Anti-Cancer Drugs 1999, **10**, pp. 903–909

Preclinical report

Synthesis and cytotoxic activity of *N*-(2-chloroethyl)-*N*-nitroureas and *N*-(2-chloroethyl)-*N*-nitrocarbamates

János Botyánszki, József Bódi, Ian J Stratford[1] and Helga Süli-Vargha
Research Group for Peptide Chemistry, Hungarian Academy of Sciences, Eötvös Lorand University, 1518 Budapest 112, POB 32, Hungary. [1]School of Pharmacy and Pharmaceutical Sciences, University of Manchester, Manchester M13 9PL, UK.

As analogs of the widely used anti-tumor agents, *N*-(2-chloroethyl)-*N*-nitrosoureas, *N*-(2-chloroethyl)-*N*-nitroureas and *N*-(2-chloroethyl)-*N*-nitrocarbamates were synthesized by nitration following the reaction of the appropriate amines or alcohols with 2-chloroethyl isocyanate. All tested compounds exert cytotoxic effect with IC_{50} values of 10^{-4} to 10^{-6} M and most of them show somewhat higher cytotoxicity in nitrogen than in air. [© **1999 Lippincott Williams & Wilkins.**]

Key words: Cytotoxicity, *N*-(2-chloroethyl)-*N*-nitro, synthesis.

Introduction

N-(2-chloroethyl)-*N*-nitrosoureas, *N,N'*-bis(2-chloroethyl)-*N*-nitrosourea (BCNU), *N*-(2-chloroethyl)-*N'*-cyclohexyl-*N*-nitrosourea (CCNU), etc., represent an important class of anti-tumor agents and are widely used in clinics. These compounds decompose rapidly under physiologic conditions, producing alkylating and carbamoylating moieties.[1] As a consequence of the rapid decomposition even in the case of targeting peptide conjugates[2] they do not selectively alkylate the DNA of the cancerous cells, but also that of the normal cells, causing general toxicity. Recently it was discovered that the carbamoylating reaction of BCNU inhibits caspase 3-mediated apoptosis, in this way decreasing the cytotoxic effect of the drug.[3]

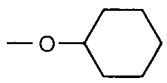
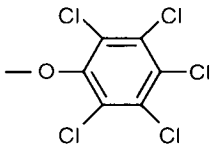
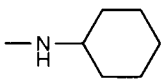
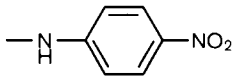
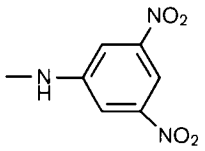
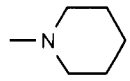
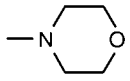
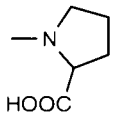
It has been long known that *N*-nitroureas and *N*-nitrocarbamates are generally more stable than *N*-nitrosoureas,[4,5] and that *N*-nitrocarbamates decompose[6] in a similar way as to the *N*-nitrosoureas producing an alkylating carbonium ion intermediate. The *N*-nitroureas generate nitroamines,[7] which in the case of *N*-(2-chloroethyl)-*N*-nitroureas would be 2-chloroethylnitroamine, theoretically also capable of alkylation. In addition, nitrocarbamates lacking carbamoylating capacity, and thus apoptosis inhibiting potency, might be even more cytotoxic than the corresponding ureas.

In the design of our *N*-(2-chloroethyl)-*N*-nitro compounds ($ClCH_2CH_2$-$N(NO_2)$-COQ) we relied on previous structure–cytotoxic activity relationships on the field of *N*-(2-chloroethyl)-*N*-nitrosourea congeners[8] (see Table 1). Thus **III b** and **III f** or **III d** and **III g** may be considered as analogs of BCNU or CCNU, respectively, which are the most frequently used nitrosoureas in clinics. With further variations of the Q group our intention was to influence the pharmacokinetic properties of the compounds. An aromatic (**III h** and **i**) or alicyclic (**III g**, **j** and **k**) group renders the molecule more lipophilic and in this way makes crossing the blood-brain barrier easier. In the case of the lactic acid derivative **III c** or the proline derivative **III l** the cytotoxic group is attached to natural carrier molecules. The reason why proline was chosen from the amino acids lies in our previous investigations, when we found that the *N*-(2-chloroethyl)-*N*-nitrosourea congener of proline amide shows a higher increase in the lifespan of L1210 leukemia-bearing mice and also has a significantly longer half-life than other amino acid derivatives or even BCNU itself.[9]

The authors thank the Hungarian National Research Fund OTKA 025838 and EORTC Screening and Pharmacology Group for their generous support.

