Eur. J. Med. Chem. 33 (1998) 609–615

Thiolysable prodrugs of 1,2-bis(methylsulfonyl)-1-(2-chloroethyl)hydrazine with antineoplastic activity

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(Received 3 November 1997; accepted 16 February 1998)

Abstract – Several 2-alkoxycarbonyl- and 2-aryloxycarbonyl-1,2-bis(methylsulfonyl)-1-(2-chloroethyl)hydrazines, conceived as thiolysable prodrugs of 1,2-bis(methylsulfonyl)-1-(2-chloroethyl)hydrazine, were synthesized and their antineoplastic activity evaluated against the L1210 leukemia in mice. In addition to producing 'cures' of mice bearing this tumor, many of the analogues were preferentially activated by glutathione (GSH) and/or glutathione S-transferase (GST), making them potentially useful in the treatment of multidrug resistant tumors with increased intracellular levels of GSH and/or GST. © Elsevier, Paris

alkylating agents / glutathione / glutathione S-transferase / bis(sulfonyl)hydrazines / drug resistance

1. Introduction

The resistance of neoplastic cells to a large number of reactive cancer chemotherapeutic agents has been shown to be correlated with increased intracellular levels of glutathione (GSH) and/or glutathione S-transferase (GST) activity [1], suggesting that the protective effects afforded by elevated thiol levels is due to a direct interaction between the sulfhydryl group and the electrophilic alkylating species. Buthionine sulfoximine (BSO) reverses this mechanism of resistance by decreasing intracellular levels of GSH through the inhibition of GSH synthesis, thereby increasing the in vitro cytotoxicity and in vivo anticancer activity of a variety of antineoplastic agents, including cyclophosphamide, melphalan and the nitrosoureas [1]. Furthermore, many neoplastic cell lines, which have not been subjected to drug selection pressures, have intrinsically high levels of GSH [2], and relatively high GST activity has been found in a variety of human tumors [3]. For example, GST activity was found to be much higher in breast carcinoma than that measured in nontumor tissues [4]. Other neoplasms such as those of the lung [5], bladder [6], colon [7], brain [8] and uterine cervix [9] also show elevated levels of GST activity compared to their nontumor counterparts. Elevated GSH levels have also been found in multiple myeloma [10] and in human gastric [11], lung [12] and ovarian [13] tumor tissues. Although the resistance of various cell lines and human tumors to a variety of therapeutic strategies has been attributed to their high non-protein thiol and GST contents, elevated thiol and/or GST levels in neoplastic cells can create a site of vulnerability which can be targeted using thiol-activated prodrugs. By such an approach, an active antineoplastic agent can be generated from a parent molecule or prodrug following reaction with a thiol such as GSH. This approach would be expected to be most successful if the generated electrophilic species had a low affinity for soft nucleophiles such as thiols. If this were not the case, the increased interception of the alkylating species by GSH/GST in cells rich in these components would negate the benefits of the increased generation of the alkylating species.

We have identified a number of 1,2-bis(sulfonyl)-1-methyl- or 1-(2-chloroethyl)-hydrazines **1** with antine-

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Abbreviations: BSO, buthionine sulfoximine; GSH, glutathione; GST, glutathione S-transferase.

$R''SO_2N(R)NHSO_2R'$

1. R', R'' = alkyl, aryl
   R = $-CH_3$, $-CH_2CH_2Cl$
2. R' = R'' = $-CH_3$; R = $-CH_2CH_2Cl$

$CH_3SO_2N(CH_2CH_2Cl)N(R)SO_2CH_3$

3. R = $-CONHCH_2CH_2Cl$
4. R = $-COCH_3$

Figure 1.

oplastic activity [14, 15]. These compounds undergo rapid spontaneous decomposition in aqueous media to generate the putative alkylating species $RN{=}NSO_2R'$. The ionization of compound **1** to give the anion $R''SO_2N(R)N^-SO_2R'$, followed by the first order elimination of the alkyl sulfinate on N-1 (the rate-determining step) is believed to be the mechanism of $RN{=}NSO_2R'$ formation. The latter reacts with nucleophiles extremely rapidly such that the rapid rate-determining elimination step controls the kinetics of alkylation [16]. The most active compound to emerge from this initial study, 1,2-bis(methylsulfonyl)-1-(2-chloroethyl)hydrazine **2**, had a half-life of 30 to 40 seconds at pH 7.4 and 37 °C and exhibited considerable host toxicity. Compound **2** was active only over a narrow dosage range; however, it produced 40% cures of mice bearing the L1210 leukemia when administered as a single intraperitoneal dose of 15 mg/kg [15]. The short biological half-life of this agent appeared to limit its distribution and therapeutic efficacy. More recently, we have synthesized compounds **3** and **4** in which the proton on N-2 has been replaced by a masking group, preventing decomposition via the described mechanism until the removal of the masking group occurs. These compounds are considerably less toxic to host animals than compound **2** and have broad-spectrum antitumor activity [17, 18]. Both of these compounds (i.e., **3** and **4**), which are prodrugs of compound **2**, produced cures of mice bearing the L1210 leukemia, and were active against a spectrum of transplanted murine and human solid tumors, with activities that compared favorably with those of the established antitumor drugs, cyclophosphamide, mitomycin C and the nitrosoureas, evaluated concomitantly [17, 18]. Thus, the active form **2**, generated by the prodrugs reported in this paper, has significant potential as an anticancer agent (see *figure 1*).

