**Acknowledgment**. Support for this study was provided by grants from the Swedish Natural Science Research Council, the Swedish Board for Technical Development, the Swedish Medical Research Council (Grant 14X-502), and Astra Research Centre.

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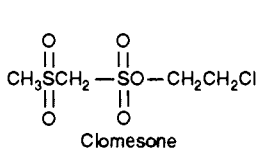
*Received December 13, 1989*

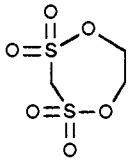
## Phenyl Selenones: Alkyl Transfer by Selenium–Carbon Bond Cleavage[1]

*Sir:*

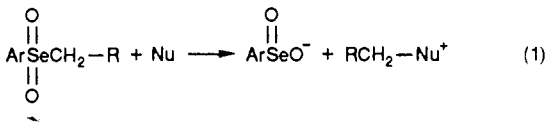
Systematic chemical modifications of nitrosoureas and triazenes led to the discovery of 2-chloroethylating antitumor agents. This class of cross-linking agents includes BCNU (carmustine), CCNU (lomustine), MeCCNU (semustine), PCNU, BIC, and MCIC.[2] They are highly active in vivo against a broad range of murine neoplasms, but have demonstrated relatively narrow clinical activity. Clomesone and Cyclodisone, derivatives of sulfonates, are examples of bifunctional 2-chloroethyl derivatives currently under active development.[3] Both have shown broad-spectrum anticancer activities and unique biological activities.[3,4]



Clomesone


Cyclodisone

Organoselenones (Se:VI) are known to undergo nucleophilic displacements, yielding seleninates and alkyl–nucleophile adducts as shown in eq 1.[5] The characteristically



(1)

high nucleophilic selectivities of organoselenones that we describe in the present report was implicit in the observations that methyl phenyl selenone is about 3 times as reactive as methyl iodide toward dimethyl sulfide,[5b] and that decyl phenyl sulfide is isolated as the sole reaction product in the treatment of decyl phenyl selenone and nonyl bromide or iodide with sodium thiophenolate in ethanolic solution.[6] These findings indicated a potential of organoselenones as biological alkylating agents. By contrast, organosulfones, analogues of selenones, generally exhibit high chemical and thermal stability,[7] and the bond cleavage between sulfur and carbon in a sulfone takes place only under exceptional circumstances.

Recently, we reported[8] the synthesis, kinetic behavior, and cytotoxicity of alkylating organoselenides, isosteres of classical nitrogen and sulfur mustards. Despite the high polarizability of the selenium atom, however, and the expectation of increased nucleophilic selectivities,[9] this class showed generally low Swain–Scott $s$ constants (with some exceptions), perhaps resulting from overly high reactivities of the ethyleneselenonium ion intermediates resulting in excessive hydrolysis. In addition, the aqueous solubility of this series was low. To date, however, there has been no reported application of organoselenone chemistry to any drug design including cross-linking antitumor agents.

The anticipation of high nucleophilic selectivity among organoselenones was particularly attractive, in view of the fact of broad antitumor activities of ethylenimines and platinating agents, which are highly selective, in contrast to nitrosoureas, which are not[9a] and have a narrow spectrum of clinical activity. We now describe alkylating organoselenones, in which the selenone moiety acts as a leaving group via Se–C breakage, that have desirable properties of slowed reactivity (compared to selenides), high selectivity (AA), and short cross-linking distance (similar to cisplatin).

