cancer, mainly lymphomas, melanomas, gliomas and a few solid tumors [1]. The clinical application of nitrosoureas however is still limited because of their delayed and cumulative toxic effects [2]. In the search for more active and less toxic analogues, nitroxyl radicals have been utilized as carriers of cytotoxic groups. The replacement of the cyclohexyl moiety in CCNU with a nitroxyl radical led to the development of the spin labelled chloroethylnitrosourea 1-(2-chloroethyl)-3-(2,2,6,6-tetramethylpiperidine-1-oxyl)-1-nitrosourea (SLCNU) [3] and other spin labeled analogues of CCNU [4]. It has been established that the nitroxyl radical moiety (spin label) can exhibit a beneficially modifying effect on the toxicity and activity of nitrosourea derivatives [4]. On the other hand halogenoethylhydrazines, halogenoethylhydrazides and halogenoethylnitrososemicarbazides possess marked antitumor effects [5, 6]. In this paper we report syntheses, antitumor activity and toxicity of a new spin labelled chloroethylnitrosourea analogue of CCNU, 4-{*N′*-[*N*-(2-chloroethyl)-*N*-nitrosocarbamoyl]hydrazono}-2,2,6,6-tetramethylpiperidine-1-oxyl (**5**), containing a hydrazone structure. The spin labelled compound **5** was prepared by three different synthetic pathways as shown in the Scheme. The conventional path through condensation of the 4-hydrazono-(2,2,6,6-tetramethylpiperidine-1-oxy) (**1**) with 2-chloroethyl isocyanate and nitrosation of intermediate urea **2** with a mixture of dinitrogen tetraoxide and sodium acetate afforded **5** in only 10% yield. In order to increase the yield and to ascertain the position of the nitroso group in the nitrosourea derivative **5**, a regio-selective method was used to transfer the chloroethyl moiety containing the nitroso group to the hydrazone **1**. Compound **5** was also synthesized by different approaches using either *N′*-hydroxysuccinimide-*N*-(2-chloroethyl)-*N*-nitrosocarbamate (**3**) or 2-chloroethylnitrosocarbamoyl azide (**4**) as regio-selective transfer reagent (see Experimental). The chemical struc-

Scheme

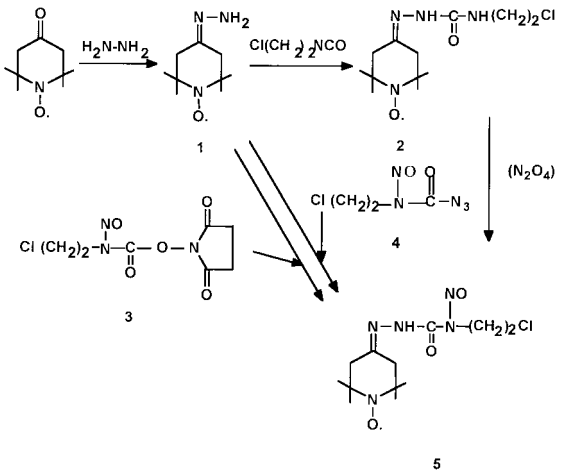

Table: In vivo activity of 5 against lymphoid leukemia L1210 and lymphocytic leukemia P388 in mice CD2F1 in comparison with CCNU and SLCNU

Compd.	L1210		P388		LD_{50} (mg/kg)
	OD (mg/kg)	ILS %	OD (mg/kg)	ILS %	
CCNU	25	646 [10]	16	182 [10]	56 [11]
SLCNU	60	713 [4]	35	542 [4]	123 [4]
5	60	831	25	735	72

OD – optical dose = daily i.p.-administered dose resulting in the maximum increase in life span. Drugs were administrated on day 1 after tumor implantation
ILS – increase in life span = [(T-C)/C] × 100, percentage of animals surviving on day 60 after tumor implantation
T – The mean survival time of the treated animals,
C – The mean survival time of the controls

ture of **5** was confirmed by elemental analysis, IR, MS and EPR spectroscopy.

Compound **5** was tested *in vivo* against lymphocytic leukemia P388 and lymphoid leukemia L1210 in mice CD2F1, according to methods described in the literature [7]. The results are presented in the Table in comparison with SLCNU and CCNU. Compound **5** prolonged the survival time of mice bearing leukemia L1210 by 831% at a dose of 60 mg/kg and leukemia P388 by 735% at a dose of 25 mg/kg. The LD_{50} for compound **5** was 72 mg/kg i. p. in mice; it showed greater cytotoxicity against leukemia L1210 and leukemia P388 than the clinically used drugs CCNU and SLCNU. Both of the compound **5** and SLCNU were less toxic than CCNU according to LD_{50} data.

In conclusion it can be pointed out that the hydrazone group in the spin labeled NU **5** influences its oncopharmacological properties.

Experimental

1. Starting compounds

4-Hydrazono-(2,2,6,6-tetramethylpiperidine-1-oxyl) (**1**) was prepared according to [8], N′-hydroxysuccinimide-N-(2-chloroethyl)-N-nitrosocarbamate (**3**) as described in [9] and 2-chloroethylnitrosocarbamoyl azide (**4**) was prepared according to [3].

