(1 H, d, H6), 6.10 (1 H, m, OH-3″), 5.85 (1 H, d, H1′), 5.60 (3 H, m, H5, H3″, OH-2′), 4.60 (1 H, d, H4′), 4.45 (1 H, m, H2′), 3.95 (2 H, s, H5′), 2.85 (1 H, t, H3′); MS m/z 257 $(M + H)^+$, 279 $(M + Na)^+$. Anal. ($C_{10}H_{12}N_2O_6$) C, H, N.

1-(3-Deoxy-3-*C*-formyl-β-D-*lyxo*-pentofuranosyl)thymine 3′,5′-*O*-Hemiacetal (34). **22** (0.16 g, 0.315 mmol) was deprotected following the general procedure with a reaction time of 7 min. The crude product was flash column chromatographed with chloroform–ethanol 6:1 on silica, to give **34** as a white solid (50 mg, 59%). A sample was recrystallized from methanol: UV λ_{max} 267 nm (ε 9395); λ_{min} 233 nm (ε 1140); ^{1}H NMR δ 11.29 (1 H, s, NH), 7.34 (1 H, s, H6), 6.13 (1 H, d, OH-3″), 5.80 (1 H, d, H1′), 5.53 (2 H, m, H3″, OH-2′), 4.59 (1 H, m, H4′), 4.44 (1 H, m, H2′), 3.96 (2 H, m, H5′), 2.83 (1 H, t, H3′), 1.74 (3 H, s, CH_3); MS m/z 271 $(M + H)^+$. Anal. ($C_{11}H_{14}N_2O_6$) C, H, N.

1-[5-*O*-(*tert*-Butyldiphenylsilyl)-2,3-epoxy-β-D-*lyxo*-pentofuranosyl]uracil (35). Dry 5′-*O*-(*tert*-butyldiphenylsilyl)uridine[23] (6.46 g, 13.39 mmol) was dissolved in dry pyridine (81 mL) and cooled to 0 °C. Methanesulfonyl chloride (2.28 mL, 29.4 mmol) in dry pyridine (26 mL) was then added with stirring over a period of 1 h, with exclusion of moisture. The orange solution was stored at 4 °C for 48 h and then poured into ice water (400 mL). The solid was filtered, washed well with water, and taken up in ethyl acetate. After washing several times with water, the mixture was dried ($MgSO_4$), filtered, and evaporated to an off-white foam (7.75 g, 91%): UV λ_{max} 258 nm (ε 9500); ^{1}H NMR δ 11.50 (1 H, s, NH), 7.75–7.40 (11 H, m, H6, Ph_2), 6.00 (1 H, d, H1′), 5.80–5.30 (3 H, m, H5, H2′, H4′), 4.30 (1 H, m, H3′), 3.95 (2 H, m, H5′), 3.35 (6 H, 2 s, SO_2CH_3), 1.00 (9 H, s, *t*Bu). This was dissolved in the minimum amount of acetone, and sodium hydroxide (1 M, 43 mL) was slowly added with stirring, while the solution was maintained by prudent additions of acetone. Stirring was continued at room temperature overnight, giving rise to a slightly less polar nucleoside as indicated by TLC. The orange solution was neutralized with hydrochloric acid (1 M), causing some precipitation. The whole was then partitioned between ethyl acetate and water and the organic layer dried ($MgSO_4$) and evaporated to an off-white foam. A sample was chromatographed on a silica column with diethyl ether–hexane 4:1 to give 35 as a white solid (4.06 g, 72%): UV λ_{max} 259 nm (ε 10590); λ_{min} 236 nm (ε 5180); ^{1}H NMR δ 11.40 (1 H, bd, NH), 7.75–7.35 (11 H, m, H6, Ph_2), 6.10 (1 H, s, H1′), 5.60 (1 H, d, H5), 4.30–3.70 (5 H, m, H2′, H3′, H4′, H5′), 1.00 (9 H, s, *t*Bu); MS m/z: 465 $(M + H)^+$, 487 $(M + Na)^+$. Anal. ($C_{25}H_{28}N_2O_5Si$) C, H, N.

Antiviral Assay Procedures. The human immunodeficiency virus (HIV) assay was based on the ability of compound to reverse HIV-mediated growth inhibition in MT-4 cells infected with the HTLV-III_B strain grown in T-cell line H9. The test involved infection of cells (1 h at 37 °C with 10 $TCID_{50}$ HIV) followed by immediate exposure to the candidate drug at concentrations of 100, 10, 1, and 0.1 μM. Mock infected cells were used as controls for all drug concentrations on the same 96-well dish, allowing a simultaneous assessment of toxicity (by growth inhibition). Triplicate wells were used for infected or uninfected cells at each drug concentration. After 5 days cell number was assessed by the uptake of a tetrazolium dye MTT into viable cells, extraction with acidified propan-2-ol, and spectrophotometric determination.

For the cytomegalovirus assay, monolayers of MRC-5 cells were formed in 24-well tissue culture panels. After 24 h the wells were infected and overlaid with 0.5% indubiose A37 medium. The candidate drug was dissolved in a suitable solvent and incorporated into the overlay medium at 10 and 100 μM. After 5 days giant cells (plaques) were visualized by methylene blue stain and examined by microscope.

The other antiviral assays were based on plaque reduction. Confluent monolayers of the appropriate cells in 50-mm diameter plastic petri dishes were infected with a suspension of the virus and overlaid with nutrient agarose in which the candidate drug was dissolved in doubling dilutions. After 5 days plaques were counted and estimated as a percentage of the control and plotted against the logarithm of the compound concentration. From this the IC_{50} was determined.

Acknowledgment. We thank the Science and Engineering Research Council for a Research Studentship (to M.J.B.). We are also indebted to the Wellcome Foundation Ltd. (Beckenham, Kent. BR3 3BS.) for providing biological testing data and additional financial support, and in particular to Dr. S. G. Rahim for useful discussions. Special thanks are due to Prof. A. S. Jones for his involvement in the initial stages of this project.

S-[2-[(2′-Carbamoylethyl)amino]ethyl] Phosphorothioate and Related Compounds as Potential Antiradiation Agents

F. Ivy Carroll,* M. B. Gopinathan, and Abraham Philip

Chemistry and Life Sciences, Research Triangle Institute, Research Triangle Park, North Carolina 27709.
Received February 5, 1990

A reinvestigation of the radiation protection activity of *S*-[2-[(2′-carbamylethyl)amino]ethyl] lithium hydrogen phosphorothioate (**4a**) revealed that this compound possessed good (70% protection at a dose of 600 mg/kg) activity. The thione and imino bioisosteres of **4**, *S*-[2-(2′-thiocarbamylethylamino)ethyl] lithium hydrogen phosphorothioate (**13a**) and *S*-[2-(2′-amidinoethylamino)ethyl] phosphorothioic acid (**18b**) showed 100% protection at doses of 300 and 150 mg/kg, respectively. The *N*-methyl (**4b**) and *tert*-butyl (**4c**) analogues of amide **4a**, the *N*-methyl (**13b**) analogue of the thioamide **13a**, the *N*-methyl (**18a**) analogue of amidine (**18b**), and the cyclic amidine *S*-[2-[[2′-(4,5-dihydroimidozoyl)ethyl]amino]ethyl] lithium hydrogen phosphorothioate (**21**) all showed 80% protection at the highest dose tested.

