Structural studies on the inactivation of γ -glutamylcysteine synthetase by the disulphide analogues of radioprotective cysteamine derivatives

Effects of aminoalkyl and hydroxyalkyl chain length and $\beta\beta$ -bis-dimethylation

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Disulphide compounds have been shown to inactivate γ -glutamylcysteine synthetase, the rate-limiting enzyme for GSH synthesis. Such compounds bind to a cysteine residue at or near the glutamate-binding site of the enzyme. This phenomenon is thought to be responsible for the synergistic toxicity of the thiophosphate radio- and chemo-protective agent WR2721 and the oxygen-radical generator 6-hydroxydopamine (2,4,6-trihydroxyphenethylamine). 6-Hydroxydopamine enhances conversion of WR2721 into its disulphide metabolite *NN'*-bis-(3-aminopropyl)cystamine, which, in turn, paralyzes the synthetase. In an effort to identify radio- and chemo-protective thiols and thiol derivatives that do not have this toxicity, we have begun to define the structure–activity relationship that governs inactivation of the enzyme by analogues of WR2721 disulphide. *NN'*-Bis(aminoalkyl)cystamines and bis(hydroxyalkyl)cystamines with an alkyl chain length of C_5 or greater are not inactivators of the synthetase. That this is not due solely to the size of these compounds is shown by the potent inactivation of the enzyme by SAPH3 disulphide, an extremely bulky cystamine analogue. $\beta\beta$ -Bis-dimethylation of the cystamine portion of the molecule also obviates inactivation. This is almost certainly due to steric interference with disulphide interchange. These findings may facilitate the safe adjunctive use of the thiol counterparts of such compounds with oxygen-radical-generating chemotherapeutic agents, and may shed light on the structure of the region of the synthetase adjacent to the glutamate-binding site.

INTRODUCTION

γ -Glutamylcysteine synthetase is the rate-limiting enzyme for GSH synthesis. Inactivation of this enzyme by disulphide compounds was first described for cystamine by Griffith *et al.* (1977). They reported the covalent nature of the inactivation, and its reversibility by dithiothreitol. Both L-glutamate and Mg^{2+} protected the synthetase from disulphide-induced inactivation (Seelig & Meister, 1982). These findings suggested that the inactivation took place by disulphide interchange, presumably with a cysteine residue, in or near the glutamate-binding site. Studies involving the use of cystamine analogues have demonstrated that the moiety ‘recognized’ by the enzyme in the inactivation process is most probably $H_3N^+-CH_2-CH_2-SS-$ (Griffith *et al.*, 1977; Seelig & Meister, 1984).

Aminoalkyl- and hydroxyalkyl-cystamines and their analogues have been proposed for adjunctive use as radio- and chemo-protective agents in cancer patients (Sweeney, 1979; Glick *et al.*, 1984). We have previously reported the synergistic toxicity of one of these agents, WR2721, with the oxygen-radical generator, 6-hydroxydopamine (2,4,6-trihydroxyphenethylamine) (Schor, 1987). This toxic effect of the two drugs used in concert appears to be due to the potentiation of the formation of the disulphide metabolite of WR2721, *NN'*-bis-(3-aminopropyl)-cystamine (Schor, 1988). Aminopropylcystamine is a potent inactivator of γ -glutamylcysteine synthetase. In hopes of identifying aminoalkyl- and hydroxyalkyl-cystamines that could be safely used in conjunction with oxygen-radical-generating chemotherapeutic agents, we have set about defining the struc-

tural characteristics of the disulphide metabolites of these agents (aminoalkyl- and hydroxyalkyl-cystamines respectively) that determine their potency as inactivators of the synthetase. In the present paper we discuss the effects of aminoalkyl and hydroxyalkyl chain length and the C-methylation of the cystamine portion of these molecules upon inactivation of γ -glutamylcysteine synthetase by the disulphide metabolites of proposed radioprotective compounds.

EXPERIMENTAL

Materials

All reagents needed for isolation and assay of γ -glutamylcysteine synthetase were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Whatman DE52 DEAE-cellulose resin was obtained from Fisher Scientific Co. (Pittsburgh, PA, U.S.A.). Agarose-linked ATP resin (*N*⁶-linked, C_8 spacer) was obtained from Sigma Chemical Co.

