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A New Family of Potential Oncostatics: 2-Chloroethylnitrososulfamides (CENS)—I. Synthesis, Structure, and Pharmacological Evaluation (Preliminary Results)

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Abstract—A new series of alkylating agents, 2-chloroethylnitrososulfamides (CENS), were developed on the model of 2-chloroethylnitrosoureas. Starting from chlorosulfonyl isocyanate, a four-step synthesis (carbamoylation–sulfamoylation, Mitsunobu alkylation, deprotection, and nitrosation) gives the title compounds in a 47–58% overall yield. The selection of the nitrosation site can be directed through an alternative route. The pharmacological evaluation shows a significant oncostatic activity towards both A549 and MCF7 cell lines. Copyright © 1996 Elsevier Science Ltd

Introduction

The essential cytotoxic effects of the alkylating agents utilized in antitumoral chemotherapy are due to the linkage of the DNA molecule by a bifunctional electrophile (e.g. chloronium ion). The 2-chloroethylnitrosoureas (CENUs),[1] a major class, present damaging cumulative side-effects such as mutagenicity, bone marrow toxicity, diabetogenesis, etc. Therefore, in the last decades, significant efforts have been made towards the synthesis of similar drugs (more active and less toxic). We chose to replace the carbonyl group in CENU with a sulfonyl group: such 2-chloroethylnitrososulfamides (CENS) would preserve the electrophilic potentialities, following the example of *N*-nitrososulfamates. These are utilized as carbonium ion donors in mild conditions.[2] Obviously, CENS cannot release in vivo carbamoylating entities (Fig. 1), which induce undesirable side-effects; moreover, *N*-nitroso, *N*-methyl-*p*-toluenesulfamide (Diazald®), is useful as a diazomethane precursor[3] and is known as a non-mutagenic compound. We describe here the synthesis and the preliminary results of the biological evaluation concerning a series of compounds containing this new pharmacophore 2-chloroethylnitrososulfamide.

Chemistry

The sulfamic acid and its reactive sulfamoylating forms (chloride, azide) can be utilized as a versatile precursor of derivatives containing the sulfamido group.[4] In the present case, however, the preparation of the sulfamide precursor *N*-2-chloroethylsulfamoyl chloride, starting from aziridine and sulfuryl chloride, was carried out in an unsatisfactory 5% yield.[5] Therefore, we have selected the alternative approach of *insertion of activated sulfamoyl group*.[6] Thus, CENSs can be synthesized in four steps starting from chlorosulfonyl isocyanate (CSI)[7] (Scheme 1). Carbamoylation and sulfamoylation—with the listed panel of aliphatic and aromatic amines—are performed by a one-pot reaction

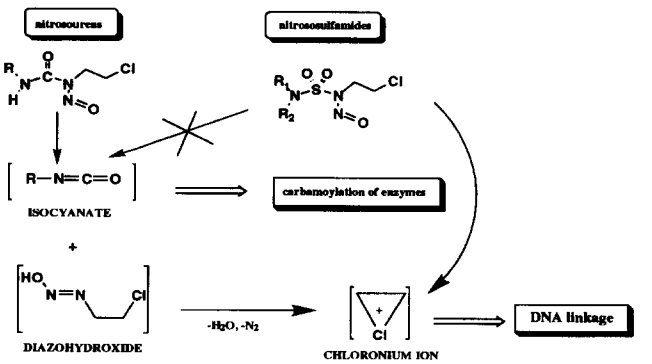

Figure 1.

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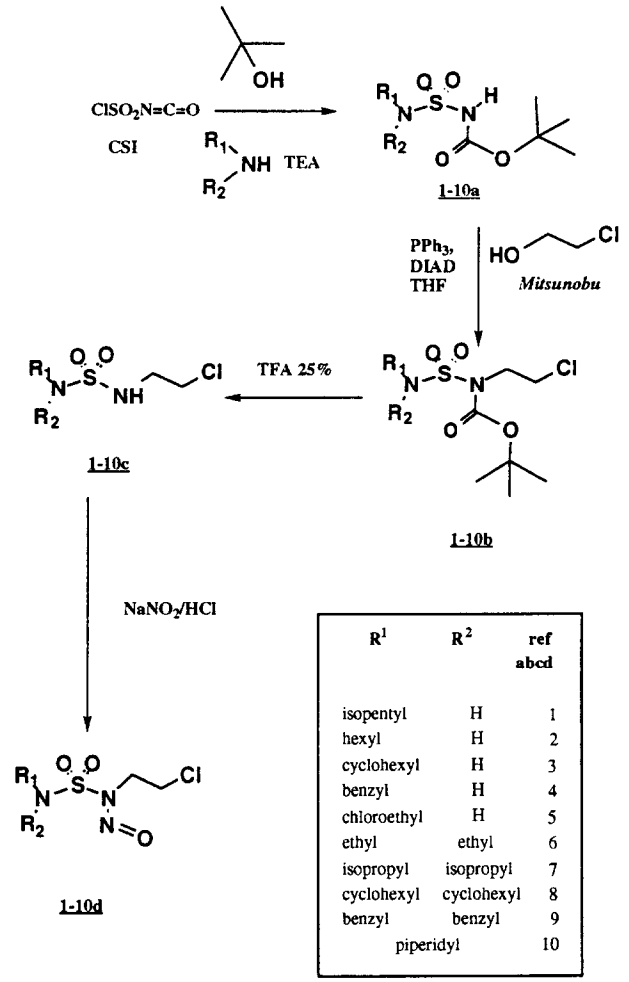
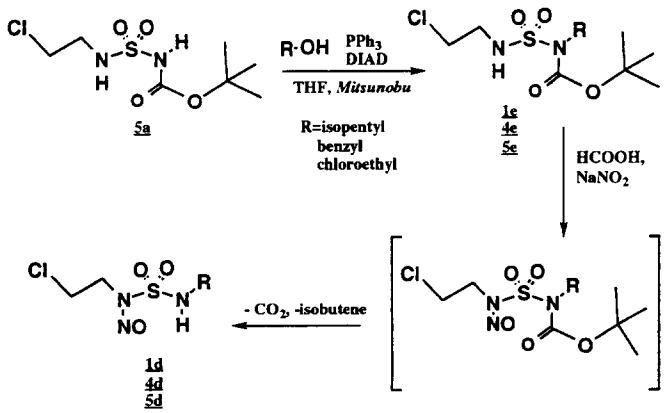
Key words: Nitrososulfamide, carboxylsulfamide, chlorosulfonyl isocyanate, Mitsunobu reaction, alkylating agent, oncostatics, chloroethylnitrosoureas, biological screening, A549, MCF7.