In 1959 the U.S. Army Medical Research and Development Command initiated a program of drug development for chemoprophylactic agents that would protect personnel against ionizing radiation. The most effective radioprotective agent developed in the 1959–1972 U.S. Army Program was *S*-[2-[3-aminopropylamino]ethyl] dihydrogen phosphorothioate (**1**, WR2721).[1-3] This compound is the phosphorothioate derivative of 2-[(3-aminopropyl)-amino]ethanethiol (**2**, WR1065). Compound **2** has been shown to be active in radioprotection,[2] and it is believed that **1** serves as a prodrug which releases **2** in tissue

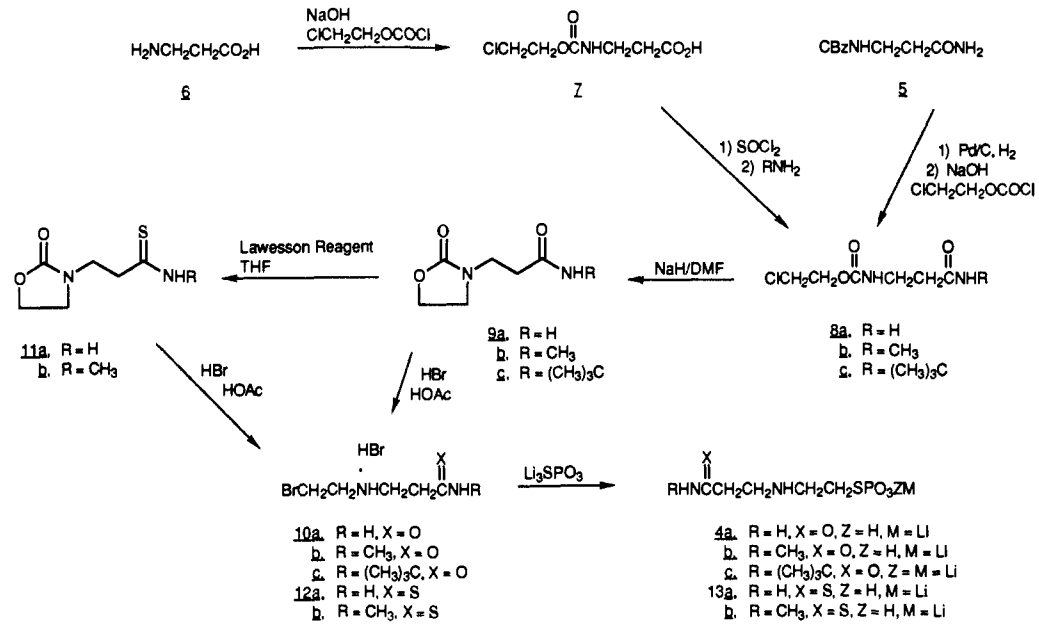
(1) Piper, J. R.; Stringfellow, C. R.; Elliot, R. D.; Johnston, T. P. *J. Med. Chem.* **1969**, *12*, 236.

(2) Sweeney, T. R. *A Survey of Compounds from the Antiradiation Drug Development Program of the U.S. Army Research and Development Command*, Walter Reed Army Institute of Research, 1979.

(3) Foye, W. O. In *Burger's Medicinal Chemistry*, 4th ed.; Wolff, M. E., Ed.; Wiley: New York, 1981; Part 3, Chapter 37, p 14.

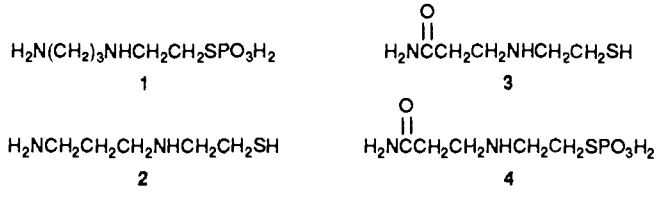
0022-2623/90/1833-2501$02.50/0

Scheme I



through the action of phosphatase enzymes.[4]

Some years ago we reported that 2-[(2′-carbamoylethyl)amino]ethanethiol (3, WR2529) showed good radioprotective activity.[5] In fact, compounds 2 and 3, which differ only in the oxidation state of the carbon next to the terminal NH_2 group, have essentially the same radioprotective activity. However, whereas conversion of 2 to its phosphorothioate, 1, resulted in a less toxic and more active compound, derivatization of 3 gave phosphorothioate 4 (WR6458) which was more toxic and therefore



could not be tested at dose levels comparable to 1.[2] Even though Sweeney has pointed out that "neither antiradiation activity nor toxicity necessarily progressed in a simple way with logical structural variation but could be highly specific",[2] the above results seem rather surprising. On the basis of the excellent antiradiation activity of 3, the close similarity of the backbone structure of 3 with 1 and 2 and the fact that the synthesis of 4 has only been reported in the patent literature,[6] we decided to prepare and reevaluate 4, as well as other amide, thioamide, and amidine analogues of 4, as radioprotective agents.

Results

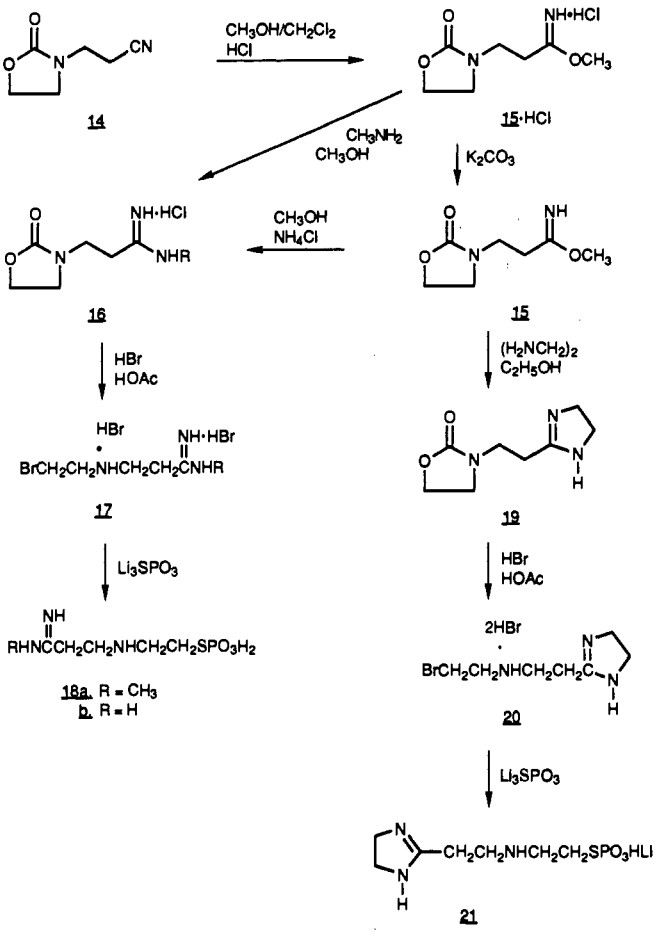
Chemistry. Scheme I outlines the synthesis of **4a** as well as its *N*-methyl and *N-tert*-butyl analogues **4b** and **4c**, respectively. Initially the key intermediate, β-[(2-chloroethoxy)carbonyl]-β-alanine amide (**8a**), was prepared from the known carbobenzoxy-β-alanine amide (**5**).[7] Thus subjection of **5** to catalytic hydrogenation to remove the carbobenzoxy protecting group followed by treatment of the liberated amine with chloroethyl chloroformate gave the intermediate **8a**. Subsequently, we found that **8a** as well as the *N*-methyl and *N-tert*-butyl analogues **8b** and **8c** was more efficiently prepared from β-alanine. Thus β-alanine (**6**) was condensed with chloroethyl chloroformate to give the β-[(2-chloroethoxy)carbonyl]-β-alanine (**7**). Reaction of **7** with thionyl chloride followed by treatment of the intermediate acid chloride with the appropriate amine or ammonia gave the amides **8a–c**. The next step in the sequence involved base-catalyzed cyclization of **8a–c** using sodium hydride in DMF to give the 3-substituted-2-oxazolidinones **9a–c**. Treatment of **9a–c** with hydrogen bromide in acetic acid under conditions similar to those used by Piper and co-workers[8] for other 3-substituted-2-oxazolidinone gave the desired bromo hydrobromides **10a–c**.