While compound **3** generates compound **2** via spontaneous decomposition in aqueous media, compound **4**,

$CH_3SO_2N(R')N(COOR)SO_2CH_3$

$\downarrow$ GSH

$CH_3SO_2N(R')NHSO_2CH_3$ + GSCOOR

Figure 2.

which is more refractory to spontaneous decomposition than compound **3**, is prone to generate compound **2** via thiolysis [18]. However, the fact that compound **4** is also activated by proteolytic enzyme-catalyzed hydrolysis in serum is not compatible with the idea that it can be used to liberate the short-lived alkylating agent, compound **2**, at a higher rate in cancer cells rich in non-protein thiols. We reasoned that preferential toxicity to tumor cells with relatively high levels of non-protein thiols could be significantly enhanced by replacing the acetyl group (**R**) in compound **4** by an alkoxy- or aryloxycarbonyl group (–COOR) to give a carbamate. Carbamate esters have been shown to be resistant to both enzymatic and spontaneous hydrolysis [19–22]. However, the nature of the carbamate linkage should make them susceptible to thiolysis, as depicted in *figure 2*.

This paper reports the synthesis of a number of 2-alkoxycarbonyl- and 2-aryloxycarbonyl-1,2-bis(methylsulfonyl)-1-methyl- and 1-(2-chloroethyl)-hydrazines which are preferentially activated by GSH and GST, making them potentially useful in the treatment of multidrug resistant tumors with increased intracellular levels of GSH and/or GST. The chloroethyl analogues were also assessed for antitumor activity against the L1210 leukemia in mice.

2. Chemistry

2-Acetyl-1,2-bis(methylsulfonyl)-1-methylhydrazine **5** was synthesized by reacting 1,2-bis(methylsulfonyl)-1-methylhydrazine [14] with acetic anhydride using a literature procedure similar to the one employed for the preparation of compound **4** [18]. 2-Alkoxycarbonyl- and 2-aryloxycarbonyl-1,2-bis(methylsulfonyl)-1-methylhydrazines (**6–9**) and the related 1-(2-chloroethyl)hydrazines (**10–17**) were prepared by reacting the appropriate alkyl or aryl chloroformate with 1,2-bis(methylsulfonyl)-1-methylhydrazine or compound **2** in an appropriate solvent in the presence of a base such as triethylamine or anhydrous sodium carbonate (see *figure 3*).

3. Results and discussion

Initially, we synthesized several 2-alkoxycarbonyl-1,2-bis(methylsulfonyl)-1-methylhydrazines and found them

$CH_3SO_2N(CH_3)N(COCH_3)SO_2CH_3$

5

$CH_3SO_2N(R)N(COOR')SO_2CH_3$

6. R = methyl; R' = methyl

7. R = methyl; R' = 2-bromoethyl

8. R = methyl; R' = ethyl

9. R = methyl; R' = p-methoxyphenyl

10. R = 2-chloroethyl; R' = methyl

11. R = 2-chloroethyl; R' = 2-chloroethyl

12. R = 2-chloroethyl; R' = vinyl

13. R = 2-chloroethyl; R' = phenyl

14. R = 2-chloroethyl; R' = p-tolyl

15. R = 2-chloroethyl; R' = p-chlorophenyl

16. R = 2-chloroethyl; R' = p-methoxyphenyl

17. R = 2-chloroethyl; R' = p-nitrobenzyl

Figure 3.

to possess a number of potential advantages over their acyl counterparts. When their stability was measured using a spectroscopic method developed in our laboratory [23], they were found to be considerably more stable in aqueous media than equivalent compounds containing acyl linkages. For example, the initial rates of hydrolysis of 2-acetyl-1,2-bis(methylsulfonyl)-1-methylhydrazine **5** and 1,2-bis(methylsulfonyl)-2-methoxycarbonyl-1-methylhydrazine **6** were 0.3% and 0.007% per minute, respectively, at pH 7.4 and 37 °C.