to give Boc-sulfamides **1–10a**. In these, the Boc group (*t*-butyloxycarbonyl) dramatically increases the acidity of the adjacent N**H** and allows an expedient alkylation under Mitsunobu conditions[8] using chloroethanol, which provides N-substituted Boc-sulfamides **1–10b**. A following treatment in an acidic organic medium furnishes deprotected sulfamides **1–10c** and a subsequent nitrosation in acidic conditions ultimately gives CENSs **1–10d** in an average yield of 80%. Yields, analytical, and other selected physicochemical and spectral data concerning these four series are reported in Tables 1–4.



R^1	R^2	ref abcd
isopentyl	H	1
hexyl	H	2
cyclohexyl	H	3
benzyl	H	4
chloroethyl	H	5
ethyl	ethyl	6
isopropyl	isopropyl	7
cyclohexyl	cyclohexyl	8
benzyl	benzyl	9
piperidyl		10

Scheme 1.



Scheme 2.

The synthesis of Boc-sulfamides **1–10a** is performed in dried dichloromethane with successive additions of *t*BuOH, and RR'NH/TEA into CSI. After recrystallization, the Boc-sulfamides are recovered in 80–95% yield. For the compounds **1–5a** derived from primary amines, both the exchangeable protons are easily discernable: the carbamic NH was revealed by NMR as a broad signal 7.5 ± 2 ppm and the other NH appears at higher field (5–7 ppm) coupling with neighbouring proton(s). The corresponding characteristic IR bands were found to be 3300 ± 25 and 3200 ± 25 cm^{-1}. The carbonyl absorption appears in the 1710–1700 cm^{-1} region and symmetric/antisymmetric elongations of the SO_2 group in the $1350 \pm 10/1350 \pm 10$ cm^{-1} regions. The fragmentation in mass spectrometry (FAB) shows the loss of a Boc or *tert*-butyl group.

The Mitsunobu reaction can be carried out in anhydrous THF as a solvent. The mixture of DEAD or DIAD (diethyl or diisopropylazodicarboxylate) and *N*-Boc-sulfamides is added to a solution of chloroethanol and PPh_3. Despite a total and fast transformation in expected chloroalkylated compounds, difficulties in elimination of phosphine oxide and hydrazinebiscarbamate decrease the yield at this step. By column chromatography, the less polar alkylated compounds **1–10b** are recovered in head fractions. The selective monoalkylation on the activated *N*-Boc position shows the difference of nucleophilic character of both NHs. The structures of **1–10b** are confirmed by the NMR spectroscopy: removal of carbamic NH signal and appearance of an A_2X_2 system at 3.6 [CH_2Cl] and 4.1 ppm [NCH_2]. A modest hypsochrom IR shift (5–10 cm^{-1}) is observed related to the C=O elongation band, as a simplification in the NH region. In MS the loss of a *tert*-butyl group is observed. The removal of the Boc group in sulfamides **1–10b** is achieved by an acidic treatment (trifluoroacetic acid). The crude *N*-2-chloroethylsulfamides **1–10c** are obtained in a quantitative yield. A subsequent purification by recrystallization gives the expected derivatives. The disappearance of the C=O absorption band in IR and the *t*Bu signal in NMR confirm the removal of the Boc group. The chloroethyl group signal appears as a triplet (3.65 ppm: CH_2Cl) quadruplet (3.4 ppm: CH_2NH) system.

The N-nitrosation reaction is performed in acidic conditions giving the desired CENS. Using TLC the presence of nitrosated sulfamides **1–10c** spots is revealed by a strong UV absorption. Acidic conditions in the subsequent workup are required to avoid denitrosation of resulting compounds.

The trisubstituted sulfamides **6–10c**, derivating from secondary amines give, obviously, the mononitrosated

derivatives. With others, variable amounts (up to 25%) of dinitrosated compounds, such as **4f**, are also collected. This one is unambigously identified by its NMR data (no exchangeable proton, triplet at 4.10 ppm (N[NO]CH_2—CH_2Cl), singlet at 4.52(N[NO]-CH_2—Ph)). The regioselectivity on *N*-chloroethyl nitrosation can be increased by using reduced amounts of sodium nitrite. In NMR the ethylene group in CENSs **1–10c** appears as an A_2X_2 system at 3.4 [CH_2Cl] and 4.15 ppm [NCH_2]. The elongation N=O IR band is observed at 1555–1585 cm^{-1}. UV characteristics for this chromophore are λ_{max} = 250–260 nm, $\varepsilon \approx 5 \times 10^3$ [cyclohexane].