Correspondence to H Süli-Vargha, Research Group for Peptide Chemistry, Hungarian Academy of Sciences, Eötvös Lorand University, POB 32, 1518 Budapest 112, Hungary.
Tel: (+36) 1 2090 555/1415; Fax: (+36) 1 3722 620;
E-mail: suline@para.chem.elte.hu

Table 1. Structure of carbamates and ureas of the general fomula: $ClCH_2CH_2-NX-CO-Q$ (**II**, X=H; **III**, X-NO_2)

Compounds (II, III)	–Q
a	$-O-CH_2CH_3$
b	$-O-CH_2CH_2-Cl$
c	$-O-CH(CH_3)-COO-CH_2CH_3$
d	
e	
f	$-NH-CH_2CH_2-C_1$
g	
h	
i	
j	
k	
l	

Materials and methods

Merck Kieselgel precoated sheets art. no. 5553 were used for TLC and Merck Kieselgel 60 art. no. 10832 were used for column chromatography. Solvent systems used are the following (v/v): *1*, chloroform: methanol 9:1; *2*, ethylacetate (EtOAc):pyridine:acetic acid:water 480:20:6:11; *3*, EtOAc:petroleum ether (pe) 1:2; *4*, EtOAc: pe 1:1; *5*, EtOAc:pyridine:acetic acid:water 60:20:6:11; *6*, EtOAc:pyridine:acetic acid:-water 120:20:6:11; *7*, EtOAc:cyclohexane 1:1; *8*, EtOAc; *9*, benzene:EtOAc 1:1; *10*, benzene; *11*, benzene:pe 1:1. Melting points were measured on a Büchi apparatus and are not corrected. Elemental analyses of all synthesized compounds were satisfactory within $\pm 0.5\%$ of the calculated values. NMR spectra were measured on a Bruker WM-250 FT-spectrometer. IR spectra were recorded on Specord IR 75 (Karl Zeiss, Jena, Germany). Voltammetry instruments used were a Metrohm Type 506 Polarecord and a Metrohm Type 663VA stand with multimode electrode.

General procedure for the synthesis of the *N*-(2-chloroethyl)-carbamoyl compounds (II)

To 0.3 mol of an alcohol or amine (**I**) in 50 ml solvent 0.33 mol of chloroethylisocyanate was added and the mixture was allowed to stand at room temperature and/or was refluxed. The reaction was monitored by TLC. When **I** had disappeared, the solvent and the isocyanate excess were evaporated *in vacuo* resulting in a solid or an oily remainder, which was crystallized from an appropriate solvent or distilled, respectively (Table 2).

General procedure for the synthesis of the *N*-(2-chloroethyl)-*N*-nitro-carbamoyl compounds (III)[5]

Method A. Acetic anhydride (3.8 ml) was cooled to 0 C and 1.3 ml (30 mmol) of 100% HNO_3 was added to it, dropwise. Then 20 mmol of **II** was added in portions (10 min) under vigorous stirring and, after the reaction was completed, the solution was poured into crushed ice. When the precipitated material was solid, the crystals were filtered off and washed with ice-water until neutral; when it was oily, it was extracted with EtOAc or ether, the organic phase was washed with brine, dried over anhydrous sodium sulphate and concentrated *in vacuo*. (Special precautions were taken in the case of **IIIf**, nitration was carried out at -20 C and the work-up procedure was performed at 0 C.)

Method B. 100% HNO_3 (3 ml) was cooled to -20 C and 4.5 mmol of **II** was added slowly under vigorous stirring, then the procedure was continued as above in Method A.

Table 2. Reaction conditions, purification and characteristics of *N*-(2-chloroethyl)carbamoyl compounds (**II**)