Furthermore, the rate of decomposition of compound **6** was not appreciably increased by levels of proteinase K or serum that enhanced the rate of activation of compound **5** almost 10-fold. However, examination of several 2-alkoxycarbonyl-1,2-bis(methylsulfonyl)-1-methylhydrazines demonstrated that the carbamate linkage was susceptible to thiolysis and GSH/GST-mediated activation. For example, the rate of activation of 2-(2-bromoethoxy)carbonyl-1,2-bis(methylsulfonyl)-1-methylhydrazine **7** was elevated approximately 18-fold in vitro by the presence of GSH (1 mM) and GST (400 μg/mL), compared to buffer alone (data not shown). Since the aim of this investigation was to synthesize agents that specifically targeted tumor cells with elevated levels of GSH and GST, 2-alkoxycarbonyl-1,2-bis(methylsulfonyl)-1-alkylhydrazines should, therefore, have significant advantages over their acyl counterparts, which are more readily activated by other mechanisms.

Table I. Relative rates of activation of 2-alkoxycarbonyl-1,2-bis(methylsulfonyl)-1-methylhydrazines in vitro in the presence of GSH and GST.

$CH_3SO_2N(CH_3)N(COOR)SO_2CH_3$ R	Relative rate
$-CH_3$	100
$-C_2H_5$	33
$-CH_2CH_2Br$	317
$-C_6H_4$-4-OCH_3	1667

2-Alkoxycarbonyl-1,2-bis(methylsulfonyl)-1-methylhydrazines (1 mM) were incubated in 1 mM potassium phosphate buffer (pH 7.4) at 37 °C in the presence of 1 mM GSH and 400 μg/mL of GST and the relative rates of activation were measured.

In the *N*-methyl series, examination of several compounds of this class demonstrated that the rate of activation by GSH/GST is highly dependent upon the nature of the 2-alkoxy- or 2-aryloxycarbonyl moiety (*table I*). In general, the more electron-withdrawing the R group, the greater the relative rate of activation by GSH/GST.

The results obtained indicated that it was possible to design analogues that are not only relatively stable in aqueous media at near-neutral pH values and in serum, but are also theoretically capable of being activated at relatively high rates in tumor cells rich in non-protein thiols. Since chloroethylating agents are, in general, more cytotoxic than methylating agents, several 2-alkoxycarbonyl- and 2-aryloxycarbonyl-1,2-bis(methylsulfonyl) 1-(2-chloroethyl)hydrazines were synthesized and their antitumor activity assessed against the L1210 leukemia in mice.

The antitumor activity of compounds **10–17** was assessed in mice bearing the L1210 leukemia. The results of these tests are summarized in *table II*. Although all of the compounds displayed significant activity against this tumor, compounds in which an aromatic ring was directly attached to the carbamate oxygen (R = aryl) produced the best results. Thus, compounds **13–16** were significantly more active than compounds **10–12** and **17**, producing in each case several 60-day 'cures' of mice bearing the L1210 leukemia. Furthermore, the aromatic compounds, **13–16**, were much less toxic than the aliphatic analogues, **10–12**, as evidenced by the significant differences in the loss of host body weight produced by these two groups of compounds. As shown in *table II*, all of the aliphatic analogues produced body weight losses of > 10.0% at their optimum daily dosage levels. Interposition of a methylene group between the aromatic ring and the carbamate nitrogen resulted in a slight lowering of

Table II. Effects of 2-alkoxycarbonyl- and 2-aryloxycarbonyl -1,2-bis(methylsulfonyl)-1-(2-chloroethyl)hydrazines on the survival time of mice bearing the L1210 leukemia.

Compound	Optimum daily dose, mg/kg [a]	Average Δ wt (%) [b]	% T/C [c]	%60-day survivors
10	60	− 10.9	200	–
11	25	− 10.7	189	–
12	12.5	− 12.4	127	–
13	30	− 1.0	234	40
14	30	− 3.0	235	20
15	30	− 1.1	75	80
16	30	− 2.5	304	40
17	30	− 8.6	222	–

[a] Administered over a range of doses once daily for six consecutive days, beginning 24 h after tumor implantation, with 5–10 mice being used per group. [b] Average percent change in body weight from onset to termination of therapy. [c] % T/C = average survival time of treated/control mice × 100; cures (60-day survivors) are listed separately and are not included in this calculation.

antileukemic activity. Thus, compound **17** produced a maximum %T/C of 222 at the optimum dosage level of 30 mg/kg per day for 6 consecutive days. However, compound **17** could be activated by a reductive mechanism, whereby lability of the carbamate moiety could be caused by the enzymatic conversion of the electron-withdrawing nitro group to the electron-releasing amino group [24].