An alternative route for the regiospecific synthesis of *N*-nitroso compounds, starting from **5a**, was also utilized (Scheme 2). Thus, the Mitsunobu alkylation is achieved with isopentanol, benzyl alcohol or chloroethanol to give, respectively, **1**, **4**, **5e**. Nitrosation in acidic conditions gives the corresponding CENS in a satisfactory yield, 75%. Therefore, the nitrosation rate is higher than the Boc-cleavage rate in this procedure.

A CENU/CENS comparative study shows immediately an essential difference concerning the same nitrosation reaction: two mononitrosed regioisomers can be obtained starting from *N,N'*-disubstituted ureas, but no double nitrosation is detected. We have explained this particularity by a six-membered cyclic structure containing an NH···N=O chelation.[9] Such intermolecular bonding doesn't exist in the CENS series. Hence further investigations are in progress.

Table 1. *N*-Boc-sulfamides

	Yield Anal. MS	mp (°C)	IR (KBr ν cm^{-1})			^{1}H NMR ($CDCl_3$, δ ppm)
			NH	C=O	SO_2	
1a	88% M = 266.3 [$C_{10}H_{22}N_2O_4S$]	118–120	3250 3300	1700	1345 1140	7.40 (s, 1H, NH Boc), 5.65 (t, 1H, NH—CH_2), 3.05 (q, 2H, NH—CH_2), 1.55 (m, 1H, CH), 1.45 (s, 9H, *t*Bu), 1.40 (m, 2H, CH_2—CH), 0.90 (d, 6H, $2CH_3$).
2a	85% M = 280.3 [$C_{11}H_{24}N_2O_4S$]	133	3350 3270	1700	1345 1135	7.65 (s, 1H, NH Boc), 5.35 (t, 1H, NH—CH_2), 3.10 (q, 2H, NH—CH_2), 1.60 (m, 2H, CH_2—CH_3), 1.5 (s, 9H, *t*Bu), 1.30 (m, 6H, $3CH_2$), 0.90 (t, 3H, CH_3).
3a	92% M = 278.3 [$C_{11}H_{22}N_2O_4S$] (FABMS < 0; NOBA): 277 [M-H]$^-$; 177 [M-Boc]$^-$.	155–157	3310	1708	1360 1150	7.10 (s, 1H, NH Boc), 5.30 (d, 1H, NH—CH), 3.30 (m, 1H, NH—CH), 2.20–2.00 (m, 10H, CH_2 Cycl), 1.45 (s, 9H, *t*Bu).
4a	94% M = 286.3 [$C_{12}H_{18}N_2O_4S$] (FABMS < 0; NOBA matrix): 285 [M-H]$^-$; 241 [M-*t*Bu]$^-$.	111–113	3298	1708	1355 1150	7.25 (m, 6H, ArH + NH Boc), 5.45 (t, 1H, NH Bn), 4.25 (d, 2H, CH_2 Bn), 1.45 (s, 9H, *t*Bu).
5a	82% M = 258.7 [$C_7H_{15}N_2O_4SCl$]	132	3325 3225	1705	1350 1140	7.35 (s, 1H, NH Boc), 5.65 (t, 1H, NH—CH_2), 3.70 (t, 2H, Cl—CH_2), 3.45 (q, 2H, N—CH_2), 1.52 (s, 9H, *t*Bu).
6a	90% M = 252.3 [$C_9H_{20}N_2O_4S$] (FABMS > 0; NOBA): 253 [M + H]$^+$; 153 [M- *t*Boc]$^+$.	84–86	3302	1712	1354 1147	6.90 (s, 1H, NH Boc), 3.75 (q, 4H, $2CH_2$), 1.50 (s, 9H, *t*Bu), 1.10 (t, 6H, 2 CH_3).
7a	95% M = 280.4 [$C_{11}H_{24}N_2O_4S$] (FABMS > 0; NOBA): 281 [M + H]$^+$; 181 [M-*t*Boc]$^+$.	127–129	3310	1724	1354 1149	6.30 (s, 1H, NH Boc), 4.95 (m, 2H, 2CH), 1.50 (s, 9H, *t*Bu), 1.35 (d, 12H, $4CH_3$).
8a	94% M = 360.5 [$C_{17}H_{32}N_2O_4S$]	147–148	3275	1740	1360 1160	6.95 (s, 1H, NH Boc), 3.45 (m, 2H, 2N—CH), 2.00–1.20 (m, 29H, CH_2 Cycl + *t*Bu).
9a	92% M = 376.4 [$C_{19}H_{24}N_2O_4S$] (FABMS < 0; GT): 375 [M-H]$^-$; 331 [M-*t*Bu]$^-$.	93–94	3304	1710	1360 1132	7.25 (m, 10H, ArH), 6.25 (s, 1H, NH Boc), 4.45 (s, 4H, $2CH_2$ Bn), 1.45 (s, 9H, *t*Bu).
10a	93% M = 264.3 [$C_{10}H_{20}N_2O_4S$] (FABMS > 0; NOBA): 265 [M + H]$^+$.	52–54	3310	1708	1360 1152	5.30 (s, 1H, NH Boc), 3.30 (t, 4H, N—CH_2), 1.70–1.50 (m, 6H, CH_2 Cycl), 1.45 (s, 9H, *t*Bu).