Compound	Reaction conditions (solvent), purification	Yield (%)	m.p. (C) or b.p. (C)/mmHg	R_f (TLC)	IR	NMR
II a[11]	3 days, r.t. (ethanol), distillation	82	102–104/14	0.86[1] 0.80[2]	neat: 3330 (NH), 1700 (CO)	ND
II b[12]	12 h, r.t.: 3 h boiling (EtOAc), distillation, crystallization (2-propranol)	72	37 112–115/0.3	0.86[1]	KBr: 3340 (NH), 1693 (CO)	^{13}C ($CDCl_3$) δ 42.0, 42.7, 43.8, 64.6, 155.6; ^1H ($CDCl_3$ δ 3.54 (2H, qq), 3.63 (2H, t), 3.69 (2H, t) 4,34 (2H, t), 5.35 (1H, br)
II c	10 h, 95 C, flash chromatography	65		0.70[1] 0.44[3]	neat: 3354 (NH), 1730 (CO)	^{13}C ($CDCl_3$) δ 14.0, 42.7, 43.7, 61.2, 69.1, 155.3, 171.3; ^1H ($CDCl_3$) δ 1.29 (3H, t), 1.48 (3H, d), 3.55-3.62 (4H, m), 4.22 (2H, qq), 5.05 (1H, qq)
II d	1 h, boiling (EtOAc), distillation	90	110–115/0.3	0.79[1] 0.73[7]	KBr: 3280 (NH), 1680 (CO), 1700sh	ND
II h[13]	2 h, boiling (EtOAc), crystallization (EtOAc: pe, 1 : 1)	18	136–138	0.66[2] 0.25[4]	(KBr), 3350 (NH), 1675 (CO)	ND
II i	12 h, r.t. (EtOAc), crystallization (acetic acid)	25	156–158	0.67[2] 0.41[4]	KBr: 3315 (NH), 1665 (CO)	ND
II j	2 h, r.t. (dioxane), crystallization (dioxane)	90	111–114	0.75[6] 0.22[4]	KBr: 3345 (NH), 1615 (CO)	ND
II k[14]	1 h, r.t. (dioxane), crystallization (dioxane)	68	149–151	0.70[6] 0.19[8]	KBr: 3300 (NH), 1615 (CO)	ND
II l	12 h, r.t. (DMF), crystallization (EtOAc)	79	145–148	0.53[5] 0.37[6]	KBr: 3375 (NH), 1710, 1600 (CO)	ND

The reaction between **III e** and cyclohexylamine

Cyclohexylamine (1.8 mmol) was added dropwise into the suspension of 2 mmol **III e** in 7 ml dimethylformamide (DMF) under stirring and ice cooling. After 10 min a solution was formed which was evaporated *in vacuo*, and the residue was applied onto a silica gel column (1.3 × 50 cm) and eluted with *11*. The fractions containing the main product with R_f 0.14 were collected, concentrated *in vacuo* and the residue was crystallized from ethanol yielding 0.5 mmol of **IV**. IR (KBr): 3300 (NH), 2850–2930 CH_2-(C_6H_{11}), 1720 (CO).

Cytotoxicity assay

For toxicity experiments the compounds were dissolved in DMSO at approximately 15 mM, and aliquots prepared and frozen at −20 C. Just before experimentation aliquots were added to the medium and diluted to give appropriate test concentrations. Chinese hamster V79 cells cultured *in vitro* were used for cytotoxicity experiments. Cells were harvested from exponentially grown cultures and seeded at 10^5 cells/well in plastic 24-well dishes. Cells were allowed to attach for 2 h and then exposed to various concentrations of drug in growth medium for 96 h at 37 C (four wells per drug concentration). Following drug exposure, the media was removed and MTT added, and cell survival assessed as described previously.[10] To assess selective hypoxic toxicity, cells were placed in specially designed glass 24-well dishes[10] and exposed to various concentrations of the drug for 3 h at 37 C under hypoxic (N_2) or aerobic conditions. Following the removal of the drug cells were allowed to proliferate for a further 3 days prior to addition of MTT and subsequent assay for survival. Data are expressed as values of IC_{50} which are the concentrations required to kill 50% of the cells under the conditions of the initial treatment (i.e. exposure to drug in air or N_2). The ratio of IC_{50} (air) versus IC_{50} (N_2) enables quantitative comparisons to be made of the O_2-dependent bioreductive activities of these compounds.

Linear sweep voltammetry technique

Polarization rate (v) 10 mV/s, base electrolyte 0.1 M KH_2PO_4/Na_2HPO_4 (pH 7.10); working electrode, hanging mercury drop electrode (drop size medium); reference electrode, Ag/AgCl/3 M KCl/base electrolyte, double junction, E_{ref} versus NHE, +207 mV; auxiliary electrode, glassy carbon electrode; 1 mM solutions of **III** in 0.1 M pH 7.1 phosphate buffer solution containing 5% ethanol. Prior to recording the voltammograms solutions were deaereted by a nitrogen gas stream of 99.95% purity for 10 min.