All compounds designated with a WR number were obtained from the Walter Reed Army Institute of Research (Bethesda, MD, U.S.A.). Their identity and purity were confirmed by n.m.r. spectroscopy. SAPH3 was synthesized and its identity and purity were assessed by the procedure of Lomis *et al.* (1988). SAPH3 and thiol compounds of the WR series were converted into their corresponding disulphides by incubation in room air at 37 °C and pH 7.4 for 18–24 h. Quantitative conversion of each thiol compound to its disulphide analogue was confirmed in each case by the use of 5,5'-dithiobis-(2-nitrobenzoic acid), as we have previously described for WR2721 (Schor, 1988). The bis-dimethyl analogues of cystamine and SAPH3 disulphide were prepared by

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Fig. 1. Structures of the cystamine and cysteamine derivatives used in the present studies

Compounds designated 'WR' were obtained from the Walter Reed Army Institute of Research. For studies of inactivation of γ -glutamylcysteine synthetase, all non-disulphide compounds were converted into their respective disulphide analogues as described in the text.

H_2O_2 oxidation of the $\beta\beta$ -dimethyl analogues of cysteamine and SAPH3 respectively. Identity and purity of these compounds were verified by i.r. spectroscopy, n.m.r. spectroscopy and m.s. The compounds were tested for inactivation of the synthetase as the hydrochloride salts. The structures of all of these compounds are shown in Fig. 1.

Male A/J mice (5–7 weeks old) were obtained from Jackson Laboratories (Bar Harbor, ME, U.S.A.). Except where noted, A/J mice were the source of enzyme for all experiments described in this paper. Male BALB/c mice (5–7 weeks old) were obtained from Zivic Miller Laboratories (Zelienople, PA, U.S.A.), and were used in one set of experiments described at the end of the Results section. All animals were housed five to a cage and fed on Wayne Rodent Blox (Wayne Research Animal Diets, Wayne, OH, U.S.A.) and water *ad libitum*.

Methods

Isolation of γ -glutamylcysteine synthetase from mouse liver. Mice were killed by cervical dislocation. The synthetase was partially purified from 100 mouse livers (approx. 1 g of liver per mouse) by the procedure of Seelig & Meister (1985), which involves $(\text{NH}_4)_2\text{SO}_4$ precipitation of the enzyme from the homogenate followed by DEAE-cellulose column chromatography. The resulting enzyme contained approx. 150 units ($\mu\text{mol/h}$) of synthetase activity/mg of protein, and was designated 'partially purified enzyme'.

For isolation of the 'pure' enzyme, the partially purified enzyme was applied to and eluted from an ATP-agarose column (Seelig & Meister, 1985). Column fractions were immediately assayed for activity, and active fractions were rapidly pooled and dialysed into 50 mM-Tris/HCl buffer, pH 7.4, to avoid inactivation

of the enzyme by Mops in the column elution buffer. The resulting enzyme contained approx. 1500 units of synthetase activity/mg of protein. Purification of the enzyme from 100 mouse livers resulted in the isolation of 30–40 units of enzyme.

Assay of γ -glutamylcysteine synthetase activity. Except where noted, synthetase activity was assessed by the assay method of Seelig & Meister (1985), which couples the generation of ADP by the synthetase to the production of NAD^+ by the actions of pyruvate kinase and lactate dehydrogenase. All assays were performed in parallel with a blank containing all of the assay components except α -aminobutyric acid, to control for the presence of non-specific ATPases in the enzyme preparation. In assays involving inactivation of the enzyme by disulphide compounds, approx. 1 unit of enzyme was used for each sample and each blank.

Because the NAD^+ -linked assay required the presence of non-substrate substances that could have interacted in some unforeseen way with the compounds being tested, or with the partially purified enzyme, we have devised a more specific assay for confirmation of direct inactivation of the synthetase by disulphide compounds. This assay was performed with 0.6 unit of γ -glutamylcysteine synthetase in 100 mM-Tris/HCl buffer, pH 8.0, containing 20 mM- MgCl_2 , 150 mM-KCl, 2 mM-EDTA, 1 mM-L-glutamic acid, 1 mM- α -aminobutyric acid and 5 mM-ATP. The reaction was stopped after 15 h by immersion in a boiling-water bath to precipitate the enzyme. Production of γ -glutamyl- α -aminobutyrate was demonstrated by amino acid analysis on a standard amino acid analyser program (Beckman). The colour constant of the dipeptide was 1.03 relative to that of lysine. The compound was eluted at 7.5 min, and was easily resolved from glutamate (12.5 min) and α -aminobutyrate (22.5 min).