Treatment of an aqueous solution of **10a** with trilithium thiophosphate followed by dilution of the aqueous reaction mixture with dimethylformamide and thorough washing of the resulting precipitate with dimethylformamide and ether yielded a solid which gave a good elemental analysis for **4a** as well as the expected ^{31}P and ^{1}H NMR resonances. Similarly, treatment of **10b** with trilithium thiophosphate followed by a dimethylformamide–ether workup and washing gave a pure sample of **4b**. In contrast, attempts to prepare **4c** by a similar procedure yielded a product which showed the expected ^{31}P and ^{1}H NMR spectra but gave elemental analyses that indicated the sample was contaminated with excess bromide. However, if 1 equiv of lithium hydroxide were added to the reaction mixture and dicyclohexylamine were added before workup, water-insoluble dicyclohexylamine hydrobromide could be removed by filtration. Dilution of the filtrate with acetonitrile provided **4c** as the pure dilithium salt.

Scheme I also gives the procedure used to prepare the thioamide compounds. Thiation of **9a–b** with 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-dithiophosphetane 2,4-disulfide (Lawesson's reagent)[9,10] in THF at 25 °C gave

(4) Knizner, S. A.; Jacobs, A. J.; Lyon, R. C.; Swenberg, C. E. *J. Pharm. Exp. Ther.* **1986**, *236*, 37 and references cited.
(5) Carroll, F. I.; Wall, M. E. *J. Pharm. Sci.* **1970**, *59*, 1350.
(6) Brois, S. J. U.S. Patent 3,501,557, March 17, 1970. Assigned to Esso Research and Engineering Company.
(7) Hanson, H. T.; Smith, E. L. *J. Biol. Chem.* **1948**, *175*, 833.
(8) Piper, J. R.; Stringfellow, C. R., Jr.; Elliott, R. D.; Johnston, T. P. *J. Med. Chem.* **1971**, *14*, 345.
(9) Fieser, M. In *Reagents for Organic Synthesis*; John Wiley and Sons: New York, 1980; Vol. 8, p 327.

Scheme II



thioamides **11a–b**, respectively. Treatment of **11a–b** with hydrogen bromide in acetic acid yielded the bromo hydrobromides **12a–b**. Phosphorothiation of **12a–b** with trilithium thiophosphate gave the target compound **13a–b**.

The amidino analogues of **4a** were prepared as outlined in Scheme II. Subjection of *N*-(2-cyanoethyl)oxazolidinone (**14**) to dry hydrogen chloride in a mixture of methylene chloride and methanol afforded the imino ester hydrochloride **15**·HCl. Compound **15**·HCl was converted to *N*-[2-(*N*-methylamidino)ethyl]oxazolidinone (**16a**) with use of methanolic methylamine. When **16a** was dissolved in acetic acid saturated with hydrogen bromide, the desired bromo dihydrobromide **17a** was obtained. Treatment of **17a** with trilithium thiophosphate in aqueous DMF gave *S*-[2-[[2'-(*N*-methylamidino)ethyl]amino]ethyl] dihydrogen phosphorothioate.

In order to prepare the unsubstituted amidino target compound **18b** and the cyclic amidino compound **21**, the imino ester hydrochloride **15**·HCl was first converted to its free base **15**. The imino ester **15** was converted to *N*-(2-amidinoethyl)oxazolidinone (**16b**) by treating **15** with ammonium chloride in methanol. When **16b** was dissolved in acetic acid saturated with hydrogen bromide, the desired bromo dihydrobromide **17b** was obtained. Phosphorothiation of **17b** with trilithium thiophosphate using conditions similar to that used for **17a** gave *S*-[2-[(2'-amidinoethyl)amino]ethyl] dihydrogen phosphorothioate (**18b**).

Treatment of **15** with ethylenediamine in ethanol provided *N*-[2-(4,5-dihydroimidazoyl)ethyl]oxazolidinone (**19**). When **19** was dissolved in acetic acid saturated with hydrogen bromide, the desired bromo dihydrobromide **20** was obtained. Treatment of **20** with trilithium thiophosphate using conditions similar to those used to prepare **18a–b** gave *S*-[2-[[2'-(4,5-dihydroimidazoyl)ethyl]amino]ethyl lithium hydrogen phosphorothioate (**21**).

Radiation-Protective Evaluation. Results for the radiation-protective properties of **4a–c**, **13a–b**, **18a–b**, and **21**, along with WR2721 and WR6458 for comparison, are listed in Table I. Test results were carried out at A. D. Little, Boston, MA, or the University of Louisville School of Medicine, Louisville, KY, according to a previously described method.[11] Test results were provided by H. A. Musallam of the Walter Reed Army Institute of Research. Tests were performed by whole-body γ-irradiation of mice 30 min after administration of the test compound intraperitoneally.

The toxicity of **4a** is in sharp contrast to the previously reported toxicity of WR6458.[2] Whereas **4a** prepared in this study showed no toxic deaths in five mice at doses of 600 mg/kg, WR6458 possessed an LD_{50} of 130 mg/kg. Moreover, **4a** was 70% protective at 600 mg/kg.

Two of the compounds showed 100% protection at the highest administered doses. Compound **13a**, the thioamide analogue of **4a**, gave 100, 40, and 10% survival at 300, 150, and 75 mg/kg, respectively, whereas compound **18b**, the amidine analogue of **4a**, led to 100, 70, and 10% survival at 150, 75, and 37.5 mg/kg, respectively. The analogues **4b**, **4c**, **13b**, **18a**, and **21** all gave 80% protection at the highest dose tested.

Discussion

Early reports (before 1959) showed that 2-aminoethanethiol [mercaptoethylamine (MEA)] and derivatives of this structure such as AET constituted the most effective class of radiation-protective compounds.[2,3,12-14] However, since MEA and AET, as well as all other compounds available at this point, had serious limitations for potential use in man, the U.S. Army Medical Research and Development Command initiated a program of drug development for chemoprophylactic agents that could protect troops against ionizing radiation. Between 1959 and 1973, approximately 4400 compounds were tested in mice.[2,15] The program was highly successful, and very substantial progress was made toward the development of a useful drug for military use. *S*-[2-[(3-Aminopropyl)amino]ethyl] dihydrogen phosphorothioate (**1**, WR2721) was the most interesting of the radioprotective agents developed.[1-3] As a part of the U.S. Army program we developed and reported that 2-[(2'-carbamoylethyl)amino]ethanethiol (**3**, WR2529) was also an interesting radioprotective agent.[5] This compound at a maximum tolerated dose (MTD) of 900 mg/kg protected 100% of mice when administered ip 15 min before irradiation.[2,5] At half MTD (450 mg/kg) survival was 90%, and at one-quarter MTD there was 70% survival. **3** has a dose-reduction factor (DRF) of 2.6 and

$$\underset{\text{MEA}}{H_2NCH_2CH_2SH} \qquad \underset{\text{AET}}{H_2NCH_2CH_2SC(O)NH_2}$$

(10) Petersen, B. S.; Schelbye, S.; Clausen, K.; Lawesson, S.-O. *Bull. Soc. Chim. Belg.* **1978**, *87*, 293.