Pharmacology

The evaluation of cytotoxicity was carried out on two types of tumoral cell lines: the A549 cell line, which possesses epithelial-like morphology, was initiated through explant culture of lung carcinoma tissue of a 58-year-old Caucasian male. The MCF7 cell line was initiated through culture of effusion of a human mammary adenocarcinoma. Nitrososulfamides **3**, **4**, **7–9**, **10d** and dinitrosed derivative **4f** were tested, with as reference **RFCNU**[10] (a substituted ribofuranosyl nitrosourea utilized in clinical chemotherapy) in accordance with the procedure described in the Experimental section.[11]

The results are reported in Figure 2. With respect to RFCNU, the cytotoxic activity of the tested CENS is staggered in five categories (Table 5). This preliminary evaluation is promising: on both tumoral cell strains, most CENS involve a significant cytotoxic effect; the lung line appears more sensitive that the mammary line. The IC_{50} is calculated for **9d** (dibenzyl derivative) and **8d** (dicyclohexyl derivative), which involve in each case the most interesting biological response (respectively 8 and 30 μg/mL).

Conclusion

The molar efficient concentrations of the most active CENS were 2.7×10^{-5} M toward the lung line (**9d**) and 10^{-4} M toward the mammary line (**8d**). Comparison with the reference compound, a 10-fold enhancement was also observed. In spite of the reduced number of compounds in the series, we can observe that this activity is in correlation with the size and the substitu-

Table 2. *N*-Boc *N*-chloroethylsulfamides

	Yield Anal. MS	mp (°C)	IR (KBr ν cm^{-1})			^1H NMR ($CDCl_3$, δ ppm)
			NH	C=O	SO_2	
1b	77% M = 328.8 $[C_{12}H_{25}ClN_2O_4S]$	foam	3350	1705	1355 1140	5.20 (t, 1H, NH—CH_2), 3.95 (t, 2H, N—CH_2), 3.63 (t, 2H, Cl—CH_2), 3.00 (q, 2H NH—CH_2), 1.60 (m, 1H, CH), 1.45 (s, 9H, *t*Bu), 1.40 (m, 2H, CH_2—CH), 0.90 (d, 6H, $2CH_3$).
2b	73% M = 342.8 $[C_{13}H_{27}ClN_2O_4S]$	foam	3350	1720	1370 1150	5.30 (t, 1H, NH), 4.40 (t, 2H, N—CH_2), 3.65 (t, 2H, Cl—CH_2), 3.00 (q, 2H NH—CH_2), 1.55 (s, 9H, *t*Bu), 1.50 (m, 2H, CH_2), 1.30 (m, 6H, $CH_2]_3$), 0.85 (t, 3H, CH_3).
3b	80% M = 340.8 $[C_{13}H_{25}ClN_2O_4S]$ (FABMS < 0; NOBA): 339 $[M\text{-}H]^-$.	50–52	3380	1710	1370 1150	5.05 (d, 1H, NH—CH), 4.02 (t, 2H, N—CH_2), 3.70 (t, 2H, Cl—CH_2), 3.30 (m, 1H, NH—CH), 2.20–1.20 (m, 10H, CH_2 Cycl), 1.48 (s, 9H, *t*Bu).
4b	72% M = 348.8 $[C_{14}H_{21}ClN_2O_4S]$ (FABMS > 0; NOBA): 349 $[M+H]^+$; 249 $[M\text{-}t\text{Boc}]^+$.	84–85	3310	1708	1356 1150	7.30 (m, 5H, ArH), 5.50 (t, 1H, NH Bn), 4.25 (d, 2H, CH_2 Bn), 4.08 (t, 2H, N—CH_2), 3.76 (t, 2H, Cl—CH_2), 1.48 (s, 9H, *t*Bu).
5b	70% M = 321.2 $[C_9H_{18}Cl_2N_2O_4S]$	62	3380	1710	1325 1150	5.80 (t, 1H, NH—CH_2), 4.10 (t, 2H, N—CH_2), 3.80–3.65 (m, 4H, 2Cl—CH_2), 3.41 (q, 2H, HN—CH_2), 1.46 (s, 9H, *t*Bu).
6b	74% M = 314.8 $[C_{11}H_{23}ClN_2O_4S]$ (FABMS > 0; GT): 315 $[M+H]^+$; 259 $[M\text{-}t\text{Bu}]^+$; 215 $[M\text{-}t\text{Boc}]^+$ 50%.	54–56		1710	1349 1152	3.90 (t, 2H, NCH_2), 3.75 (q, 4H, $NCH_2]_2$), 3.65 (t, 2H, Cl—CH_2), 1.50 (s, 9H, *t*Bu), 1.15 (t, 6H, $2CH_3$).
7b	71% M = 342.8 $[C_{13}H_{27}ClN_2O_4S]$ (FABMS > 0; NOBA): 343 $[M+H]^+$; 287 $[M\text{-}t\text{Bu}]^+$.	65–66		1712	1365 1149	4.94 (m, 2H, 2CH), 3.90 (t, 2H, N—CH_2), 3.63 (t, 2H, Cl—CH_2), 1.48 (s, 9H, *t*Bu), 1.33 (d, 12H, $4CH_3$).
8b	70% M = 423.0 $[C_{19}H_{35}ClN_2O_4S]$	103–104		1735	1350 1145	3.95 (t, 2H, N—CH_2), 3.65 (t, 2H, Cl—CH_2), 3.45 (m, 2H, 2N—CH), 1.80–1.30 (m, 29H, CH_2 Cycl + *t*Bu).
9b	70% M = 438.9 $[C_{21}H_{27}ClN_2O_4S]$ (FABMS > 0; NOBA): 439 $[M+H]^+$; 366 $[M\text{-}Ot\text{Bu}]^+$.	106–108		1708	1350 1145	7.30 (m, 10H, 2ArH), 4.30 (s, 4H, $2CH_2$ Bn), 3.93 (t, 2H, N—CH_2), 3.65 (t, 2H, Cl—CH_2), 1.54 (s, 9H, *t*Bu).
10b	77% M = 326.8 $[C_{12}H_{23}ClN_2O_4S]$ (FABMS < 0; GT): 325 $[M\text{-}H]^-$; 225 $[M\text{-}Boc]^-$.	92–94		1715	1361 1164	3.95 (t, 2H, N—CH_2), 3.66 (t, 2H, Cl—CH_2), 3.30 (t, 4H, N—CH_2 Cycl), 1.65–1.55 (m, 6H, CH_2 Cycl), 1.52 (s, 9H, *t*Bu).