Treatment of γ -glutamylcysteine synthetase with putative inactivators. Each reaction mixture (400 μl) contained 1 unit of enzyme and the compound being tested (0.025–1 mM) in 50 mM-Tris/HCl buffer, pH 7.4. For each assay, two reaction mixtures were prepared, one for the sample cuvette and one for the blank cuvette. All assays were performed in duplicate. In addition, all experiments contained a control assay containing enzyme alone. Control and experimental reaction mixtures were incubated at 37 °C for 15 min, conditions used by previous authors in studying inactivation of the synthetase by other compounds (Griffith *et al.*, 1977; Schor, 1988). The samples were placed on ice, and immediately assayed for synthetase activity as described above. Addition of the incubation mixture to the assay solution containing Mg^{2+} and L-glutamate prevents continuation of the inactivation reaction beyond the 15 min of incubation (Seelig & Meister, 1982; Schor, 1988).

RESULTS

Equivalence of partially purified and pure enzyme with respect to inactivation of the synthetase by disulphide compounds

The complete purification of γ -glutamylcysteine synthetase from mouse liver resulted in a 10-fold increase in specific activity, but a 5-fold loss of yield over the partially purified enzyme. As is shown in Fig. 2, there was no significant difference ($P > 0.05$; Student's *t* test) between the partially purified and pure enzymes when the degree of inactivation by various WR-series compounds was measured by the NAD^+ -linked assay. All of the results presented in this paper were generated with one or other of these preparations. For completeness, the Figure and Table legends indicate which of these preparations was used for each set of experiments.

Fig. 2. Comparison of the degree of inactivation of partially purified (▨) and pure (■) γ -glutamylcysteine synthetase by various compounds in the WR series

All agents (1 mM) were incubated with the enzyme (1 unit) for 15 min at 37 °C. Residual activity after incubation with each compound is shown as the percentage of the activity of a control sample of enzyme incubated under the same conditions. Except where noted, in this and all subsequent Figures the mean and standard deviation are shown.

Fig. 3. Inactivation of the synthetase by WR2721 disulphide as demonstrated by direct determination of the formation of γ -glutamyl- α -aminobutyric acid in the presence of the enzyme

Residual activity after incubation of the enzyme (1 unit; partially purified) with WR2721 disulphide is shown as the percentage of the activity of a control sample of enzyme incubated under the same conditions. This assay eliminates the possibility that apparent inactivation of the synthetase might be due to the presence of 'linked' substrates in the conventional assay.

Direct demonstration of synthetase inactivation by disulphide compounds in the absence of linked substrates

Because the NAD⁺-linked assay is an indirect measure of synthetase activity, it is possible that the disulphide compounds interfere with the assay, rather than truly inactivating the synthetase itself. We therefore devised a direct assay for the dipeptide product of the synthetase reaction by using amino acid analysis, to demonstrate the specificity and robustness of this phenomenon.

Fig. 3 shows the concentration-dependence of inactivation of the synthetase by *NN*'-bis-(3-aminopropyl)cystamine, the disulphide metabolite of WR2721, as assessed by using the direct

Fig. 4. Effect of alkyl chain length upon inactivation of γ -glutamylcysteine synthetase by hydroxyalkylcystamines (○) and aminoalkylcystamines (●)

All compounds were added at a concentration of 1 mM, and incubated with the enzyme (1 unit; pure) at 37 °C for 15 min. Residual activity after incubation with each compound is shown as the percentage of the activity of a control sample of enzyme incubated under the same conditions. Cystamine itself (▨) is shown on this graph as having a 'chain length' of zero.

assay. These results cannot be compared quantitatively with the indirect assay, because the lengthy reaction time required for the direct assay is beyond the maximum time for linear kinetic progress of the reaction. They do, however, unequivocally demonstrate that the synthetase reaction itself, and not the linked reactions of the indirect assay, is blocked in a concentration-dependent fashion by the presence of aminopropylcystamine in the preincubation mixture.