2. 4-{N′-[N-(2-chloroethyl)-N-nitrosocarbamoyl]hydrazono}-2,2,6,6-tetramethylpiperidine-1-oxyl (**5**)

2.1. By N′-hydroxysuccinimide-N-(2-chloroethyl)-N-nitrosocarbamate (**3**)

(2,2,6,6-Tetramethylpiperidine-1-oxyl)-hydrazine 0.184 g (1 mM) was dissolved in DMF (2 ml at 0–5 °C). The mixture was stirred vigorously for 15 min and *N′*-hydroxysuccinimide-*N*-(2-chloroethyl)-*N*-nitrosocarbamate 0.23 g (1 mM) was added. After stirring of 3 h the mixture was poured into ice/H_2O (70 ml). The received yellow crude product was extracted with $(C_2H_5)_2O$ (3×10 ml). The combined extracts were successively washed with 10 citric acid (2×10 ml), 10% $NaHCO_3$ (2×10 ml) and a saturated solution of NaCl (2×10 ml). The organic layers were dried over anh. $MgSO_4$ and filtered. The solvent was evaporated under reduced pressure and the residue was crystallized twice from $(C_2H_5)_2O$/n-hexane as pale yellow crystals, yield 72%.

2.2. By 2-chloroethylnitrosocarbamoyl azide (**4**)

(2,2,6,6-Tetramethylpiperidine-1-oxyl)-hydrazine 0.184 g (1 mM) was dissolved in anh. pyridine (15 ml at 0 °C). A solution of 2-chloroethylnitrosocarbamoyl azide 0.178 g (1 mM) dissolved in 2 ml dry $(C_2H_5)_2O$ (3 ml) was added dropwise. The mixture was stirred at 0 °C for 3 h and was poured into iceH_2O. The organic layer was separated and the aqueous layer was extracted with $(C_2H_5)_2O$ (3×10 ml). All $(C_2H_5)_2O$-extracts were combined, washed with 2 N HCl (2×10 ml), 10% $NaHCO_3$ (2×10 ml) and a saturated solution of NaCl (2×10 ml), dried over anh. $MgSO_4$ and filtered. The solvent was evaporated under reduced pressure. The semisolid residue was crystallized twice from $(C_2H_5)_2OH$/n-hexane as pale yellow crystals, yield 58%.

M.p. 113–115 °C (dec.); Rf: 0.71 [$CHCl_3/CH_3OH$ (v/v 9 : 1)]; Ms m/z: 318 (M^+), 210 (M^+-108), 180 (M^+-138); IR (KBr): (3320, 1720, 1458, 1340 cm^{-1}); $C_{12}H_{21}ClN_5O_3$ (318.8); Calcd.: C 45.21, N 21.97, H 6.63; Found: C 44.5, N 21.5, H 6.4.

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Antimicrobial activity of some Nepalese medicinal plants

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Medicinal plants from Nepal are very popular as a source of pharmacologically active compounds. The local people of Nepal have rich folklore about the traditional use of different parts of various plants. The present paper describes the antimicrobial activity of a number of Nepalese medicinal plants used as remedies against various diseases.

Dried and powdered plant material (5 g each) was extracted successively with dichloromethane, methanol and 50% aqueous methanol in a soxhlet extraction apparatus. Evaporation of the solvent followed by drying in vacuo provided crude extracts. The plants and their parts used for extraction and the amount of extract obtained under different extraction conditions are summarized in Table 1. The extracts were screened for antimicrobial activity against Gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus* and *Micrococcus flavus*), Gram-negative bacteria (*Proteus mirabilis*, *Serratia marcescens* and *Escherichia coli*) and the yeast species *Candida maltosa* using a modified disc diffusion method [1, 2].

Growth of the fungal species *C. maltosa* was only inhibited by the dichloromethane and methanol extracts of *Zanthozylum armatum* with MIC values of 500 and 1000 μg/disc, respectively.

Both extracts as well as the methanol extract of *Rhododendron anthopogon* showed weak activity against different gram-negative bacteria (MIC between 500 and 2000 μg/disc) while the aqueous methanol extracts of *Bergenia ligulata*, *Bombax cieba*, *Dipsacus mytis*, *Rh. anthopogon* and *Salvia coccinia* were slightly active against *P. mirabilis* only (MIC 2000 μg/disc).

Most of the extracts were active against the Gram-positive bacteria (see Table 2). The most pronounced activity was shown by the dichloromethane extract of *Maharanga bicolor* with a MIC value of 0.25 μg/disc for all the three Gram-positive bacteria included into the experiment. Generally, the dichloromethane extracts showed the highest activity followed by the methanol extracts while methanol-water extracts were less active with the exception of *B. ligulata* were the methanol extract was most active. The dichloromethane extract did not show any activity against *B. subtilis* and *M. flavus* and moderate activity against *S. aureus*. Divergent results were obtained with extracts of *Parnassia nubicola* with highest activity of dichloro-