tion of the linked amine, relating to the global lipophilic character of each molecule. For example, toward A549 cells, the measure of log *P* (octanol–water) was 1,2 for the most active derivative **9d** and 0.02 for the less active **4d**. The lipohydrophilic nature of alkyl group on the amine seems the determining factor which guides the choice of the most appropriate substituents. For the chemical point of view, the great versatility of chlorosulfonyl isocyanate in the described strategy can allow the preparation of a therapeutic vectors library. This work is in progress and will be the subject of further publications.

Experimental

Chemistry

Melting points were determined in open capillary tubes on a thermotechnal apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer spectrophotometer. Microanalyses were performed in the microanalysis laboratory of ENSCM (Montpellier). Ultraviolet spectra were recorded on a Cary 1186 spectrophotometer. ^{1}H NMR were determined with an AC 250 Bruker spectrometer. Chemical shifts are expressed in parts per million, with TMS as reference. The multiplicity is indicated as: s (singlet), d (doublet), t (triplet), q (quadruplet), m (multiplet) , l (large) and combination of these signals. Fast atom bombardment mass spectra (FABMS) were recorded in positive or negative mode on a JEOL DX 300 spectrometer using G (glycerol), GT (thioglycerol) or NOBA (nitrobenzylic alcohol) matrix. Thin-layer chromatography (TLC) was performed on silica gel $60F_{254}$ (Merck). In a homogeneous series, releved R_f are $X_d > X_b > X_a > X_c$ (dichloromethane or ether:pentane). Column chromatography was performed with silica gel 60. All solvents used for the reactions were anhydrous.