(11) Klayman, D. L.; Grenan, M. M.; Jacobus, D. P. *J. Med. Chem.* **1969**, *12*, 510.

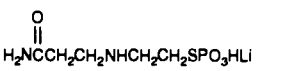
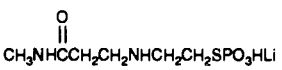
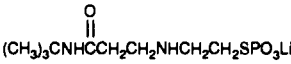
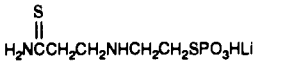
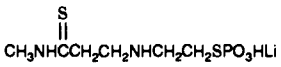
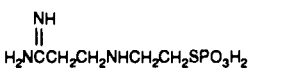
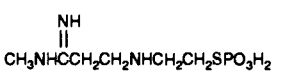
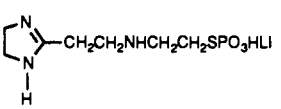
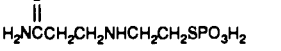
(12) Klayman, D. L.; Copeland, E. S. In *Drug Design*; Ariens, E. J., Ed.; Academic Press: New York, 1975; Vol. VI, Chapter 2.

(13) Foye, W. O. In *Medicinal Chemistry*; Burger, A., Ed.; Wiley: New York, 1970; p 1669.

(14) Thomson, J. F. In *Radiation Protection in Mammals*; Reinhold: New York, 1962.

(15) Grant, G. A.; Leach, K. Search for Radioprotective Compounds, Part XIX. Report 827 to Defense Research Establishment, Ottawa, Ottawa, Ontario.

Table I. Radiation-Protective Properties in Mice[a]

compd	structure	approximate LD_{50}, mg/kg	drug dose, mg/kg	vehicle of administration	radiation dose, GY	30-day survival, %
4a		>600[b]	600 300 150	80% H_2O 20% C_2H_5OH	10	70 10 0
4b		>600	600 300	H_2O	10	80 20
4c		>300	300 150 75	H_2O	9.5	80 60 40
13a		>300	300 150 75	H_2O	9.5	100 40 10
13b		>250	150 75 37.5	80% H_2O 20% C_2H_5OH	10	80 0 0
18b		>150	150 75 37.5	85% H_2O 15% C_2H_5OH[c]	9.5	100 70 10
18a		>150	150 75 37.5	85% H_2O 15% C_2H_5OH[c]	9.5	80 50 30
21		>100	100 75 37.5	H_2O	9.5	80 20 0
WR2721[d]	$H_2NCH_2CH_2CH_2NHCH_2CH_2SPO_3H_2$	1000	600 300 150 75		10	100 100 40 40
WR6458[d]		130	50 25		10	0 0

[a] Antiradiation tests in groups of 10 mice against lethal γ-radiation: 9.5 or 10 GY from ^{60}CO source 30 min after intraperitoneal dosing. [b] No toxic deaths observed in five mice at this dose. [c] Contains 0.3% of methyl cellulose. [d] Taken from ref 2.

is second only to **1** in terms of this indicator of protective activity.[16] Both monkeys and miniature swine were protected with **3**.[17]

In the introduction we pointed out that WR2721 is the phosphorothioate derivative of the parent thiol, **2** (WR1065), which is active in radioprotection. It is believed that **1** serves as a prodrug which releases **2** in tissue through the action of phosphatase enzymes.[4] A comparison of **2** with its amide analogue **3** showed the two compounds to have essentially the same radioprotective activity.[2] However, whereas conversion of **2** to its phosphorothioate, **1**, resulted in a less toxic and more active compound, surprisingly, derivatization of **3** gave a phosphorothioate, **4** (WR6458), with no activity and high toxicity. These unexpected results led us to synthesize and reevaluate **4** as a radiation protective agent.

The results shown in Table I show that in contrast to the earlier data,[2] compound **4** was not toxic at the highest dose level when tested as its lithium salt **4a** and that the compound was 70% protective at 600 mg/kg. Since it is unlikely that there would be any difference in the toxicity of the free acid **4** and its lithium salt **4a**, this data implies the original sample of **4** was either contaminated with toxic impurities or does not have the structure shown. Moreover, the amide analogs **4b** and **4c** both showed 80% protection at 600 and 300 mg/kg, respectively. Thus the activity of **4a** is retained when either small (CH_3) or large [$(CH_3)_3C$] substitutions are made on the terminal nitrogen. These results suggested that **4a–c** represent a new lead structure for the design of potential useful radiation protection agents.

Because of the highly specific nature of the activity and toxicity of the phosphorothioates,[2] the classical medicinal chemistry methods such as the Hansch approach is not very useful for optimization of the lead compounds. In cases like this, bioisosterism can be an aid in the process of developing lead compounds.[18-24] The amide carbonyl of **4a** provides a site for introducing isosteric groups while retaining the complete sulfur–carbon–nitrogen backbone structure of **4a** as well as **1** and **2**. Compound **1** and **2** differ from **3** and **4** in that they contain a basic terminal NH_2 function whereas **3** and **4** possess a neutral amide NH_2 group.

Since the thione sulfur (=S) and the imino nitrogen (=NH) are well precedented as isosteres of carbonyl oxygen (=O), we chose to synthesize and evaluate the sulfur

(16) Yuhas, J. M. *Radiat. Res.* **1970**, *44*, 621.
(17) Davidson, D. E.; Grenan, M. M.; Sweeney, T. R. In *Radiation Sensitizers. Their Use in the Chemical Management of Cancer*; Brady, L. W., Ed.; Mansson Publishing USA: Chicago, IL, 1980; p 309.
(18) Burger, A. In *Medicinal Chemistry*, 3rd ed.; Wiley-Interscience: New York, 1970; p 201. (b) Schatz, V. B. In *Medicinal Chemistry*, 2nd ed.; Burger, A., Ed.; Wiley-Interscience: New York, 1970; p 201.
(19) Lipinski, C. A. *Ann. Rep. Med. Chem.* **1986**, *21*, 283.
(20) Foye, W. O. In *Principles of Medicinal Chemistry*; Lea and Febiger: Philadelphia, 1970.
(21) Korolkovas, A. In *Essentials of Molecular Pharmacology: Background for Drug Design*; Wiley: New York, 1970.
(22) Ariens, E. J. In *Drug Design*; Ariens, E. J., Ed.; Academic Press: New York, 1971; Vol. I.
(23) Rudinger, J. In *Drug Design*; Ariens, E. J., Ed.; Academic Press: New York, 1971; Vol. II Chapter 9.
(24) Thornber, C. W. *Chem. Soc. Rev.* **1979**, *8*, 563.

isosteres **13a–b** and the imino isosteres **18a–b**, and **21**. The terminal amino in **13a–b** would be neutral similar to **4a**. In contrast, the terminal amino in **18a–b** and **21** would be strongly basic and more like **1**. The thioamide congeners **13a–b** would possess greater lipid solubility than their amide counterparts (**4a–b**). As a result of the size, electronegativity and other properties of sulfur, the double bond tautomers such as **13aA** will make a greater contribution to the structure than their amide analogues.

$$HN{=}C(SH)CH_2CH_2NHCH_2CH_2SPO_3H_2$$
13aA

Examination of the data in Table I shows that the conversion of **4a** to the thione analogue **13a** and the imino analogue **18b** provide compounds which are more protective than the lead compound **4a**. Conversion of **4b** to its thione and imino analogues **13b** and **18a**, respectively, gave compounds that showed protection equal to **4b** at one-half the dose level used for **4b**. The cyclic amidine analogue **21** showed activity similar to **18a**.