Table I presents the results of chemical kinetic parameters and antiproliferative activities of a sulfone and aryl haloalkyl selenones 1–6 and closely related nitrogen, sulfur, and selenium compounds. In the alkylation reactions of 1–6, 4-(4-nitrobenzyl)pyridine (NBP) was used as a model biologic nucleophile that somewhat resembles the N7 site of guanine.[9a] The reactions were carried out at 37 °C in aqueous acetone in the presence of Tris-HCl buffer at pH 7.4 as described in the previous report.[7] Experimental first-order rate constants, $k'_{nbp}$, were obtained from the plots of log (percent remaining alkylating species) vs time, where NBP was present in pseudo-first-order excess. AA is a parameter of nucleophilic selectivity, which is the

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**Table I.** Chemical Kinetic Parameters and Cytotoxicity Studies of Aryl Haloalkyl Selenones and Closely Related Compounds

| no. | p-X-$C_6H_4M(O_2)CH_2CH_2Y$ X | M | Y | $n^a$ | $10^3k'_{nbp}$,[b] $min^{-1}$ | $T_{1/2}$,[c] h | AA,[d] % | $IC_{50}$,[e] μM L1210 | CCRF-CEM |
|---|---|---|---|---|---|---|---|---|---|
| 1 | H | S | Cl | 1 | <0.12 | >96 | >90 | 2.3 | 2.25 |
| 2 | H | Se | H | 1 | 2.48 | 4.67 | 75 | 5.0 | 10.0 |
| 3 | H | Se | Cl | 1 | 1.38 | 8.37 | 132 | 0.55 (0.51[f]) | 0.15 |
| 4 | $NO_2$ | Se | Cl | 1 | 10.38 | 1.08 | 140 | 0.9 | 0.4 |
| 5 | $OCH_3$ | Se | Cl | 1 | 1.25 | 9.25 | 100 | 0.36 (0.31[f]) | 0.14 |
| 6 | H | Se | $CH_2OMs$ | 1 | 1.21 | 9.55 | 74 | 1.2 (2.5[f]) | 0.9 |
| 7 | $C_6H_5SeCH_2CH_2Cl$[g] | | | 1 | 371 | 0.031 | 23 | | 8.0 |
| 8 | $(ClCH_2CH_2)_2Se$[g] | | | 2 | 2865 | 0.004 | 22 | | 0.75 |
| 9 | $C_6H_5SeO_2H$ | | | 0 | | | | 3.0 | 6.0 |
| 10 | Clomesone | | | 1 | 1.23 | 9.40 | 87 | 3.0[h] (2.2[f]) | |
| 11 | Chlorambucil | | | 2 | 1.49 | 7.75 | 108 | | |

[a] The number of alkylating equivalents/mole based on the actual number of alkylating group(s) showing significant reaction with 4-(4-nitrobenzyl)pyridine (NBP). [b] Pseudo-first-order rate constants of NBP alkylation at 37 °C in aqueous acetone (52.2:47.8, v/v) containing 40.6 mM NBP, 17.4 mM Tris-HCl buffer, pH 7.4, and 0.087 or 0.174 mM alkylating agent. [c] Half-lives of NBP alkylation. [d] AA (%) is the percentage of the extent of NBP alkylation (absorbance at 560 nm of the final alkyl–NBP adduct with triethylamine alkalinization) at the end of the reaction relative to mechlorethamine hydrochloride (=100%), correction made for the number of alkylating equivalents/mole, *n*. [e] Drug concentration required to express 50% growth inhibition against L1210 murine leukemic cells and CCRF-CEM human lymphoblast cells in vitro after 48 h incubation. [f] $IC_{50}$ against L1210/L-PAM, L1210 resistant to L-PAM. [g] From ref 8. [h] 3.6 μM from ref 4b.

relative preference of an electrophilic aliphatic species toward the stronger of two (or more) nucleophiles in competition reactions.[8,9a] AA (%) is specifically defined as the percentage of the extent of the alkyl–NBP adduct formed at the end of the alkylation (absorbance at 560 nm of the alkyl–NBP product with triethylamine alkalinization) relative to mechlorethamine hydrochloride (=100%), with correction made for the number (*n*) of alkylating equivalents/mole. The *n* number assigned to each compound was based on the number of alkylating group(s) showing significant reaction with NBP. That is why, for example, *n* = 1 was given to the selenones **3–6** and Clomesone (**11**), which are bifunctional in structure. Since $k'_{nbp}$ for $ArSeO_2$ ≫ $k'_{nbp}$ for Cl, where the two terms are reactivities of arylselenones and chloride leaving groups toward NBP, AA (%) due to C–Cl scission was concluded to have contributed less than 10% to the observed AAs of **3–5**. Cell culture studies of **1–10** on murine leukemic cell lines, L1210 and L1210/PAM, and a human leukemic lymphoblastoid CCRF-CEM line followed the literature procedure,[8] except that 0.9% saline was used instead of DMSO to dissolve the test compounds.