Table 3. *N*-Chloroethylsulfamides

	Yield Anal. MS	mp (°C)	IR (KBr ν cm^{-1})		^{1}H NMR ($CDCl_3$, δ ppm)
			NH	SO_2	
1c	98% M = 228.7 $[C_7H_{17}ClN_2O_2S]$	69–70	3300	1320 1140	4.65 (s br, 1H, NH-*i*Pn), 3.95 (t, 1H, NH—CH_2), 3.55 (t, 2H, Cl—CH_2), 3.25 (q, 2H NH—CH_2), 2.45 (t, 2H, NH—CH_2*i*Pn), 1.55 (m, 1H, CH), 1.30 (m, 2H, CH_2—CH), 0.77 (d, 6H, $2CH_3$).
2c	96% M = 242.7 $[C_8H_{19}ClN_2O_2S]$ (FABMS < 0; NOBA) 239, $[M\text{-}H]^-$.	72–73	3300	1370 1135	4.70 (t, 1H, NH hex), 4.25 (t, 1H, NH—CH_2), 3.70 (t, 2H, Cl—CH_2), 3.40 (q, 2H NH—CH_2), 3.05 (q, 2H, NH—CH_2 hex), 1.55 (m, 2H, CH_2CH_3), 1.30 (m, 6H, $CH_2]_3$), 0.90 (m, 3H, CH_3).
3c	98% M = 240.7 $[C_8H_{17}ClN_2O_2S]$	96–98	3300 3270	1360 1152	4.60 (d, 1H, NH—CH), 3.70 (t, 2H, Cl—CH_2), 3.60 (q, 2H, NH—CH_2), 3.25 (m, 1H, NH—CH), 2.20–1.20 (m, 10H, CH_2 Cycl).
4c	94% M = 248.7 $[C_9H_{13}ClN_2O_2S]$ (FABMS > 0; NOBA): 249 $[M+H]^+$.	97	3300	1365 1168	7.55 (s, 5H, ArH), 4.70–4.55 (2t, 2H, 2NH), 4.25 (d, 2H, Bn—CH_2), 3.68 (t, 2H, Cl—CH_2), 3.40 (q, 2H, N—CH_2).
5c	91% M = 221.1 $[C_4H_{10}Cl_2N_2O_2S]$ (FABMS > 0; NOBA): 222 $[M+H]^+$.	97–98	3300 3250	1315 1130	4.75 (t, 2H, 2NH—CH_2), 3.65 (t, 4H, 2Cl—CH_2), 3.35 (q, 4H, 2 HN—CH_2).
6c	97% M = 214.7 $[C_6H_{15}ClN_2O_2S]$	foam	3330	1325 1150	4.80 (t, 1H, NH), 3.63 (t, 2H, Cl—CH_2), 3.35 (q, 4H, $2NCH_2$), 3.30 (q, 2H, NCH_2), 1.15 (t, 6H, $2CH_3$).
7c	97% M = 242.7 $[C_8H_{19}ClN_2O_2S]$ (FABMS > 0; NOBA): 343 $[M+H]^+$.	92–94	3325	1320 1140	5.15 (t, 1H, NH), 4.95 (m, 2H, 2CH), 3.68 (t, 2H, Cl—CH_2), 3.45 (q, 2H, N—CH_2), 1.35 (d, 12H, $4CH_3$).
8c	98% M = 322.9 $[C_{14}H_{27}ClN_2O_2S]$	124–125	3330	1315 1145	4.40 (t, 1H, NH), 3.62 (t, 2H, Cl—CH_2), 3.25 (q, 2H, N—CH_2), 3.15 (m, 2H, 2N—CH), 1.75–1.00 (m, 20H, CH_2 Cycl).
9c	95% M = 338.8 $[C_{16}H_{19}ClN_2O_2S]$ (FABMS > 0; NOBA): 339 $[M+H]^+$; 248 $[M\text{-}Bn]^+$.	< 50	3300	1360 1160	7.45 (m, 10H, 2ArH), 4.45 (t, 1H, NH), 4.35 (s, 4H, $2CH_2$ Bn), 3.60 (t, 2H, Cl—CH_2), 3.25 (q, 2H, N—CH_2).
10c	100% M = 226.7 $[C_7H_{15}ClN_2O_2S]$ (FABMS > 0; NOBA): 227 $[M+H]^+$.	52	3320	1350 1155	4.62 (t, 1H, NH), 3.64 (t, 2H, Cl—CH_2), 3.40 (q, 2H, N—CH_2), 3.32 (t, 4H, N—CH_2 Cycl), 1.62–1.50 (m, 6H, CH_2 Cycl).

Sulfamoylation. A soln of 0.05 mol of *N*-chlorosulfonyl (*tert*-butyl) carbamate was prepared by addition of *tert*-butanol (4.8 mL in 25 mL of dried dichloromethane) into a solution of CSI (7.1 g in the same solvent). This reagent was slowly added at 0 °C into a solution containing a primary (or secondary) amine (0.05 mol in 100 mL dichloromethane) and 1.1 equiv of triethylamine. The reaction was achieved in 45 min. The medium was diluted with 100 mL dichloromethane, washed with two fractions of HCl 0.1 N, dried and concd in vacuo. The crude residue was then purified by column chromatography (yield 85–95%).

Substitution. A soln of *N*-*t*-butyloxycarbonyl, *N'*-alkyl or *N',N'*-dialkyl sulfamide (0.03 mol) and diethyl (diisopropyl) azodicarboxylate (0.03 mol; 5.22 or 6.06 g) in THF (25 mL) was added dropwise (20 min, 5 °C) to a solution of equimolar quantities of triphenylphosphine (7.6 g) and chloroethanol (2.4 g; 2 mL) in THF (25 mL). The reaction medium was stirred under an atmosphere of dry nitrogen for about 45 min. TLC reveals the formation of a substituted compound (UV, ninhydrin) less polar than its precursor. Oxidoreduction compounds were removed by filtration after precipitation into diethylether. The filtrate was concentrated and the crude residue was purified by column chromatography eluted with dichloromethane. Chloroalkylated sulfamides were recovered in 70–80% yield.

Deprotection. A soln of trifluoroacetic acid (50% in dried dichloromethane; 3 equiv) was added dropwise into a stirred soln of substituted *N*-Boc, *N*-chloroethylsulfamide (0.2 mol) in dried dichloromethane (15 mL) at 0 °C. The reaction medium was stirred during 2 h,