Conclusion

A reexamination of the radiation protection activity of *S*-[2-[(2'-carbamoylethyl)amino]ethyl] dihydrogen phosphorothioate (**4**) revealed that in contrast to a previous report[2] this compound showed good radiation protection activity. Bioisosteric replacement of the carbonyl oxygen of **4a** with sulfur or nitrogen led to compounds **13a** and **18b**, respectively, which were highly protective. The *N*-methyl derivatives of **4b**, **13b**, and **18a**, respectively, all showed good activity. In addition the *N*-*tert*-butylamide analogue **4c** and the cyclic amidine derivative **21** show good radiation protective activity.

The high activity of **13a** and **18b** in the standard assay indicates that these compounds have potential as radiation-protective agents and should be evaluated in larger animals. In addition the good activity of all the remaining amide, thioamide, and amidine analogues of **4a** suggest that further bioisosteric modification of **4a** might lead to more useful compounds.

Experimental Section

Melting points were determined on a Koffler hot stage. Infrared (IR) spectra were recorded on a Perkin-Elmer 457 spectrophotometer. Ultraviolet spectra were run on a Varian model 2290 spectrophotometer. Proton magnetic resonance (^{1}H NMR) spectra were obtained on a Bruker 250 spectrometer. Chemical shifts were reported in δ values relative to tetramethylsilane (Me_4Si). Carbon and phosphorus magnetic resonance spectra were determined at 22.4 and 36.2 MHz, respectively, on a JEOL FX-90Q spectrometer. Microanalyses were performed by Galbraith Laboratories, Knoxville, TN and Atlantic Microlab Inc., Atlanta, GA. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within 0.4% of the calculated values.

β-[(2-Chloroethoxy)carbonyl]-β-alanine (7). Chloroethyl chloroformate (7.15 g, 0.5 mol) and a solution of NaOH (20 g, 0.5 mol) in 100 mL of H_2O were added simultaneously over a period of 1 h to a stirred solution of β-alanine (**6**) (44.5 g, 0.5 mol) and NaOH (20 g, 0.5 mol) in 500 mL of H_2O. The pH of the reaction mixture was maintained between 7–8 during the addition. After stirring for 4 h at room temperature, the reaction mixture was extracted with ether. The mixture was extracted with ethyl acetate after acidification of the aqueous layer to pH 2–3 with 6 N HCl. The ethyl acetate solution was washed with NaCl solution and dried over Na_2SO_4. The residue after removal of the solvent was recrystallized from ethyl acetate–hexane to give 92.9 g (93%) of **7**: mp 69–70 °C; ^{1}H NMR ($CDCl_3$) δ 2.46 (t, 2, CH_2CO_2), 3.47 (m, 2, NCH_2), 3.67 (t, 2, CH_2Cl), and 4.34 (t, 2, OCH_2). Anal. ($C_6H_{10}ClNO_4$): C, H, Cl, N.

***N*-Methyl-β-[(2-chloroethoxy)carbamoyl]-β-alanine Amide (8b).** A solution of **7** (50 g, 0.26 mol) in 350 mL of $CHCl_3$ was heated to reflux with $SOCl_2$ (61 mL) for 1 h. The solvents were removed under reduced pressure, and the residue was dissolved in 50 mL of toluene and again evaporated to dryness under reduced pressure to provide the acid chloride of **7**. A solution of methylamine (15.9 g, 0.52 mol) in 200 mL of $CHCl_3$ was added dropwise to a solution of the acid chloride in 350 mL of $CHCl_3$ at dry ice–acetone temperature over a period of 1.5 h. When the addition was complete, the mixture was allowed to come to room temperature and was stirred overnight. The precipitated methylamine hydrochloride was removed by filtration and washed with $CHCl_3$. The combined $CHCl_3$ solution was washed with saturated NaCl solution and evaporated to dryness. The resulting solid was recrystallized from $CHCl_3$–hexane to give 35.2 g (66%) of **8b**: mp 125–126 °C; ^{1}H NMR ($CDCl_3$) δ 2.42 (t, 2, CH_2CO), 2.81 (d, 3, NCH_3), 3.46 (m, 2, NCH_2), 3.66 (t, 2, CH_2Cl), and 4.29 (t, 2, OCH_2). Anal. ($C_7H_{13}ClN_2O_3$): C, H, Cl, N.

***N*-[2-(*N'*-Methylcarbamoyl)ethyl]oxazolidinone (9b).** To a suspension of hexane washed NaH [(50% suspension), 6.8 g, 0.16 mol] in 200 mL of dry DMF was added a solution of **8b** (33.28 g, 0.16 mol) in 100 mL of DMF over a period of 1 h. The precipitated NaCl obtained after stirring overnight was separated by filtration, and the solution was evaporated to an oil under reduced pressure. The product was purified by silica gel column chromatography using 20% MeOH–CH_2Cl_2 as the eluant to give 16.5 g (94%) of **9a**: ^{1}H NMR (CD_3OD) δ 2.44 (t, 2, CH_2CO), 2.71 (s, 3, NCH_3), 3.51 (t, 2, $NHCH_2$), 3.62 (t, 2, NCH_2 oxazolone), and 4.31 (t, 2, CH_2O). Anal. ($C_7H_{12}N_2O_3{\cdot}0.25H_2O$): C, H, N.

A 25.4-g (0.12 mol) run gave 16.2 g (77%) of **9b**.

2-Substituted-ethyl Bromide Hydrobromides. General Procedure. A solution of the oxazolidinone in excess acetic acid saturated with HBr was stirred at room temperature overnight. The excess HBr and some acetic acid was removed under reduced pressure. Dilution of the remaining solution with 10 mL of MeOH followed by careful addition of 100 mL of ether gave the desired product. The final purification was achieved by recrystallization from the appropriate solvent.

2-[*N*-[2'-(Methylcarbamoyl)ethyl]amino]ethyl Bromide Hydrobromide (10b). Recrystallization from MeOH–ether gave 32% of **10b**: mp 124–126 °C; ^{1}H NMR (CD_3OD) δ 2.67 (t, 2, CH_2CO), 2.75 (s, 3, NCH_3), 3.31 (t, 2, CH_2N), 3.53 (t, 2, NCH_2) and 3.72 (t, 2, CH_2Br). Anal. ($C_6H_{14}Br_2N_2O$): C, H, Br, N.

2-[(2-Carbamoylethyl)amino]ethyl Bromide Hydrobromide (10a). Recrystallization from MeOH–ether gave 60% of **10a**: mp 144–145 °C; ^{1}H NMR (CD_3OD) δ 2.72 (t, 2, CH_2CH_2CO), 3.34 (t, 2, $NHCH_2CO$), 3.52 (t, 2, $NHCH_2CH_2Br$), and 3.72 (t, 2, $NHCH_2CH_2Br$). Anal. ($C_5H_{12}Br_2N_2O$): C, H, Br, N.

2-[*N*-[2'-(*tert*-Butylcarbamoyl)ethyl]amino]ethyl Bromide Dihydrobromide (10c). Recrystallization from MeOH–ether gave 65% of **10c**: mp 131–132 °C; NMR (MeOH-d_4) δ 1.34 [s, 9, $C(CH_3)_3$], 2.68 (t, 2, CH_2CO), 3.55 (t, 2, $BrCH_2CH_2N$), and 3.76 (t, 2, $BrCH_2CH_2$). Anal. ($C_9H_{21}Br_3N_2O$): C, H, Br, N.