Effect of alkyl chain length upon inactivation of γ -glutamylcysteine synthetase by aminoalkyl- and hydroxyalkylcystamines

Our previous studies had shown inactivation of the synthetase by aminopropylcystamine at concentrations that were attainable *in vivo* (Schor, 1988). In an effort to identify compounds that do not inactivate the synthetase, we examined the effect of alkyl chain length upon the synthetase inactivation by other aminoalkyl and hydroxyalkyl derivatives of cystamine.

Increasing alkyl chain length decreases the degree of inactivation of the synthetase caused by incubation with these compounds at a concentration of 1 mM (Fig. 4). The results are similar for the aminoalkyl and hydroxyalkyl compounds. Although not strictly of the same structural class, the results for cystamine are plotted in Fig. 4 as a chain length of zero.

In order to test whether the size alone of the longer-chain-length compounds precluded their binding to the synthetase, we examined the effects of SAPH3 disulphide, an extremely bulky cystamine analogue, upon synthetase activity. Fig. 5 (● symbols) shows the concentration-dependence of enzyme inactivation by SAPH3 disulphide. SAPH3 disulphide is a more effective inactivator of the synthetase than is cystamine (■ symbols), the most effective inactivator described hitherto (Griffith *et al.*, 1977).

Effect of the terminal moiety upon inactivation of γ -glutamylcysteine synthetase by aminoalkyl- and hydroxyalkylcystamines

For a chain length of two carbon atoms, both the aminoalkyl and hydroxyalkyl compounds were inactivators of the synthetase

Fig. 5. Comparison of cystamine (■), SAPH3 disulphide (●) and their respective $\beta\beta$ -bis-dimethyl analogues (□ and ○) with regard to inactivation of the synthetase

A representative experiment is shown. The activity of 1 unit of enzyme was designated '100 % control activity', and results shown represent the residual activity after incubation with each compound. All compounds were incubated with the enzyme (1 unit; partially purified) at 37 °C for 15 min.

Fig. 6. Concentration-dependence of the inactivation of the synthetase by WR3123 (○) and WR884 disulphide (●)

These compounds differ only in their terminal moiety (hydroxy and amino respectively). Each compound was incubated with the synthetase (1 unit; partially purified) at 37 °C for 15 min. Residual activity after incubation with each compound is shown as the percentage of the activity of a control sample of enzyme incubated under the same conditions.

at a concentration 1 mM. We have assessed the concentration-dependence of this inactivation for each of these compounds. As shown in Fig. 6, *NN'*-bis-(2-hydroxyethyl)cystamine (WR3123) is more potent than *NN'*-bis-(2-aminoethyl)cystamine (WR884 disulphide) at all concentrations below 1 mM. At a concentration of 1 mM both compounds completely inactivate the enzyme.

Effect of $\beta\beta$ -bis-dimethylation of cystamine and SAPH3 upon inactivation of γ -glutamylcysteine synthetase

The effect of dimethylation of cystamine and its derivatives at the β -carbon atoms upon synthetase activity has been the subject of speculation (Seelig & Meister, 1984), but, to our knowledge, has not been tested directly. Dimethylation of the carbon atoms on either side of the disulphide moiety of cystamine leads to a

Table 1. Effects of dialysis, disulphide reduction and the presence of glutamate in the incubation mixture upon the inactivation of γ -glutamylcysteine synthetase

Each compound was incubated with the synthetase (1 unit; partially purified) for 15 min at 37 °C. The activity of the treated sample relative to a control sample incubated under the same conditions was assessed after completion of incubation ('Drug alone'). The return of activity was assessed after completion of dialysis (24 h) or immediately after the addition of 2-mercaptoethanol. In the case of glutamate treatment, 9 mM-glutamate was included in the initial incubation solution, and activity of the treated sample relative to a control sample incubated under the same conditions was assessed after completion of incubation ['L-Glutamate (9 mM)']. In each case a representative result is shown.

	Activity (% of control)		
	WR2721 disulphide (1 mM)	WR3123 (1 mM)	SAPH3 disulphide (0.1 mM)
Drug alone	0	0	0
Dialysis	0	0	0
2-Mercaptoethanol (10 mM)	35	18	19
L-Glutamate (9 mM)	50	Not done	60

marked decrease in the capacity of the compound to inactivate the enzyme (Fig. 5, □ symbols). In a like manner, dimethylation of the analogous carbon atoms of SAPH3 disulphide largely blocks the inactivation of the synthetase (Fig. 5, ○ symbols).