Results and discussion

The general synthetic route for the *N*-(2-chloroethyl)-*N*-nitrocarbamoyl compounds is shown in Scheme 1.

Accordingly, first an alcohol or an amine **I** was reacted with chloroethylisocyanate resulting in an *N*-(2-chloroethyl)carbamoyl compound **II**, which after nitration gave the appropriate nitro compound **III** (see Scheme 1, and Tables 2 and 3).

Several attempts were made for the synthesis of the BCNU analog **III f**, which was successful when special attention was paid to the cooling and work-up procedures. However, the NMR spectrum showed a minimal acetic acid contamination and perhaps consequently the oily compound decomposed over several days in the refrigerator.

In the preparation of **III g** and **III h** we did not obtain one main product, since presumably both urea nitrogens, and in the latter case the aromatic ring, too, were nitrated simultaneously. The same problem that also occurred in the synthesis of different *N*-(2-chloroethyl)-*N*-nitrosoureas was solved using *N*-(2-chloroethyl)-*N*-nitrosocarbamic acid active esters[15] for the acylation of the appropriate amines. For an analog reaction we prepared *N*-(2-chloroethyl)-*N*-nitrocarbamic acid pentachlorophenyl ester (**III e**) and let it react with cyclohexyl amine to get the CCNU analog **III g**. In this reaction a multi-component mixture was formed, which beside the starting materials contained pentachlorophenol and dicyclohexyl-urea (identified on TLC), and *N*-cyclohexyl-carbamic acid pentachlorophenyl ester (**IV**), which was isolated and identified by

$$\underset{\mathbf{I}}{ClCH_2CH_2NCO + H-Q} \rightarrow \underset{\mathbf{II}}{ClCH_2CH_2NH-CO-Q} \rightarrow \underset{\mathbf{III}}{.ClCH_2CH_2-N(NO_2)-CO-Q}$$

$$Q: -NH-R, O-R, -OAr, -NH-Ar, -NRR'$$

Scheme 1.

Table 3. Reaction conditions and characteristics of *N*-(2-chloroethyl)-*N*-nitrocarbamoyl derivatives (**III**)

Compound	Reaction method, purification	Yield (%)	m.p. (°C) or b.p. (°C)/mmHg	R_f (TLC)	IR	NMR
III a[11]	**A**, 1 h, distillation	51	92–93/0.3	0.87[2] 0.53[3]	neat: 1777 (CO), 1583 ($N-NO_2$)	^{13}C ($CDCl_3$), δ 13.8, 39.7, 49.6, 64.8, 149.9; ^1H ($CDCl_3$), δ 1.39 (3H, t), 3.78 (2H, t), 4.4 (4H, m)
III b	**A**, 30 min, distillation	46	150–155/0.3	0.51[3]	neat: 1778 (CO), 1750 (sh), 1584 ($N-NO_2$)	^{13}C ($CDCl_3$), δ 39.7, 40.7, 49.8, 67.5, 149.5; ^1H ($CDCl_3$), δ 3.80 (4H, m), 4.44 (2H, t), 4.55 (2H, t)
III c	**B**, 10 min	59		0.58[3]	neat: 1780, 1748 (CO), 1590 ($N-NO_2$)	^{13}C ($CDCl_3$), δ 13.9, 16.6, 39.3, 49.8, 72.1; ^1H ($CDCl_3$), δ 1.30 (3H, t), 1.61 (3H, d), 3.81 (2H, t), 4.25 (2H, qq), 4.47 (2H, t), 5.22 (1H, qq)
III d	**B**, 10 min, precipitation from methanol/water	73	37–38	0.68[4] 0.57[9]	KBr: 1775 (CO), 1583 ($N-NO_2$)	^{13}C ($CDCl_3$), δ 23.1, 25.0, 31.0, 39.8, 49.7, 78.2, 149.5; ^1H ($CDCl_3$), δ 1.25–2.0 (10H, m), 3.37 (2H, t), 4.41 (2H, t), 4.94 (1H, m)
III e	**A**, 24 h, r.t. crystallization (2-propanol)	72	145–146	0.89[1]	KBr: 1790 (CO), 1590–1610 ($N-NO_2$)	
III f	**A**, 40 min, column chromatography[4]	33		0.59[4]	neat: 1720 (CO), 1557 ($N-NO_2$), 3395 (NH)	^1H (DMSO), δ 3.53 (2H, m), 3.70 (2H, d), 3.79 (2H, t), 4.27 (2H, t), 8.89 (NH)
III i	**B**, 10 min	79	83–85 (dec)	0.58[4]	KBr: 1709 (CO), 3294 (NH), 1545 ($N-NO_2$)	^1H ($CDCl_3$), δ 3.84 (2H, t), 4.69 (2H, t) 8.80 (2H, d), 8.86 (1H, t), 10.67 (NH)
III k	**B**, 10 min, column chromatography	68		0.43[4] 0.1[10]	KBr: 1705 (CO), 1550 ($N-NO_2$)	ND