2-[(2'-Thiocarbamoylethyl)amino]ethyl Bromide Hydrobromide (12a). Recrystallization from MeOH–ether gave 64% of **12a**: mp 116–118 °C; ^{1}H NMR (DMSO-d_6) δ 2.92 (t, 2, CH_2CH_2CS), 3.3 (t, 2, NCH_2CH_2CS), 3.48 (t, 2, CH_2CH_2N), and 3.71 (t, 2, CH_2CH_2Br). Anal. ($C_5H_{12}Br_2N_2S$): C, H, Br, N, S.

2-[*N*-[2'-(Methylthiocarbamoyl)ethyl]amino]ethyl Bromide Hydrobromide (12b). Recrystallization from MeOH–ether gave 65% of **12b**: mp 166–169 °C; ^{1}H NMR (CD_3OD) δ 3.1 (s, 3, CH_3), 3.15 (t, 2, CH_2CH_2CS), 3.54 (m, 4, $CH_2N^+H_2CH_2$), and 3.75 (t, 2, CH_2CH_2Br). Anal. ($C_6H_{14}Br_2N_2S$): C, H, Br, N.

2-[*N*-[2'-(Methylamidino)ethyl]amino]ethyl Bromide Dihydrobromide (17a). Recrystallization from MeOH–ether afforded 80% of **17a** as colorless crystals: mp 155 °C dec; ^{1}H NMR (CD_3OD) δ 2.96 (s, 3, NCH_3), 3.05 [t, 2, $CH_2CH_2C(NH)$], 3.49–3.64 (m, 4, CH_2CH_2), and 3.8 (t, 2, CH_2CH_2Br). Anal. ($C_6H_{16}Br_3N_3$): C, H, Br, N.

2-[(2'-Amidinoethyl)amino]ethyl Bromide Dihydrobromide (17b). Recrystallization from CH_3OH–ether afforded 78% of **17b** as colorless crystals: mp 158–160 °C; ^{1}H NMR (DMSO-d_6) δ 2.96 [t, 2, $-CH_2C(NH)-$], 3.5 (b, 4, $-CH_2NHCH_2-$), 3.79 (t, 2, CH_2Br), 8.8 (br s, NH), and 9.23–9.65 (br s, NH). Anal. ($C_5H_{14}Br_2N_3$): C, H, Br, N.

2-[*N*-[2'-(4,5-Dihydroimidazoyl)ethyl]amino]ethyl Bromide Dihydrobromide (20). Recrystallization from methanol

afforded 71% of **20** as colorless crystals: mp 169–173 °C; ^{1}H NMR (DMSO-d_6) δ 3.04 [t, 2, CH_2(C=N)], 3.44 (m, 4, CH_2NHCH_2), 3.79 (t, 2, $BrCH_2$), and 3.85 (s, 4, =NCH_2CH_2NH). Anal. ($C_7H_{16}Br_3N_3$): C, H, Br, N.

***S*-[2-[[2′-(*N*-Methylcarbamoyl)ethyl]amino]ethyl] Lithium Hydrogen Phosphorothioate (4b).** To a stirred solution of trilithium thiophosphate[25] (0.696 g, 3 mmol) in 3 mL of H_2O was added bromo compound **10b** as a solid. After all the solid dissolved, 1.5 mL of DMF was added, and stirring was continued for 3.5 h. At this point a ^{31}P NMR spectrum indicated that all the trilithium thiophosphate had reacted. A small amount of solid was removed by centrifugation. The clear supernatant was diluted with 30 mL of DMF while maintained in a water bath at 20 °C. The mixture was stirred for 30 min and again centrifuged. The supernatant was separated by decantation. The solid was stirred with DMF–ether (1:1) and centrifuged. The residue obtained was stirred with 200 mL of ether and separated by filtration. This solid was dried under a stream of N_2 to give 0.47 g (63%) of **4b**: ^{1}H NMR (D_2O) δ 2.67 (t, 2, CH_2CH_2CO), 2.75 (s, 3, NCH_3), 2.96 (m, 2, CH_2CH_2S), 3.24 and 3.27 (d t, 4, CH_2NCH_2); ^{31}P NMR (D_2O) 16.11 ppm (t, J = 13 Hz). Anal. ($C_6H_{14}LiN_2O_4PS\cdot{}^1/_3\,H_2O$): C, H, N, P, S.

This experiment was repeated at 0.1 mol scale three times giving yields of 60, 80, and 88%.

β-[(2-Chloroethoxy)carbonyl]-β-alanine Amide (8a). Method A (from 7). Compound **8a** was prepared by a procedure similar to that described for **8b**. Thus, acid **7** (65 g, 0.33 mol) was converted to the acid chloride and treated dropwise with a solution of NH_3 (11.3 g, 0.66 mol) in 30 mL of THF and stirred overnight. The mixture was diluted with water, and the organic layer was separated and washed with saturated sodium chloride solution and dried (Na_2SO_4). The residue obtained after removal of the solvents was recrystallized from EtOAc to give 38.5 g (60%) of **8a**: mp 67–68 °C; ^{1}H NMR (CD_3OD) δ 2.44 (t, 2, CH_2CH_2CO), 3.41 (t, 2, NCH_2CH_2CO), 3.68 (t, 2, $ClCH_2CH_2O$), and 4.28 (t, 2, $ClCH_2CH_2O$). Anal. ($C_6H_{11}ClN_2O_3$): C, H, Cl, N.

Method B (from 5). A solution of **5**[7] (33 g, 0.015 mol) in 50 mL of MeOH and 50 mL of THF was hydrogenated over 5% Pd/C for 1 h at which time the hydrogenolysis was complete. The catalyst was removed by filtration, and the filtrate was evaporated to dryness. The resulting residue was suspended and stirred in 50% MeOH–H_2O, and chloroethyl chloroformate (2.12 g) and a solution of NaOH (0.6 g in 4 mL H_2O) were added simultaneously over a period of 20 min. After 2 h, the mixture was extracted with EtOAc. The organic layer was washed with NaCl solution and dried (Na_2SO_4). The residue, after removal of the solvent, was recrystallized from EtOAc to give 2.2 g (75%) of **8a**: mp 67–68 °C; ^{1}H NMR (CD_3OD) identical with a sample prepared by method A.

***N*-(2-Carbamoylethyl)oxazolidinone (9a).** Compound **9a** was prepared by a procedure analogous to that described for **9b**. Thus, 20.1 g (0.1 mol) of **8a** was treated with 4.8 g (0.15 mol) of NaH suspension (50% oil suspension) in DMF. Workup and recrystallization from a $CHCl_3$–hexane mixture gave 12.6 g (77%) of **9a**: mp 105–106 °C; ^{1}H NMR ($CDCl_3$) δ 2.55 (t, 2, CH_2CH_2CO), 3.57 (t, 2, NCH_2CH_2CO), 3.70 (t, 2, OCH_2CH_2N), and 4.33 (t, 2, OCH_2CH_2N). Anal. ($C_6H_{10}N_2O_3$): C, H, N.