Effects of glutamate, 2-mercaptoethanol and dialysis upon the inactivation of γ -glutamylcysteine synthetase by WR3123, SAPH3 disulphide and WR2721 disulphide

We have previously shown that, as is the case for cystamine (Griffith *et al.*, 1977), inactivation of the synthetase by WR2721 disulphide is (a) not reversible by dialysis, (b) at least partially preventable by the inclusion of glutamate in the reaction mixture, and (c) largely reversible by treatment with 10 mM-dithiothreitol (Schor, 1988). We performed similar experiments with WR2721 disulphide-treated, WR3123-treated and SAPH3 disulphide-treated partially purified enzyme to assess the likelihood of mechanistic similarity between their modes of enzyme inactivation and that of WR2721 disulphide.

Table 1 shows the results of these experiments with concentrations of each agent that would be expected to inactivate the synthetase completely. In no case does dialysis against 50 mM-Tris/HCl buffer, pH 7.4, restore activity to the inactivated enzyme. Inactivation by all three compounds is attenuated by inclusion of L-glutamate (9 mM) in the incubation mixture. Inactivation by all of the compounds is partially reversed by treatment with 2-mercaptoethanol.

Verification of these results with pure enzyme from A/J mice was precluded by the lack of availability of mice from the Jackson Laboratories. We have therefore examined the effects of dialysis, 2-mercaptoethanol treatment and incubation with L-glutamate upon inactivated pure enzyme from BALB/c mice. We have compared these results with those obtained with partially purified enzyme from A/J mice in Table 2. The pure BALB/c-mouse enzyme appeared to be inactivated to a lesser extent by WR2721 disulphide than was the partially purified A/J-mouse enzyme. With both WR2721 disulphide and WR3123, dialysis did not reverse the inactivation of either enzyme preparation. Both enzyme preparations gave comparable degrees of return of activity with 2-mercaptoethanol treatment. In both cases the degree of return was greater for WR2721 disulphide-inactivated

Table 2. Comparison of partially purified enzyme from A/J-mouse liver and pure enzyme from BALB/c-mouse liver: effects of dialysis, disulphide reduction and the presence of glutamate in the incubation mixture upon the inactivation of γ -glutamylcysteine synthetase

The experimental conditions for the data shown here are given in the legend to Table 1. In each case a representative result is shown.

	Activity (% of control)			
	WR2721 disulphide		WR3123	
	A/J mouse partially purified enzyme	BALB/c-mouse pure enzyme	A/J-mouse partially purified enzyme	BALB/c-mouse pure enzyme
Drug alone	0	33	0	0
Dialysis	0	33	0	0
2-Mercaptoethanol (10 mM)	35	67	18	25
L-Glutamate (9 mM)	50	41	Not done	90

enzyme than for WR3123-inactivated enzyme. With WR2721 disulphide, L-glutamate affords comparable degrees of protection from inactivation of both enzyme preparations.

DISCUSSION

We have previously described inactivation of γ -glutamylcysteine synthetase by the disulphide metabolite of the chemoprotective free-radical scavenger WR2721 [*NN'*-bis-(3-aminopropyl)cystamine *S*-phosphate; Schor, 1988]. The formation of this metabolite is potentiated in the presence of compounds that generate oxygen radicals. This finding places caveats on the use of WR2721 as a 'protector' of normal cells from oxygen-radical-forming drugs such as bleomycin. We have therefore begun to construct a structure-activity relationship for the inactivation of γ -glutamylcysteine synthetase by the disulphides of free radical-scavenging analogues of WR2721.

It appears from the present study that two structural alterations of these compounds most profoundly affect their ability to react with the synthetase. Increasing the alkyl chain length to five or more carbon atoms prevents inactivation of the synthetase by compounds in this series. In addition, dimethylation of the cystamine moiety on the β -carbon atoms greatly decreases the potency of such compounds as inactivators of this enzyme.