Synthesis of the organoselenones **2–6** was achieved by oxidations of the corresponding selenides at room temperature.[10] The preparative methods for 2-chloroethyl and 3-mesylpropyl selenides were reported previously.[8,11] The oxidants were potassium permanganate in water for **2** in methylene chloride and *m*-chloroperbenzoic acid (mCPBA) in ether for **3–6** in dichloromethane.[10] The oxidation reactions of selenides into selenones gave less than 60% yields isolated from silica gel column chromatography. This is due primarily to the difficulty of oxidizing the selenium atom in monooxidized selenoxides,[10] as well as to the complication that selenoxide intermediates are prone to syn elimination forming an alkene and a selenenic acid.[12] Although methanol would seem to be suitable as the reaction solvent,[13] its nucleophilicity toward selenones in the presence of mCPBA[6] and methanolysis with 2-chloroethyl selenides limited its use.

In the data of NBP alkylation reactions in Table I, clear distinctions between 2-chloroethyl phenyl sulfone (**1**) and the selenone analogue **3** were found in terms of reactivity and probable reaction mechanisms. This suggests that the sulfone **1** undergoes nucleophilic substitution via C–Cl cleavage because of the similarity of its $k'_{nbp}$ to that of simple alkyl chlorides. The selenone **3** with a half-life of 502 min and 132% of AA for the NBP alkylation instead reacts by Se–C breakage. This derives from the fact that ethyl phenyl selenone (**2**) with no classical alkylating group produced 75% AA with a half-life of 280 min, confirming the chemistry of aryl selenones as electrophilic substrates. The selenones **3**, **5**, **6** showed alkylating reactivities, in terms of both rates and extents (AA) of alkylation comparable to those of Clomesone (**10**) and Chlorambucil (**11**). In the case of 2-chloroethyl 4-nitrophenyl selenone (**4**), reactivity was approximately 8 times greater than either the unsubstituted analogue **3** or Clomesone. This accelerating electronic effect of the *p*-nitro group in the series of the selenones **3–5** is opposite to that observed for the selenides, analogues of **7**, in the same NBP alkylation reactions.[8,11]

The possibly greater reactivities and nucleophilic selectivities of **3** and **4**, over **5**, argues in favor of initial charge separation at the Se-C bond: positive selectivity-reactivity relationships have been observed in examples of unimolecular reactions in which carbonium ion formation is rate limiting.[14] This is certainly also the case for mustard-type agents that in general are highly selective and act initially through internal nucleophilic displacement to produce their charged, highly reactive intermediates. The aquation reactions of cisplatin are analogous. A positive reactivity-selectivity relationship may also be anticipated because the properties of nucleophilicity and nucleophilic selectivity (electrophilicity) are highly correlated with thermodynamic parameters.[9b,c] The high AAs of **3–5** relative to that of classical (2-chloroethyl)nitrosoureas implies the expectation of greater alkylation of the strongly nucleophilic N7 position of guanine of DNA and, at the same time, less competing reactions on the weakly nucleophilic O6 site of guanine and other, possibly extraneous intracellular nucleophilic sites.[9]

In vitro testing of **3–5**, with high AA values, indicated potent antiproliferative activities against L1210, L1210/L-PAM, and CCRF-CEM cell lines. All $IC_{50}$ values (μM) of the selenones **3–5** were less than 1.0, while Clomesone