In general, compounds **10–17** were less active against the L1210 leukemia than their aminocarbonyl [17] and acyl [18] counterparts. However, since the L1210 leukemia may not possess elevated levels of GSH/GST, this system may not be the optimum one for evaluation of the clinical potential of this class of agents. To test the validity of the hypothesis that compounds **10–17** were capable of being preferentially activated by GSH and/or GST, we have measured their rates of activation in 1 mM phosphate buffer (pH 7.4, 37 °C) alone, in 1 mM phosphate buffer containing 5 mM GSH, and in 1 mM phosphate buffer containing 5 mM GSH and 17.5 U/mL of GST (*table III*). In general, the activation rate followed the order, hydrolysis < thiolysis (i.e., GSH alone) < thiolysis catalyzed by GST. Perhaps the most interesting compound to emerge from this series was the 4-nitrobenzyl analogue **17**, which was found to undergo little or no hydrolysis in phosphate buffer, an extremely slow rate of thiolysis, but a relatively high rate of activation when thiolysis was catalyzed by GST. The rate of activation in the presence of 5 mM GSH, a concentration in the range reported for various cell lines [25], and

Table III. Rates of activation of 2-alkoxycarbonyl- and 2-aryloxycarbonyl-1,2-bis(methylsulfonyl)-1-(2-chloroethyl) hydrazines (**10–17**) under different conditions.

Compound	Decomposition rate (nmole/mL/min)		
	Hydrolysis	5 mM GSH	5 mM GSH + 17.5 U/mL GST
10	0.03	0.02	0.00
11	0.02	0.02	0.08
12	0.07	2.07	4.70
13	0.06	0.37	1.80
14	0.02	0.45	1.52
15	0.06	0.92	5.33
16	0.14	0.41	1.80
17	BLD [a]	0.06	20.5

[a] BLD = below the level of detection.

17.5 U/mL of GST was 340-fold greater than that in the presence of 5 mM GSH alone. Of the compounds examined (**10–17**), compound **17** was unique in the magnitude of this activation. It was difficult to arrive at clearcut structure–activity correlations based on the data presented in *table III*; however, in the aryl carbamate series (**13–16**), antitumor activity against the L1210 leukemia parallels their uncatalyzed thiolysis rates, supporting our hypothesis that these compounds act as thiolysable prodrugs of compound **2**.

4. Conclusion

In summary, 2-alkoxycarbonyl- (or 2-aryloxycarbonyl-) 1,2-bis(methylsulfonyl)-1-(2-chloroethyl)-hydrazines represent a new series of compounds that have the potential to exploit a site of vulnerability in neoplastic cells. Some of the factors associated with the increased resistance of neoplastic cells to a variety of alkylating agents, i.e., the increased intracellular levels of GSH and GST, are expected to increase sensitivity to these thiol-activated prodrugs. Stability studies on the synthesized agents have already confirmed that the 2-alkoxycarbonyl moiety is considerably more susceptible to thiolysis and GSH/GST-mediated activation than to spontaneous or serum-catalyzed activation. These properties make this class of agents potential new candidates for clinical development.

5. Experimental protocols

5.1. Synthesis

Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. Proton

magnetic spectra were recorded on a Varian EM-390 spectrometer with tetramethylsilane as an internal standard. Elemental analyses (C, H, N) were performed by the Baron Consulting Co., Orange, CT and were within ± 0.4% of the calculated values for all compounds reported unless stated otherwise.

2-Acetyl-1,2-bis(methylsulfonyl)-1-methylhydrazine ***5***. This compound was prepared by reacting 1,2-bis(methylsulfonyl)-1-methylhydrazine [14] with acetic anhydride using a procedure similar to the one described for the synthesis of compound **4** [18]; recrystallization solvent: ethanol; yield: 44.6%; m.p.: 80–81 °C; ^{1}H NMR (acetone-d_6): δ 3.4, 3.3 and 3.2 (9H, 3*s*, 2 CH_3SO_2 and NCH_3), 2.4 (3H, *s*, $COCH_3$); Anal. $C_5H_{12}N_2O_5S_2$ (C, H, N).

1,2-Bis(methylsulfonyl)-2-(methoxycarbonyl)-1-methylhydrazine ***6***. A mixture of 1,2-bis(methylsulfonyl)-1-methylhydrazine [14] (1.00 g, 0.005 mol), anhydrous sodium carbonate (1.9 g, 0.018 mol), methyl chloroformate (1.23 g, 0.013 mol) and acetone (30 mL) was heated under reflux for 18 h. The reaction mixture was filtered and the filtrate evaporated to dryness in vacuo. The residue, a thick oil, was stirred with methanol (5 mL) and cooled in ice. The solid that separated was filtered and recrystallized from ethanol – petroleum ether (0.63 g, 48.9%); m.p.: 88–89 °C; ^{1}H NMR ($CDCl_3$): δ 4.0 (3H, *s*, OCH_3), 3.5, 3.4, 3.1 (9H, 3*s*, 2 CH_3SO_2 and NCH_3); Anal. $C_5H_{12}N_2O_6S_2$ (C, H, N).