Two possible explanations could account for the lack of inactivation by compounds with long alkyl chains: (1) steric constraints prevent these larger compounds from binding near the glutamate-binding site of the enzyme; or (2) increases in the alkyl chain length beyond that which is optimal for binding to the enzyme decrease the number of possible non-covalent interactions of the compound with the enzyme. As regards the first possibility, the inactivation of the synthetase by the bulky SAPH3 disulphide makes it unlikely that steric considerations alone are responsible for this situation. On the other hand, improper positioning of the secondary amine of the compound, analogous to the amine moieties of cystamine, would be expected to lead to a block of enzyme inactivation, since the amine moieties of cystamine have been shown to be required for enzyme inactivation (Griffith *et al.*, 1977; Seelig & Meister, 1984).

The effect of $\beta\beta$ -bis-dimethylation of cystamine and SAPH3 disulphide upon synthetase inactivation is almost certainly related to steric hindrance, since the covalent binding of these compounds to the enzyme is most probably mediated via disulphide interchange (Seelig & Meister, 1982; Schor, 1988), and the methylated carbon atoms are adjacent to the cystamine disulphide moiety.

The mechanism of inactivation of the synthetase by the cystamine analogues tested in the present work appears to be, at

least in part, the same as that described for cystamine (Griffith *et al.*, 1977). The inability of dialysis to reverse this inactivation implies that the interaction is a covalent one. The ability of glutamate to block substantially interaction of the drugs with the synthetase is strong evidence for an interaction in or near the normal glutamate-binding site. In all cases the inactivation is at least partially reversible by 2-mercaptoethanol, indicating that disulphide interchange plays a role in inactivation by each of these drugs. It appears that 2-mercaptoethanol is much less effective in this regard than is dithiothreitol (Schor, 1988).

The inter-drug variability in the degree to which glutamate obviates the inactivation (WR3123 > SAPH3 disulphide > WR2721 disulphide) is probably due to differences in the relative affinity or 'fit' of each drug for the glutamate-binding site, although we cannot rule out the possibility that its effect upon WR3123-mediated inactivation differs between the two enzyme preparations shown in Table 2. The variability in the degree of reversal of inactivation by 2-mercaptoethanol (WR2721 disulphide > WR3123 = SAPH3 disulphide) is most likely due to differences in the ability of each drug to block the action of 2-mercaptoethanol upon the inactivated enzyme. This could be the result of steric hindrance by the rest of the drug molecule (e.g., for SAPH3) or of tight non-covalent association of the rest of the drug molecule with adjacent moieties (e.g., for WR3123). Such tight association might require the use of denaturing or salting agents to permit access of 2-mercaptoethanol to the drug-enzyme disulphide. These experiments do not completely exclude the possibility that some of these drugs have additional mechanisms of inactivation of the synthetase.

It remains to be seen whether alkyl chain elongation and/or $\beta\beta$ -bis-dimethylation of radio- and chemo-protective cystamine analogues prevents their adjunctive toxicity with oxidizing agents such as 6-hydroxydopamine. One would predict that the potentiation by oxidizing agents of disulphide formation would not lead to paralysis of GSH synthesis in the case of long-chain alkyl and/or methylated derivatives.

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REFERENCES

- Glick, J. H., Glover, D., Weiler, C., Norfleet, L., Yuhas, J. & Kligerman, M. M. (1984) *Int. J. Radiat. Oncol. Biol. Phys.* **10**, 1777–1780
- Griffith, O. W., Larsson, A. & Meister, A. (1977) *Biochem. Biophys. Res. Commun.* **79**, 919–925
- Lomis, T. J., Siuda, J. F. & Shepherd, R. E. (1988) *J. Chem. Soc. Chem. Commun.* 290–292
- Schor, N. F. T. (1987) *Cancer Res.* **47**, 5411–5414
- Schor, N. F. (1988) *Biochem. Pharmacol.* **37**, 1751–1762
- Seelig, G. F. & Meister, A. (1982) *J. Biol. Chem.* **257**, 5092–5096
- Seelig, G. F. & Meister, A. (1984) *J. Biol. Chem.* **259**, 3534–3538
- Seelig, G. F. & Meister, A. (1985) *Methods Enzymol.* **113**, 379–392
- Sweeney, T. R. (1979) A Survey of Compounds from the Antiradiation Drug Development Program of the U.S. Army Medical Research and Development Command, Walter Reed Army Institute of Research, Washington

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