Table 4. *N*-Nitroso, *N*-chloroethylsulfamides

	Yield Anal. MS	mp (°C)	IR (KBr ν cm^{-1})			^1H NMR ($CDCl_3$, δ ppm)
			NH	C=O	SO_2	
1d	71% M = 257.7 $[C_7H_{16}ClN_3O_3S]$	foam	3260	1575	1355 1150	4.55 (s br, 1H, NH-*i*Pn), 4.00 (t, 2H NH—CH_2), 3.55 (t, 2H, Cl—CH_2), 2.45 (t, 2H, NH—CH_2-*i*Pn), 1.55 (m, 1H, CH), 1.30 (m, 2H, CH_2—CH), 0.77 (d, 6H, $2CH_3$).
2d	77% M = 271.7 $[C_8H_{18}ClN_3O_3S]$	foam	3265	1580	1350 1155	4.60 (t, 1H, NH hex), 4.05 (t, 2H N—CH_2), 3.55 (t, 2H, Cl—CH_2), 3.00 (q, 2H, NH—CH_2 hex), 1.40 (m, 2H, CH_2—CH_3), 1.35 (m, 6H, $CH_2]_3$), 0.95 (m, 3H, CH_3).
3d	73% M = 269.7 $[C_8H_{16}ClN_3O_3S]$ (FABMS < 0; GT): 239 $[M\text{-}NO]^-$; 161 $[RNSO_2]^-$; 63	55	3270	1580	1360 1150	4.60 (d, 1H, NH—CH), 3.90 (t, 2H, N—CH_2), 3.54 (t, 2H, Cl—CH_2), 3.25 (m, 1H, NH—CH), 2.20–1.20 (m, 10H, CH_2 Cycl).
4d	62% M = 277.7 $[C_9H_{12}ClN_3O_3S]$ (FABMS < 0; GT): 247 $[M\text{-}NO]^-$; 211.	foam		1580	1352 1164	7.58 (m, 5H, ArH), 5.60 (t, 1H, NH exch), 4.25 (d, 2H, Ph—CH_2), 4.00 (t, 2H, N—CH_2), 3.45 (t, 2H, Cl—CH_2).
5d	51% M = 250.1 $[C_4H_9Cl_2N_3O_3S]$	foam	3250	1550	1320 1140	4.65 (t, 1H, NH), 4.45 (t, 2H, ON—N—CH_2), 3.75 (m, 4H, 2Cl—CH_2), 3.65 (m, 2H, HN—CH_2).
6d	75% M = 243.7 $[C_6H_{14}ClN_3O_3S]$ (FABMS > 0; NOBA): 244 $[M+H]^+$.	foam		1576	1356 1166	4.05 (t, 2H, ON—N—CH_2), 3.47 (t, 2H, Cl—CH_2), 3.35 (q, 4H, 2N—CH_2), 1.25 (t, 6H, $2CH_3$).
7d	77% M = 271.7 $[C_8H_{18}ClN_3O_3S]$	foam		1580	1364 1154	4.00 (t, 2H, ON—N—CH_2), 3.90 (m, 2H, 2*i*PrCH), 3.47 (t, 2H, Cl—CH_2), 1.25 (d, 12H, $4CH_3$).
8d	80% M = 351.9 $[C_{14}H_{26}ClN_3O_3S]$ (EIMS$^+$) 322 $[M\text{-}NO]^+$, 20%; 241 $[R_2NSO_2]^+$ 10%; 180 $[R_2N]^+$ 20%.	64–65		1580	1375 1175	3.95 (t, 2H, N—CH_2), 3.40 (t, 2H, Cl—CH_2), 3.30 (m, 2H, 2N—CH), 1.75–1.00 (m, 20H, CH_2 Cycl).
9d	84% M = 367.8 $[C_{16}H_{18}ClN_3O_3S]$	43–44		1580	1360 1154	7.25 (m, 10H, 2ArH), 4.60 (s, 4H, $2CH_2$ Bn), 4.05 (t, 2H, N—CH_2), 3.51 (t, 2H, Cl—CH_2).
10d	88% M = 255.7 $[C_7H_{14}C_lN_3O_3S]$ (FABMS > 0; NOBA): 256 $[M+H]^+$; 225 $[M\text{-}NO]^+$; 148 $[R_2NSO_2]^+$; 63.	52–53		1575	1348 1162	4.50 (t, 2H, N—CH_2), 4.10 (t, 2H, Cl—CH_2), 3.52 (t, 4H, N—CH_2 Cycl), 1.80–1.50 (m, 6H, CH_2 Cycl).

concd under red. pres. and coevaporated with diethyl ether. The residue was recrystallized in a AcOEt:hexane mixture (yield 95%).

Nitrosation. To a stirred solution of *N*-(2-chloroethyl) sulfamide (0.01 mol) in anhydrous formic acid (10 mL) were added, by fractions, dried sodium nitrite (2 equiv) at 0 °C during 30 min. Then the reaction medium was poured into ice and extracted by dichloromethane, washed with a saturated hydrogen carbonate solution, dried and evapd under red. pres. The recrystallization (petroleum ether) afforded the resulting CENS in a 75% yield.