1,2-Bis(methylsulfonyl)-2-(2-bromoethoxycarbonyl)-1-methylhydrazine ***7***. To a stirred solution of 1,2-bis(methylsulfonyl)-1-methylhydrazine [14] (2.02 g, 0.01 mol) and 2-bromoethyl chloroformate (3.49 g, 0.019 mol) in acetone (100 mL) was added triethylamine (1.45 g, 0.014 mol) in portions over a period of 15 min. The reaction mixture was stirred for an additional 18 h, filtered and the filtrate evaporated to dryness in vacuo. The residue was taken up in chloroform (100 mL) and washed with water (3 × 20 mL). The chloroform layer was dried over anhydrous magnesium sulfate, filtered and the filtrate evaporated to dryness. The residue was triturated with petroleum ether until a solid separated. It was filtered and recrystallized from ethanol (1.52 g, 43.0%); m.p.: 81–82 °C; ^{1}H NMR ($CDCl_3$): δ 4.7 and 3.6 (4H, 2*t*, CH_2CH_2Br), 3.5, 3.3 and 3.1 (9H, 3*s*, 2 CH_3SO_2 and NCH_3); Anal. $C_6H_{13}BrN_2O_6S_2$ (C, H, N).

1,2-Bis(methylsulfonyl)-2-(ethoxycarbonyl)-1-methylhydrazine ***8***. This compound was prepared from 1,2-bis(methylsulfonyl)-1-methylhydrazine [14] and ethyl chloroformate using a procedure similar to that described for compound **6**; recrystallization solvent: ethanol–petroleum ether; yield: 51.0%; m.p.: 89–90 °C; ^{1}H NMR ($CDCl_3$): δ 4.4 (2H, *q*, OCH_2), 3.5, 3.3 and 3.1 (9H, 3*s*, 2 CH_3SO_2 and NCH_3), 1.4 (3H, *t*, $OCCH_3$); Anal. $C_6H_{14}N_2O_6S_2$ (C, H, N).

1,2-Bis(methylsulfonyl)-2-[(4-methoxyphenoxy)carbonyl]-1-methylhydrazine ***9***. To a stirred solution of 1,2-bis(methylsulfonyl)-1-methylhydrazine [14] (0.50 g, 0.0025 mol) and 4-methoxyphenyl chloroformate (0.63 g, 0.0033 mol) in acetone (30 mL) was added triethylamine (0.29 g, 0.0029 mol) and the mixture stirred for 1 h. The reaction mixture was filtered and the filtrate evaporated to dryness in vacuo. The residue was triturated with petroleum ether (5 mL) and the petroleum ether layer decanted. Column chromatography of the residue on silica gel (70–270 mesh, 60 Å, $CHCl_3$), followed by crystallization from ethanol gave 0.14 g (16.1%) of the title compound; m.p.: 101–102 °C; ^{1}H NMR (acetone-d_6): δ 7.3 and 7.0 (4H, 2*d*, aromatic H), 3.9 (3H, *s*, OCH_3), 3.6, 3.4 and 3.2 (9H, 3*s*, 2 CH_3SO_2 and NCH_3); Anal. $C_{11}H_{16}N_2O_7S_2$ (C, H, N).

1,2-Bis(methylsulfonyl)-1-(2-chloroethyl)-2-(methoxycarbonyl)hydrazine ***10***. To a stirred solution of compound **2** [15] (1.25 g, 0.005 mol) and methyl chloroformate (2.46 g, 0.026 mol) in acetone (35 mL) was added triethylamine (1.45 g, 0.014 mol). The reaction mixture was stirred for an additional 16 h, filtered and the filtrate evaporated to dryness in vacuo. The residue was taken up in ethyl acetate (100 mL) and washed with water (3 × 15 mL). The ethyl acetate layer was dried over anhydrous magnesium sulfate, filtered and the filtrate evaporated to dryness in vacuo. Column chromatography of the residue on silica gel (70–270 mesh, 60 Å, $CHCl_3$), followed by crystallization from chloroform–petroleum ether gave 0.45 g (29.2%) of the title compound; m.p.: 87–88 °C; ^{1}H NMR (acetone-d_6): δ 4.0 (3H, *s*, OCH_3), 3.7–4.2 (4H, *m*, CH_2CH_2Cl), 3.5 and 3.3 (6H, 2*s*, 2 CH_3SO_2); Anal. $C_6H_{13}ClN_2O_6S_2$ (C, H, N).