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Table II. Antitumor Activity of 2-Chloroethyl Phenyl Selenone (3): Survey of in Vitro Cytotoxicities against Human Cell Lines

| tumor type | NCI screen[a] (NSC 619534) | av $IC_{50}$, $\mu M \pm SD$ | (range) |
|---|---|---|---|
| leukemia | CCRF-CEM,[a] CCRF-SB, MOLT-4, RPMI 8226, K-562 | 0.45 ± 0.14 | (0.32–0.63) |
| colon | COLO 320 DM, HCT-15, COLO 205, HCT 116, HCC 2998, KM20L2, KM12 | 2.3 ± 1.6 | (0.8–5.0) |
| melanoma | LOX, SK-MEL-2, SK-MEL-28, SK-MEL-5, UACC-257, M19-MEL, Malme-3M, UACC-62 | 3.7 ± 2.3 | (1.3–7.9) |
| NSCLC[b] | H23, H460, HOP-18, HOP-19, HOP-62 | 4.3 ± 2.0 | (1.0–6.3) |
| renal | SN12 KI, SN12 C, UO-31, A498 | 4.6 ± 4.0 | (1.3–10.0) |
| CNS | TE671, SF-268, SF-295, U251, SNB19, SNB78, SF-539 | 6.0 ± 5.2 | (0.8–15.8) |
| ovarian | A2780, OVCAR5, SKOV-3, IGROVI | 7.8 ± 6.6 | (0.4–15.8) |

| tumor type | BACTEC screen[c] |
|---|---|
| NSCLC | SKLU 1 (0.06 μM), SKMES 1 (0.15 μM), CALU 3 (2.2 μM) |
| colon | COLO 320 (1.2 μM), HT29 (1.9 μM), OM-1 (>4 μM) |
| breast | Hs578t (0.9 μM), MCF-7M (>4 μM) |

[a] Using a modified, automated nitrotetrazolium spectrophotometric assay of cell viability. Cell lines are listed in order of decreasing sensitivity. [b] NSCLC = non-small cell lung carcinoma. [c] Using $^{14}CO_2$ production from [$^{14}$C]glucose; 1-h drug exposure. Data obtained by Dr. Daniel Von Hoff, University of Texas Health Science Center at San Antonio.

exhibited values of 3.0 (3.6[4b]) and 2.2 μM on L1210 and L1210/L-PAM, respectively.

In Table II are summarized the results of NCI in vitro screening of 3 (NSC 619534) by the nitrotetrazolium assay of cell viability (Dr. Nathaniel H. Greenberg, personal communication). Leukemias clearly are more sensitive than other cell types, as found for classical cross-linking alkylating agents. In a separate evaluation using $^{14}CO_2$ production (BACTEC) as an indicator of viability, however, 3 showed low IC50s against 2 of 3 human lung cancer lines (Dr. D. Van Hoff, personal communication).

It is of interest that the organoselenones against L1210 and CCRF/CEM cell lines appeared to show greater cytotoxicities with *slowed* alkylating reactivities. A plot of $IC_{50}$ vs $\sigma_p$ (Figure 1) shows parallelism for the two cell lines, in which $r$ and slope values were 0.974 and 0.36 for L1210 cells, and 0.982 and 0.46 for CCRF-CEM cells. The slopes in Figure 1 suggest that both human and murine leukemic cell lines expressed similar relative sensitivities to the organoselenones. A notable finding was that the selenones in cell culture studies retained essentially undiminished sensitivity to L1210/L-PAM cells, a L1210 subline resistant to L-PAM. Such a property of a lack of cross-resistance to L-PAM makes these Se-based haloethylating agents worthy of further development, considering that cyclophosphamide and ciplatin have limited and complete cross-resistance to L1210/L-PAM, respectively.[16] In the case of 3-(mesyloxy)propyl phenyl selenenone (6) carrying