elemental analysis and IR spectroscopy (Scheme 2). Although the pentachlorophenoxy group is a much better leaving group than ethoxy or methoxy, the same reaction happened as in the case of ethyl *N*-nitrocarbamates and anilin, when phenylcarbamates[1] are formed.

A similar reaction took place when the active ester **III e** reacted with 2-chloroethyl-amine resulting in bis(2-chloroethyl)-urea and pentachlorophenol.

Nitroaromatic compounds are often used in cancer chemotherapy either as radiosenzitizers or as bio-reductive prodrugs.[16] To our knowledge, there are no reports on the use of *N*-nitro compounds, although in theory they might also be reduced by the action of nitroreductases. Thus, *N*-(2-chloroethyl)-*N*-nitroureas can also be considered as bioreductive prodrugs of the *N*-(2-chloroethyl)-*N*-nitrosoureas. Therefore, we measured the reduction potentials of some *N*-nitro derivatives and the results summarized in Table 4 show that most of them are in the optimal potential range (between −300 and −450 mV) determined for hypoxic selectivity of nitro-aromatics.[17]

In a preliminary cytotoxicity assay (Table 4) all compounds showed activity in the 10^{-3} M concentration range, with the exception of **III d**, which is the most effective with a value of 3×10^{-6} M. This value is similar to that previously found for CCNU in this cell line (Stratford and Smith, unpublished observations). There is no significant difference in the cytotoxicity between the two types of compounds, ureas and carbamates, following the exposure of cells for 96 h. However, the 16- and 8-fold differences in 3 h versus. 96 h aerobic toxicity for **III k** and **III l** may indicate that these compounds have greater aqueous stability. Most of the compounds show somewhat higher cytotoxicity in nitrogen than in air, which is favourable for killing cells in the hypoxic area inside the tumours.

Conclusion

The aim of our work was to investigate whether the *N*-(2-chloroethyl)-*N*-nitrourea and *N*-(2-chloroethyl)-*N*-nitrocarbamate analogs of *N*-(2-chloroethyl)-*N*-nitrosoureas, the well-known anti-tumor agents, possess similar cytotoxic activity. Not all theoretically possible analogs of BCNU and CCNU were synthesized, because the synthetic method, which allows the introduction of the *N*-(2-chloroethyl)-*N*-nitrosocarbamoyl group into an amine in one step with the aid of an active ester, cannot be used for the synthesis of *N*-(2-chloroethyl)-*N*-nitroureas and carbamates. However, according to our original assumption, all *N*-(2-chloroethyl)-*N*-nitroureas and carbamates which were tested exerted a cytotoxic effect that increases with the incubation time, indicating significant aqueous stability of these agents. Furthermore, the presence of a nitro group in the structure renders the compounds as targets for reductive enzymes, although the known bioreductive prodrugs contain C-nitro and not N-nitro moieties. The

$$\underset{\textbf{III e}}{ClCH_2CH_2-N(NO_2)-CO-OC_6Cl_5} + H_2N-C_6H_{11} \rightarrow \underset{\textbf{IV}}{C_6H_{11}-NH-CO-OC_6Cl_5}$$

$$\textbf{IV} + H_2N-C_6H_{11} \rightarrow \underset{\textbf{V}}{C_6H_{11}-NH-CO-NH-C_6H_{11}} + HOC_6Cl_5$$

Scheme 2.

Table 4. Reduction potentials and cytotoxicity of *N*-(2-chloroethyl)-*N*-nitro compounds

Compounds	IC_{50} (mM)			IC_{50} ratio (air/N_2)	E_p vs NHE (mV) [(i_p/μA)]
	96 h, air	3 h, air	3 h, N_2		
III a	0.13	0.3	0.2	1.5	−293 (0.768)
III b	0.3	0.55	0.3	1.8	−283 (0.820)
III c	0.25	0.45	0.3	1.5	−293 (0.728)
III d	0.003	0.008	0.008	1.0	−403 (0.600)
III k	0.15	2.5	1.0	2.5	−553 (0.544)
III l	0.1	0.8	0.4	2.0	−693 (0.312)

presented reduction potentials seem to be appropriate for the reductive enzymes, and also a slight difference could have been observed in the cytotoxicities of the *N*-nitro compounds in aerobic and anaerobic conditions.