***S*-[2-[(2′-Carbamoylethyl)amino]ethyl] Lithium Hydrogen Phosphorothioate (4a).** Compound **10a** (0.31 g, 0.0012 mol) was added to a stirred solution of trilithium thiophosphate (0.23 g, 0.001 mol) in 1 mL of water. After dissolution, 0.5 mL of DMF was added, and the mixture was stirred for 2 h. After successive addition of 5 mL of DMF and 4 mL of ether, the mixture was stirred for another 30 min. The solution was filtered, washed first with a mixture of DMF and ether (1:1, 50 mL) and finally with 150 mL of absolute ethanol. On drying in vacuo overnight 180 mg (76%) of **4a** was obtained as a white solid: ^{1}H NMR (D_2O) δ 2.77 (t, 2, CH_2CH_2CO), 3.5 (m, 2, SCH_2CH_2), and 3.4 (m, 4, $CH_2N^+H_2CH_2$); ^{31}P NMR (H_2O) 15.63 ppm (t, J = 13 Hz). Anal. ($C_5H_{12}LiN_2O_4PS$): C, H, N, P, S.

A 0.024-mol run gave an essentially quantitative yield of **4a**.

(25) We thank Dr. J. R. Piper, Southern Research Institute, for providing information on the synthesis, stability and storage of $LiSPO_3$.

***N*-*tert*-Butyl-β-[(2-chloroethoxy)carbamoyl]-β-alanine Amide (8c).** A solution (19.6 g, 0.1 mol) of **7** in 200 mL of $CHCl_3$ was heated to reflux with $SOCl_2$ (26 mL) for 1 h. The solvents were removed under reduced pressure. The residue was dissolved in 50 mL of dry toluene and again evaporated to dryness under reduced pressure to provide the acid chloride of **8c**. The acid chloride dissolved in 25 mL of $CHCl_3$ was added to a solution of 8 g (0.11 mol) of *tert*-butylamine and 8.8 g (0.11 mol) of pyridine in 80 mL of $CHCl_3$ at dry ice–acetone temperature over a period of 30 min. The reaction mixture was stirred overnight, and the temperature was allowed to reach 23 °C. The resulting precipitate was separated by filtration, and the solution was washed with saturated NaCl solution and dried (Na_2SO_4). The residue obtained on evaporation was crystallized from EtOAc–hexane to give 17.16 g (69%) of **8c**: mp 46–48 °C; ^{1}H NMR ($CDCl_3$) δ 1.34 [s, 9, $C(CH_3)_3$], 2.68 (t, 2, CH_2CO), 3.56 (t, 2, NCH_2CH_2CO), 3.70 (dd, 2, OCH_2CH_2N), and 4.32 (dd, 2, OCH_2CH_2N). Anal. ($C_{10}H_{19}ClN_2O_3$): C, H, Cl, N.

This experiment was repeated two times on a 0.4-mol scale to give 78–81% yield.

***N*-[2-(*N*′-*tert*-Butylcarbamoyl)ethyl]oxazolidinone (9c).** To a stirred hexane-washed suspension of NaH (0.25 g, 0.005 mol) in 5 mL of DMF was added a solution of 1.25 g (0.005 mol) of **8c** in 5 mL of DMF. After 16 h the reaction was diluted with CH_2Cl_2. The precipitated NaCl was removed by filtration and washed with CH_2Cl_2. The filtrate and washings were evaporated under reduced pressure, and the resulting oily residue was chromatographed on silica gel (100 g) with 10% MeOH–CH_2Cl_2 as eluent. The pure fraction was evaporated to dryness and the solid obtained recrystallized from CH_2Cl_2–hexane to give 0.68 g (64%) of **9c**: mp 118–119 °C; NMR ($CDCl_3$) δ 1.35 [s, 9, $C(CH_3)_3$], 2.42 (t, 2, CH_2CO), 3.54 (t, 2, NCH_2CH_2CO), 3.65 (dd, 2, OCH_2CH_2N), 4.30 (dd, 2, OCH_2CH_2). Anal. ($C_{10}H_{18}N_2O_3$): C, H, N. This experiment was repeated twice on a 0.25-mol scale to give 65% and 78% yields.

***S*-[2-[[2′-(*N*′-*tert*-Butylcarbamoyl)ethyl]amino]ethyl] Dilithium Phosphorothioate (4c).** To a stirred solution of 20.43 g (0.05 mol) of **10c** and 2.12 g (0.05 mol) of $LiOH\cdot H_2O$ in 60 mL of H_2O at 25 °C was added 8.24 g (0.045 mol) of $Li_3PSO_3\cdot 4.2H_2O$. When a clear solution was obtained, the mixture was diluted with 30 mL of DMF and stirring was continued for 4 h. Dicyclohexylamine (40 g) was added, and the mixture was stirred an additional 2 h. The mixture was filtered, and the filtrate was diluted successively with CH_3CN (600 mL) and ether (1 L). The resulting precipitate was collected, redissolved in 30 mL of H_2O and diluted with 300 mL of DMF followed by ether (600 mL). The precipitate was collected by filtration and washed thoroughly with ether and dried to give 9.25 g (67%) of **4c**: mp >295 °C dec; ^{1}H NMR (D_2O) δ 1.32 [s, 9, $C(CH_3)$], 2.38 (t, 2, CH_2CO), 2.83 (m, 6, $CH_2NHCH_2CH_2S$); ^{31}P NMR (D_2O) 16.42 ppm (t, J = 10 Hz). Anal. ($C_9H_{19}N_2Li_2O_4PS\cdot 0.5H_2O$).

***N*-(2-Thiocarbamoylethyl)oxazolidinone (11a).** A mixture of 25 g (0.17 mol) of **9a** and 36.8 g (0.09 mol) of Lawesson's reagent[9,10] was stirred with 790 mL of freshly distilled THF for 2 h at which time the mixture became clear. This mixture on partial concentration under reduced pressure followed by dilution with hexane and cooling gave a precipitate which was collected by filtration and washed with cold ether. Recrystallization from MeOH–ether gave 14.5 g (54%) of **10a**: mp 141–142 °C; ^{1}H NMR (DMSO-d_6) δ 2.69 (t, 2, CH_2CH_2CS), 3.52 (m, 4, CH_2NHCH_2), and 4.2 (t, 2, CH_2CH_2O). Anal. ($C_6H_{10}N_2O_2S$): C, H, N, S.

***S*-[2-[(2′-Thiocarbamoylethyl)amino]ethyl] Lithium Hydrogen Phosphorothioate (13a).** To a stirred solution of 5.46 g (0.026 mol) of $Li_3SPO_3\cdot 4.2H_2O$ in 130 mL of water was added 8.58 g (0.0294 mol) of **12a**. Dimethylformamide (65 mL) was added to the solution, and the mixture was stirred for 2.75 h, diluted with 280 mL of CH_3CN and placed in the refrigerator (4 °C) for 6 days. The pale yellow crystals were separated by filtration, washed with CH_3CN (420 mL) and ether (420 mL), and dried under vacuum for 6 h to give 5.1 g (65%) of **13a**: mp 73–83 °C; ^{1}H NMR (D_2O) δ 2.94–3.09 [m, 4, $CH_2CH_2C(S)$], 3.39–3.53 (m, 4, $-SCH_2CH_2NH-$); ^{31}P NMR (D_2O) δ 15.63 (J = 13 Hz). Anal. ($C_5H_{12}LiN_2O_3PS_2\cdot 3H_2O$): C, H, N, P, S.