1,2-Bis(methylsulfonyl)-1-(2-chloroethyl)-2-(2-chloroethoxycarbonyl)hydrazine ***11***. To a stirred solution of compound **2** [15] (1.25 g, 0.005 mol) and 2-chloroethyl chloroformate (1.00 g, 0.007 mol) in dry acetonitrile (10 mL) was added triethylamine (1.20 g, 0.012 mol). The reaction mixture was stirred for an additional 18 h, filtered and the filtrate evaporated to dryness in vacuo. The residue was triturated with petroleum ether (2 × 10 mL) and the petroleum ether layer was decanted each time. The residue was taken up in ethyl acetate (100 mL) and washed with dilute hydrochloric acid (3 × 10 mL), followed by water (2 × 10 mL). The ethyl acetate layer was dried over anhydrous magnesium sulfate, filtered and the filtrate evaporated to dryness on a rotary evaporator. Column chromatography of the residue on silica gel (70–270 mesh, 60 Å, $CHCl_3$), followed by crystallization from chloroform–petroleum ether gave 0.58 g (32.5%) of the title compound; m.p.: 73–74 °C; ^{1}H NMR ($CDCl_3$): δ 4.6 (2H, *t*, OCH_2), 3.6–4.1 (6H, *m*, CH_2Cl and NCH_2CH_2Cl), 3.5 and 3.2 (6H, 2*s*, 2 CH_3SO_2); Anal. $C_7H_{14}Cl_2N_2O_6S_2$ (C, H, N).

1,2-Bis(methylsulfonyl)-1-(2-chloroethyl)-2-(vinyloxycarbonyl)hydrazine ***12***. Compound **12** was prepared by reacting compound **2** [15] with vinyl chloroformate using a procedure similar to the one described for compound **11**; recrystallization solvent: ethanol; yield: 39.4%; m.p.: 85–86 °C; ^{1}H NMR ($CDCl_3$): δ 7.0–7.3 (1H, *m*, CH = C), 4.8–5.3 (2H, *m*, C = CH_2), 3.6–4.2 (4H, *m*, CH_2CH_2Cl), 3.5 and 3.2 (6H, 2*s*, 2 CH_3SO_2); Anal. $C_7H_{13}ClN_2O_6S_2$ (C, H, N).

1,2-Bis(methylsulfonyl)-1-(2-chloroethyl)-2-(phenoxycarbonyl)hydrazine ***13***. Compound **13** was prepared by reacting compound **2** [15] with phenyl chloroformate using a procedure similar to the one described for compound **11.** However, the reaction time was decreased from 18 h to 3 h; recrystallization solvent: ethanol; yield: 27.0%; m.p.: 75–76 °C; ^{1}H NMR ($CDCl_3$): δ 7.1–7.6 (5H, *m*, aromatic H), 3.6–4.2 (4H, *m*, CH_2CH_2Cl), 3.5 and 3.2 (6H, 2*s*, 2 CH_3SO_2); Anal. $C_{11}H_{15}ClN_2O_6S_2$ (C, H, N).

1,2-Bis(methylsulfonyl)-1-(2-chloroethyl)-2-[(4-tolyloxy)carbonyl]hydrazine ***14***. Compound **14** was prepared by reacting compound **2** [15] with 4-tolyl chloroformate using a procedure similar to the one employed for compound **11**; recrystallization solvent:

ethanol; yield: 31.2%; m.p.: 85–86 °C; ^{1}H NMR ($CDCl_3$): δ 7.3 and 7.1 (4H, 2*d*, aromatic H), 3.7–4.2 (4H, *m*, CH_2CH_2Cl), 3.5 and 3.2 (6H, 2*s*, 2 CH_3SO_2), 2.4 (3H, *s*, $ArCH_3$); Anal. $C_{12}H_{17}ClN_2O_6S_2$ (C, H, N).

1,2-Bis(methylsulfonyl)-1-(2-chloroethyl)-2-[(4-chlorophenoxy) carbonyl]hydrazine ***15***. Compound **15** was prepared by reacting compound **2** [15] with 4-chlorophenyl chloroformate using a procedure similar to the one employed for compound **11**; recrystallization solvent: ethanol; yield: 54.4%; m.p.: 124–125 °C; ^{1}H NMR (acetone-d_6): δ 7.5and 7.3 (4H, 2*d*, aromatic H), 3.9–4.2 (4H, *m*, CH_2CH_2Cl), 3.6 and 3.3 (6H, 2*s*, 2 CH_3SO_2); Anal. $C_{11}H_{14}Cl_2N_2O_6S_2$ (C, H, N).

1,2-Bis(methylsulfonyl)-1-(2-chloroethyl)-2-[(4-methoxyphenoxy) carbonyl]hydrazine ***16***. Compound **16** was prepared by reacting compound **2** [15] with 4-methoxyphenyl chloroformate using a procedure similar to the one employed for compound **11**; recrystallization solvent: ethanol; yield: 30.0%; m.p.: 119–121 °C; ^{1}H NMR ($CDCl_3$): δ 7.1and 6.9 (4H, 2*d*, aromatic H), 3.6–4.2 (4H, *m*, CH_2CH_2Cl), 3.8 (3H, *s*, OCH_3), 3.5 and 3.2 (6H, 2*s*, 2 CH_3SO_2); Anal. $C_{12}H_{17}ClN_2O_7S_2$ (C, H, N).