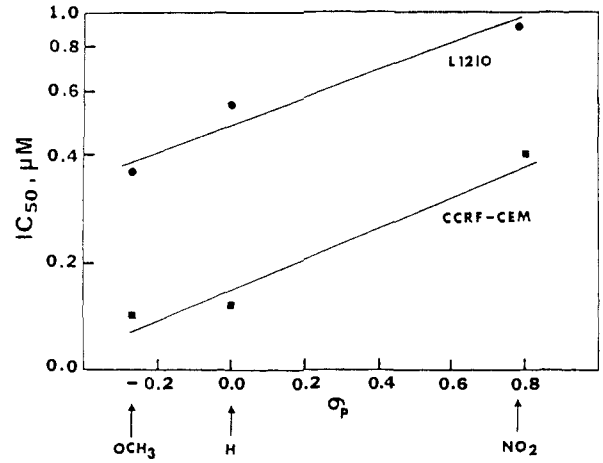


**Figure 1.** Correlation of molar antiproliferative activities ($IC_{50}$, μM) and Hammett constants ($\sigma_p$) of para-substituted aryl 2-chloroethyl selenones 3–5 against L1210 (●) and CCRF-CEM (■) cells.

an *n*-propyl alkylating arm, less potent cytotoxicity than the 2-chloroethylating selenones 3–5 and cross-resistance to L1210/L-PAM were found despite the comparable NBP alkylating reactivity. This less effective antiproliferative activity of the *n*-propyl analogue 6 appears to be consistent with other observations of increased halo side chain length causing reductions in biological activity.[17]

In the disk-diffusion colony formation assay (Dr. T. H. Corbett, Wayne State University) of the 2-chloroethyl selenones 3–5 on L1210, mouse colon adenocarcinoma #7, human colon HCT8, and a normal-like cell (IMC), the selenones were found to be similarly cytotoxic against all lines, with a shallow dose–response that is characteristic of clinically effective agents.

It is known that enormous differences exist in the toxicities of selenium compounds depending on the oxidation states of the Se atom. Selenites, $Se(IV)O_3^{2-}$, or selenates, $Se(VI)O_4^{2-}$, are far more toxic than selenides containing bivalent selenium.[11,18] In agreement with this fact, a comparison between 2-chloroethyl phenyl selenone (3) and selenide 7 reveals the markedly altered chemical and biological activities between the two oxidation states. The half-life, AA, and $IC_{50}$ of 112 s, 23%, and 8.0 μM for the selenide 7 contrast with 502 min, 132%, and 0.15 μM for the selenone 3. This is attributable to "umpolung"[19] that reversed the nucleophilic Se in 7 into the electrophilic Se in 3. This umpolung solves the valency restriction problem encountered in organoselenium alkylating agent development that requires two selenium atoms to make bifunctional mustard-like alkylating agents, with the exception of bis(2-chloroethyl) selenide 8.[8,11] Compound 8 was found to be extremely reactive and cytotoxic as presented in Table I.[8]

Since benzeneseleninate is a highly probable metabolite, as given in eq 1, benzeneselenic acid (9) was evaluated in L1210 and CCRF-CEM cell lines. The $pK_a$ of 9 is 4.70, slightly weaker than benzoic acid (4.20).[20] The $IC_{50}$ data of 9 demonstrate that the seleninate metabolite is not responsible for the enhanced cytotoxicity of 3 nor possibly,

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in the case of 4 and 5. However, the $IC_{50}$ of 9 in L1210 cells was equicytotoxic to Clomesone.[4b]

In summary, we have identified alkylating organoselenones as potential lead compounds for development as potential antitumor agents, based on their novel selenium–carbon bond cleavage, useful reactivities, good solubilities, high nucleophilic selectivities, and highly potent antiproliferative activities against human and murine leukemic cell lines. Noteworthy is the finding of a lack of cross-resistance to L1210/L-PAM by 2-chloroethyl arylselenones. An areneseleninate, a probable metabolite from an arylselenone, did not account for the enhanced cell growth inhibition, which is probably related to cross-link formation.

**Acknowledgment**. We are indebted to Thomas H. Corbett at Wayne State University for the disk diffusion assay, to Daniel Von Hoff for the BACTEC screen, and to Daniel P. Griswold, Jr., at the Southern Research Institute for the L1210 cell lines.