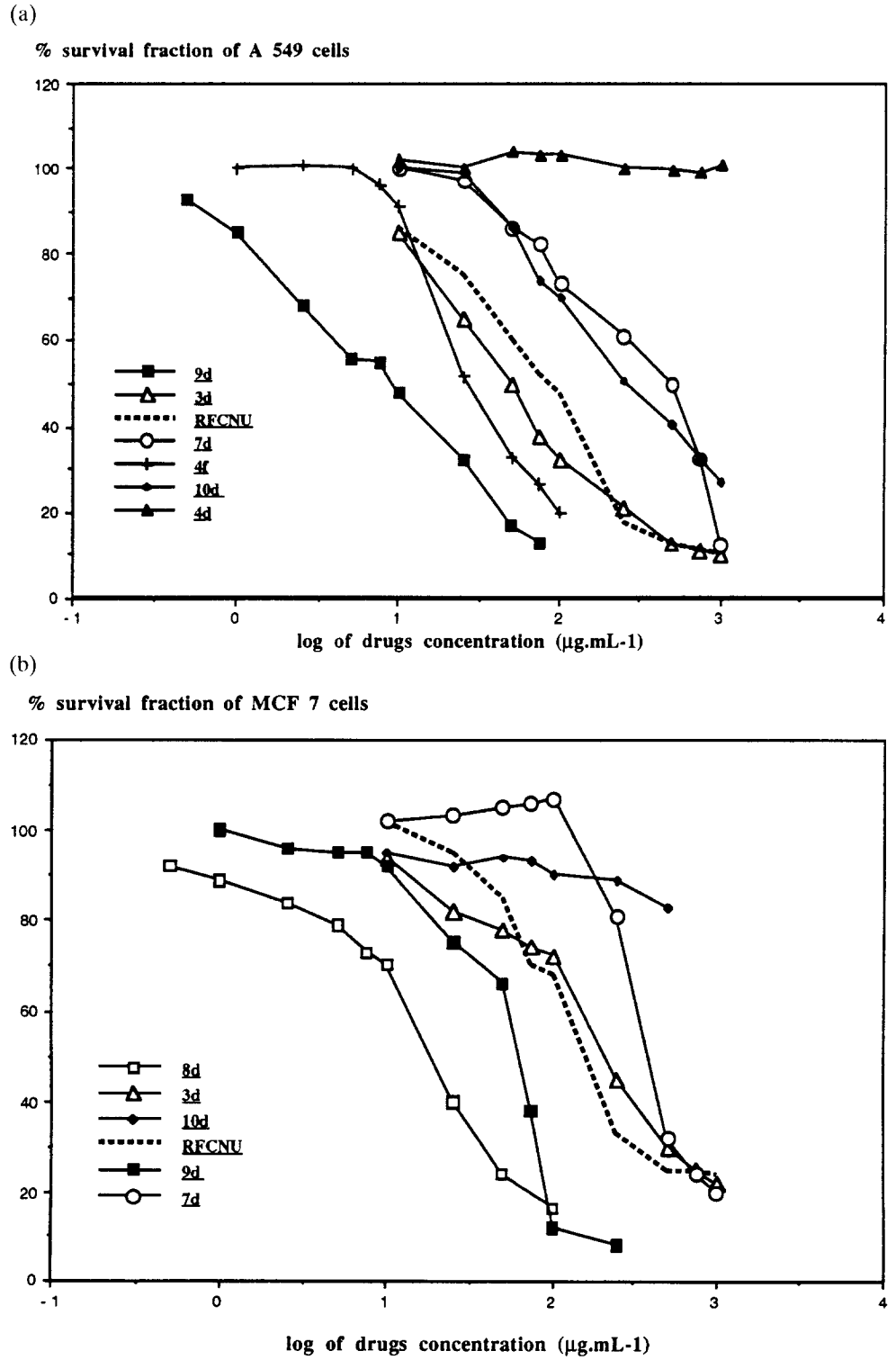


Figure 2. Cytotoxicity results.

Table 5. Cytotoxic features CENS vs. RFCNU towards tumoral lines

	+++	+	±	−	0
A549 (IC_{50})	**9d** (8 μg/mL)	**4f** (25 μg/mL) **3d** (25 μg/mL)	Ref (80 μg/mL)	**10d** (250 μg/mL) **7d** (500 μg/mL)	**4d** (inactive)
MCF 7 (IC_{50})	**8d** (30 μg/mL)	**9d** (60 μg/mL)	Ref (160 μg/mL) **3d** (200 μg/mL)	**7d** (400 μg/mL)	**10d** (inactive)

Pharmacology

Materials and reagents. RPMI 1640, phosphate-buffer saline (PBS), Hepes, $NaHCO_3$ and gentamycin were obtained from Seromed. 3-Methyl-(dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT), dimethylsulfoxide, trypsin EDTA and D-glucose were purchased from Sigma Chemicals. Culture flasks and 96 multiwell plates for MTT tests were purchased from Nunc Lab.

A549 and MCF7 cells were obtained from the American Type Culture Collection at the 73th and 143th passages, respectively (Rockville, MD; ATCC CLL 185).

Cell culture. The cells were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum containing 3.5 g/L of D-glucose, 2.38 g/L of Hepes, 2 g/L of $NaHCO_3$ and gentamycin 0.01% w/v. The cells were kept in a humidified incubator with 5% CO_2. Under these conditions, the doubling time of the cell culture was 24 h for A549 and 30 h for MCF7 cells. The cells were routinely subcultured once a week.

Cell cytotoxicity assay. The CENS were dissolved in DMSO and then added culture medium to obtain the desired drug concentration. The DMSO concentration did not exceed 2% (v/v) of the culture medium.

The cells were harvested from culture and maintained in exponential growth phase. Cell suspensions were distributed into 96-well culture plates at 500 cells:well. After 48 h of incubation, 0.05 mL of nitrososulfamide solution at the indicated concentration was added in 0.15 mL of culture medium per well. For the control cell, 0.05 mL of culture medium supplemented with 0.2% DMSO (v/v) was added. The plates were then incubated for 4 h at 37 °C and the CENS-containing media were removed. Then the cells were resuspended in 0.2 mL of fresh medium and further incubated for 72 h at 37 °C. Cell survival was measured by MTT as previously described with slight modifications. Briefly, 0.05 mL of MTT solution at 1 mg/mL in PBS was added in each well and the cells were incubated for 4 h at 37 °C. The medium was removed and 0.15 mL of DMSO per well. The plates were then agitated on an orbital shaker for 30 min to solubilize the formazan grain. The absorbance of each well was measured at 540 nm with a microplate reader (Titerpek Uniskan). Data were collected from six similarly treated wells and the toxicity was defined as the surival fractions of the cells that was determined by the formula: survival fraction = A of treated cells/A of control cells.

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