These preliminary results indicate that the *N*-(2-chloroethyl)-*N*-nitrocarbamoyl compounds represent a new group of potential anti-cancer agents which deserve further investigation.

Acknowledgments

We thank Dr T Dankházi for the sweep voltammetry measurements. Special thanks to Dr HR Hendriks (EORTC-NDDO) who arranged the co-operation.

References

1. Brundrett RB. Chemistry of nitrosoureas. Intermediacy of 4,5-dihydro-1,2,3-oxadiazole in 1,3-bis(2-chloroethyl)-1-nitrosourea decomposition. *J Med Chem* 1980; **23**: 1245–7.
2. Jeney A, Kopper L, Nagy P, Lapis K, Süli-Vargha H, Medzihradszky K. Antitumor action of *N*-(2-chloroethyl)-*N*-nitrosocarbamoyl derivatives of biologically active polypeptide hormone fragments. *Cancer Chemother Pharmacol* 1986; **16**: 129–32.
3. Peták I, Mihalik R, Bauer P, Süli-Vargha H, Sebestyén A, Kopper L. BCNU is a caspase-mediated inhibitor of drug-induced apoptosis. *Cancer Res* 1998; **58**: 614–8.
4. Thiele J, Lachman A. Ueber Nitroharnstoff, Nitrourethan, und Nitramid. *Justus Liebigs Ann Chem* 1895; **288**: 267–311.
5. White EH, Grisley DW Jr. The preparation and decomposition of certain *N*-nitroamides and *N*-nitrocarbamates. *J Am Chem Soc* 1961; **83**: 1191–6.
6. White EH, Field KW. Deamination of aliphatic amines in ethanol. *J Am Chem Soc* 1975; **97**: 2148–53.
7. Kniphorst LCE. The nitration of symmetrical aryl-alkyl-ureas. *Recl Trav Chim Pays-Bas* 1925; **44**: 693–27.
8. Johnston TP, McCaleb GS, Opliger PS, Montgomery JA. The synthesis of potential anticancer agents XXXVI. *N*-nitrosoureas. II. Haloalkyl derivatives. *J Med Chem* 1966; **9**: 892–911.
9. Süli-Vargha H, Bódi J, Mészáros M, Medzihradszky K. Decomposition of *N*-(2-chloroethyl)-*N*-nitrosocarbamoyl amino acid amides. *J Med Chem* 1988; **31**: 1492–5.
10. Stratford IJ, Stephens MA. The differential hypoxic toxicity of bioreductive agents determined *in vitro* using the MTT assay. *Int J Radiat Oncol Biol Phys* 1989; **16**: 973–6.
11. Curry HM, Mason JPh. Carbamates and *N*-nitrocarbamates. *J Am Chem Soc* 1951; **73**: 5043–6.
12. Brintzinger H, Pfannstiel K. Notiz über Nitrosoalkylcarbamidsäureester. *Chem Ber* 1948; **81**: 378–80.
13. Lown JW, McLaughlin LW, Chang Y-M. Mechanism of action of 2-haloethylnitrosoureas on DNA and its relation to their antileukemic properties. *Bioorg Chem* 1978; **7**: 97–110.
14. Tsujihara K, Ozeki M, Morikawa T, Yoshihisa A. A new class of nitrosoureas. I. Synthesis and antitumor activity of 1-(2-chloroethyl)-3,3-disubstituted-1-nitrosoureas having a hydroxyl group at the beta-position of the substituents. *Chem Pharm Bull* 1981; **29**: 2509–15.
15. Martinez J, Oiry J, Imbach JL, Winternitz F. Activated *N*-nitroscarbamates for regioselective synthesis of *N*-nitrosoureas. *J Med Chem* 1982; **25**: 178–82.
16. Adams GE, Stratford IJ. Bioreductive drugs for cancer therapy: the search for tumor specificity. *Int J Radiat Oncol Biol Phys* 1994; **29**: 231–8.
17. Sinhababu AK, Thakker DR. Prodrugs of anticancer agents. *Adv Drug Delivery Rev* 1996; **19**: 241–73.

(Received 12 August 1999; revised form accepted 3 September 1999)