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## Naltrindole 5′-Isothiocyanate: A Nonequilibrium, Highly Selective δ Opioid Receptor Antagonist

*Sir:*

Opioid ligands are among the best-known classes of physiologically active agents that interact with multiple subpopulations of opioid receptors.[1,2] Receptors that recognize such ligands have been categorized into three major types, the best documented of which are named μ, κ, and δ. The enkephalins, endorphins, and dynorphins represent some of the classes of the endogenous peptides that interact with these multiple receptor types.

Efforts to develop opioid receptor affinity labels (nonequilibrium opioid ligands) have been pursued as biochemical and pharmacologic probes to address the problem of receptor multiplicity and receptor isolation.[3] However, until very recently there were no reports of nonequilibrium, δ-selective opioid receptor antagonists that are active in vivo. Ligands such as fentanyl isothiocyanate (FIT) and its 3-methyl analogue (SUPERFIT) are useful affinity labels, but they display no nonequilibrium antagonist activity in vivo.[4] A recently designed affinity label, [D-Ala$^2$,Leu$^5$,Cys$^6$]enkephalin (DALCE), has been reported to bind covalently to δ receptors by a thiol–disulfide exchange mechanism and it appears to possess a pharmacologic profile in vivo consistent with nonequilibrium δ opioid receptor antagonism.[5,6]

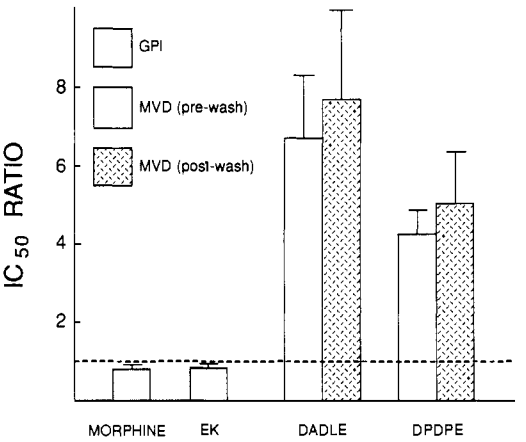
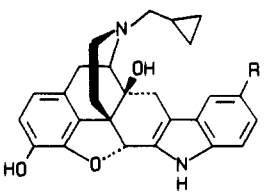



**Figure 1.** The antagonism of μ-, κ-, and δ-selective agonists by NTII (100 nM) in GPI and MVD preparations. Morphine and ethylketazocine (EK) were tested in the GPI, and the δ-selective ligands (DADLE and DPDPE) were evaluated in the MVD. The prewash $IC_{50}$ values were obtained by determining the agonist $IC_{50}$ in the presence of NTII prior to washing. Postwash $IC_{50}$ values were obtained by thoroughly washing the preparation that was incubated with NTII and then determining the agonist $IC_{50}$. The $IC_{50}$ ratio was calculated by dividing the $IC_{50}$ of the standard agonist in the NTII-treated preparation by the $IC_{50}$ of the agonist alone in the same tissue. The dashed line represents a ratio of 1 (no change).

Here we report on the design and synthesis of the first nonpeptide, nonequilibrium δ opioid receptor antagonist, naltrindole 5′-isothiocyanate (1, NTII), that exhibits high pharmacologic selectivity both in vitro and in vivo.




1 R = N=C=S
2 R = H
3 R = $NO_2$
4 R = $NH_2$

The design of NTII (1) was based on the attachment of the isothiocyanate group to naltrindole[7] (2, NTI), a highly selective, reversible δ opioid receptor antagonist. The isothiocyanate group is capable of reacting under physiologic conditions with $NH_2$, imidazole, and thiol groups.[8] Its reactivity with water or hydroxyl groups is negligible. The isothiocyanate group has been employed successfully in the design of a number of opioid receptor affinity labels.[3]

Target compound 1 was prepared by reacting naltrexone with (*p*-nitrophenyl)hydrazine to form 5′-nitroindole 3, which was reduced to 5′-amino derivative 4 and then treated with thiophosgene.[9]

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