1,2-Bis(methylsulfonyl)-1-(2-chloroethyl)-2-[(4-nitrobenzyloxy) carbonyl]hydrazine ***17***. Compound **17** was prepared by reacting compound **2** [15] with 4-nitrobenzyl chloroformate using a procedure similar to the one employed for compound **11**; recrystallization solvent: ethanol; yield: 22.9%; m.p.: 132–133 °C; ^{1}H NMR (acetone-d_6): δ 8.3and 7.8 (4H, 2*d*, aromatic H), 5.6 (2H, *s*, Ar CH_2), 3.6–4.2 (4H, *m*, CH_2CH_2Cl), 3.6 and 3.3 (6H, 2*s*, 2 CH_3SO_2); Anal. $C_{12}H_{16}ClN_3O_8S_2$ (H, N); C: calc. 33.53; found 34.35.

5.2. *Antitumor activity*

The ascites cell form of leukemia L1210 was obtained from the Frederick Cancer Research Facility, Division of Cancer Treatment Tumor Repository of the National Cancer Institute, and was maintained by serial passage in tissue culture. Every 8 weeks, tumor cells were injected intraperitoneally into 5 donor CD_2F1 mice 8–10 weeks of age and were allowed to grow for 7 days. The peritoneal fluid was withdrawn, and the suspension was centrifuged for 5 min at 1600g. The supernatant was decanted, and 10^5 cells/mL were seeded into 10 mL of RPMI 1640 medium supplemented with 10% fetal bovine serum and 1% L-glutamine (200 mM) and once again maintained in culture. To assay for antitumor activity, 0.1 mL of the cell suspension containing 10^5 leukemia cells was injected into each recipient mouse. Test compounds were administered over a wide range of dosage levels (12.5–60 mg/kg), beginning 24 h after tumor implantation, and continued once daily for 6 consecutive days. Each drug was administered intraperitoneally as a solution in 100% dimethyl sulfoxide, in a volume not exceeding 0.025 mL. In each experiment, animals were distributed into groups of 5 mice of comparable weight and maintained throughout the course of the experiment on Purina Laboratory Chow pellets and water ad libitum. Control tumor-bearing mice given comparable volumes of vehicle were included in each experiment. Mice were weighed during the course of the experiments, and the percentage change in body weight from onset to termination of therapy was used as an indication of drug toxicity. Determination of the sensitivity of neoplasms to these agents was based upon the prolongation of survival time afforded by the drug treatments.

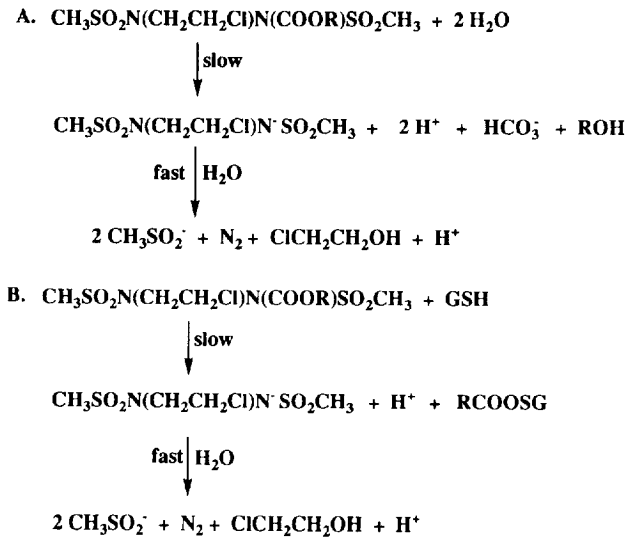

Figure 4.

5.3. *Decomposition studies*

The rate of decomposition of various compounds in the presence and absence of GSH and/or GST was studied by following the acidification of weakly buffered (1 mM potassium phosphate, pH 7.4, at 37 °C) solutions of phenol red (20 mg/L) spectrophotometrically at 560 nm. Since sulfonic acids and carbonic acid are essentially 100% and greater than 90% ionized, respectively, at pH 7.4, and since compound **2** would decompose rapidly after the rate-determining activation step, preventing the accumulation of intermediates, the assumed stoichiometries used to calculate the decomposition rates of the drugs were as follows: 3 mol of protons released during the hydrolytic decomposition of the 2-alkoxycarbonyl- (or 2-aryloxycarbonyl-) 1,2-bis(methylsulfonyl)-1-(2-chloroethyl)hydrazines and 2 mol of protons generated during the GSH and GSH/GST initiated decomposition of 2-alkoxycarbonyl- (or 2-aryloxycarbonyl-) 1,2-bis (methylsulfonyl)-1-(2-chloroethyl) hydrazines as shown in *figure 4*.

All mixtures were adjusted to pH 7.4 prior to drug addition, and the assay was calibrated using HCl standards. Calibrations were carried out for all of the reaction mixtures tested to allow for changes in sensitivity resulting from differences in buffering capacity that were due to other additives (i.e., GST/GSH). The assay was approximately linear over a range of H^+ additions of 0 to 100 μmol/L. Drugs were added in 10 μL of 100 mM solutions in DMSO to 0.99 mL of assay buffer with rapid mixing to prevent precipitation. The initial rate of decomposition was measured using 1.0 mM drug and 0 to 5.0 mM GSH in the presence or absence of 400 μg/mL of equine liver GST (79 units/mg, Sigma).

References

[1] Stewart D.J., Evans W.K., Cancer Treat. Rev. 16 (1989) 1–40.

[2] Mitchell J.B., Phillips T.L., DeGraff W., Carmichael J., Rajpal R.K., Russo A.J., Int. J. Rad. Oncol. Biol. Phys. 12 (1986) 1143–1146.

[3] Di Ilio C., Federici G., Glutathione transferase in human tumors and human cancer cell lines. In: Viña J. (Ed.), Glutathione: Metabolism and Physiological Functions, CRC Press, Boca Raton, FL, 1990, pp. 117-124.

[4] Di Ilio C., Sacchetta P., Del Boccio G., La Rovere G., Federici G., Cancer Lett. 29 (1985) 37–42.

[5] Di Ilio C., Del Boccio G., Aceto A., Casaccia R., Mucilli F., Federici G., Carcinogenesis 9 (1988) 335–340.

[6] Whyte W.L., Pong R.S., Buckpitt A.R., Biochem. Pharmacol. 33 (1984) 1813-1816.

[7] Siegers C.P., Younes-Bose H., Hoppenkamps R., Younes M., J. Cancer Res. Clin. Oncol. 107 (1984) 238–241.

[8] Polidoro G., Di Ilio C., Sacchetta P., Del Boccio G., Federici G., Int. J. Biochem. 16 (1984) 741–746.

[9] Shiratori Y., Soma Y., Maruyama H., Sato S., Takano A., Sato K., Cancer Res. 47 (1987) 6806–6809.

[10] Mulcahy R.T., Bailey H.H., Gipp J.J., Cancer Chemother. Pharmacol. 34 (1994) 67–71.

[11] Barranco S.C., Townsend Jr. C.M., Weintraub B., Beasley E.G., McClean K.K., Shaeffer J., Liu N.H., Schellenberg K., Cancer Res. 50 (1990) 3614–3618.

[12] Cook J.A., Pass H.I., Iype S.N., Friedman N., DeGraff W., Russo A., Mitchell J.B., Cancer Res. 51 (1991) 4287–4294.

[13] Green J.A., Vistica D.T., Young R.C., Hamilton T.C., Rogan A.M., Ozols R.F., Cancer Res. 44 (1984) 5427–5431.

[14] Shyam K., Hrubiec R.T., Furubayashi R., Cosby L.A., Sartorelli A.C., J. Med. Chem. 30 (1987) 2157-2161.

[15] Shyam K., Penketh P.G., Divo A.A., Loomis R.H., Patton C.L., Sartorelli A.C., J. Med. Chem. 33 (1990) 2259–2264.

[16] Penketh P.G., Shyam K., Sartorelli A.C., J. Med. Chem. 37 (1994) 2912–2917.

[17] Shyam K., Penketh P.G., Loomis R.H., Rose W.C., Sartorelli A.C., J. Med. Chem. 39 (1996) 796–801.

[18] Shyam K., Penketh P.G., Divo A.A., Loomis R.H., Rose W.C., Sartorelli A.C., J. Med. Chem. 36 (1993) 3496–3502.

[19] Saari W.S., Schwering J.E., Lyle P.A., Smith S.S., Engelhardt E.L., J. Med. Chem. 33 (1990) 97–101.

[20] Verbiscar A.J., Abood L.G., J. Med. Chem. 13 (1970) 1176–1179.

[21] Firestone R.A., Pisano J.M., Falck J.R., McPhaul M.M., Krieger M.J., J. Med. Chem. 27 (1984) 1037–1043.

[22] Alexander J., Cargill R., Michelson S.R., Schwam H., J. Med. Chem. 31 (1988) 318–322.

[23] Penketh P.G., Shyam K., Patton C.L., Sartorelli A.C., Anal. Biochem. 238 (1996) 46–49.

[24] Teicher B.A., Sartorelli A.C., J. Med. Chem. 23 (1980) 955–960.

[25] Lehninger A.L., Biochemistry (2nd ed.), Worth Publishers, New York, 1975, p.795.