***N*-[2-(*N*′-Methylthiocarbamoyl)ethyl]oxazolidinone (11b).** Compound **11b** was prepared by a procedure similar to that described for **11a**. Thus, a solution of **9b** (13.1 g, 0.08 mol) in

freshly distilled THF (300 mL) under N_2 was stirred for 2 h with Lawesson's reagent (16.8 g, 0.04 mol) to give 9.0 g (62%) of **11b**; mp 138–139 °C; ^{1}H NMR ($CDCl_3$) δ 2.99 (t, 2, CH_2CH_2CS), 3.16 (s, 3, NCH_3), 3.61 (t, 2, NCH_2CH_2CS), 3.71 (t, 2, NCH_2CH_2O), and 4.32 (t, 2, NCH_2CH_2O); mass spectrum calculated for $C_7H_{12}N_2O_2S$ *m/e* 188.0620, found 188.0622. Anal. ($C_7H_{12}N_2O_2S$): C, H, N, S.

***S*-[2-[[2'-(*N*-Methylthiocarbamoyl)ethyl]amino]ethyl] Lithium Hydrogen Phosphorothioate (13b).** To a stirred solution of 0.12 g (0.001 mol) of trilithium thiophosphate in 5 mL of water was added 0.340 g (0.0012 mol) of **12a** at room temperature. When all the solid was dissolved, DMF (2.5 mL) was added and stirring was continued for 2 h. The mixture was diluted with DMF (5 mL) followed by ether (20 mL). The thick jelly-like precipitate was stirred for 1 h. The partially granulated precipitate was washed with a 1:1 mixture of DMF and ether (50 mL) followed by ether (250 mL). The white solid was dried for 40 min to give 0.280 g (96%) of **13b**: ^{1}H NMR (D_2O) δ 2.86 (t, 2, CH_2CH_2CS), 3.02 (t, 2, CH_2CH_2S), 3.39 (t, 2, NCH_2CH_2CS), and 3.49 (t, 2, CH_2CH_2S); ^{31}P NMR (D_2O) 15.57 ppm (t, J = 13 Hz). Anal. ($C_6H_{14}LiN_2O_3PS_2 \cdot 1.5\ H_2O$): C, H, N, P, S.

This experiment was repeated on a 0.24 molar scale to give 94% of **13a**.

***N*-(2-Cyanoethyl)oxazolidinone (14).** The title compound was prepared by modification of a reported procedure.[26] To a vigorously stirred mixture of 2-oxazolidinone (87 g, 1 mol), 50% sodium hydroxide (w/w, 4 g), and toluene (250 mL) maintained at 55–60 °C; acrylonitrile (106 g, 2 mol) was added dropwise over a period of 30 min. The mixture was then stirred at 80 °C for 1 h and refluxed for 3 h. Additional acrylonitrile (50 mL) was added towards the end of the reaction to drive it to completion. Excess acrylonitrile was removed by distillation, and the residue was left at room temperature overnight. The mixture was filtered, cooled in an ice bath, and acidified with concentrated H_2SO_4 to pH ~1. The salt was removed by filtration, and the filtrate was dried over anhydrous $MgSO_4$. The residue obtained after removal of the solvent under vacuum was dried under high vaccum overnight to give 117 g (84%) of **14** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 2.68 (t, 2, CH_2CH_2CN), 3.57 (t, 2, NCH_2CH_2), 3.75 (t, 2, NCH_2CH_2O), and 4.39 (t, 2, CH_2CH_2O).

The product obtained was sufficiently pure to be used in the next step.

***N*-[2-(*N*-Methylamidino)ethyl]oxazolidinone Hydrochloride (16a).** A stirred solution of **14** (10 g, 0.07 mol) in dry CH_2Cl_2 (20 mL) and dry methanol (4.6 mL, 114 mmol) was saturated with dry HCl at −10 to −5 °C. After saturation, the mixture was stirred at −5 to 2 °C for 6 h and concentrated to dryness to give **15**·HCl as a white solid. Compound **15**·HCl was dissolved in dry methanol (90 mL) and cooled in a dry ice bath. To the stirred mixture at −40 °C (partial precipitation occurred) was added in one portion methylamine (5.6 g, 0.18 mol). Dry methanol (10 mL) was added to facilitate stirring, and stirring was continued for 30 min at −30 to −10 °C. The mixture was evaporated, and the resulting white solid was recrystallized from methanol–ether to give 10.7 g (72%) of **16a** as colorless needles: mp 169–172 °C; ^{1}H NMR (DMSO-d_6) δ 2.6 [t, 2, $-CH_2C(NH)-$], 2.78 (s, 3, $NHCH_3$), 3.52 [t, 2, $-CH_2CH_2C(NH)-$], 3.65 (t, 2, OCH_2CH_2), 4.26 (t, 2, OCH_2), 8.7 (br s, NH), 9.5 (br s, NH), and 10.1 (br s, NH). Anal. ($C_7H_{14}ClN_3O_2$): C, H, Cl, N.

***S*-[2-[[2'-(*N*-Methylamidino)ethyl]amino]ethyl]-phosphorothioic Acid (18a).** To a stirred solution of $Li_3SPO_3 \cdot 4.7H_2O$ (7.7 g, 0.036 mol) in water (167 mL) was added compound **17a** (13.9 g, 0.038 mol). After dissolution, DMF (83.5 mL) was added, and the stirring was continued for 2 h. After refrigeration overnight, the product was filtered and washed first with DMF (300 mL) and then ether (1.6 L). The solid was dissolved in water (120 mL) and treated with acetonitrile until crystals separated. After the mixture was cooled in an ice bath for 5 h, the colorless crystals were separated by filtration, washed with acetonitrile (330 mL) and ether (330 mL), and dried in vacuo overnight to give 7 g (67%) of **18a**: mp 111–113 °C; ^{1}H NMR (D_2O) δ 2.95–3.05 [m, 7, $CH_2CH_2C(N=)NCH_3$], and 3.39–3.46 (m, 4, SCH_2CH_2NH); ^{31}P NMR (D_2O) δ 15.57 (J = 13 Hz). Anal. ($C_6H_{16}N_3O_3PS \cdot 3H_2O$) C, H, N, P, S.

***N*-[2-(Methoxyimino)ethyl]oxazolidinone (15).** A stirred solution of **14** (100 g, 0.68 mol) in a mixture of dry CH_2Cl_2 (200 mL) and dry methanol (46 mL, 1.14 mol) was saturated with dry HCl at −10 to 5 °C and was stirred at 0 to 2 °C for 6 h. The mixture was poured into an ice-cold solution of K_2CO_3 (300 g in 1 L of H_2O) along with stirring and cooling. After stirring for 10 min, the mixture was extracted with chloroform. The dried (Na_2SO_4) chloroform extract was evaporated. The residue was dried under high vacuum overnight to give 100 g (82%) of **15** as a pale yellow oil. This product was sufficiently pure to use in the next step. An analytical sample was prepared by column chromatography (SiO_2, 5% CH_3OH–CH_2Cl_2): ^{1}H NMR (DMSO-d_6) δ 2.44 [t, 2, $CH_2C(NH)$], 3.36 [t, 2, $-CH_2CH_2C(NH)-$], 3.58 (m, 5, OCH_3, NCH_2CH_2O), 4.23 (br m, 2, OCH_2), and 8.1 (s, 1, NH). Anal. ($C_7H_{12}N_2O_3$): C, H, N.

***N*-(2-Amidinoethyl)oxazolidinone Hydrochloride (16b).** To a stirred solution of **15** (12.2 g, 0.071 mol) in dry methanol (40 mL) was added 3.8 g (0.071 mol) of NH_4Cl. The reaction was slightly exothermic. After the reaction was stirred for 1 h, ethyl ether was added to the point of turbidity, and the mixture was left in the refrigerator overnight. After filtration, the mother liquor was diluted with more ether (crystals separated) and cooled in an ice–salt bath for 4 h. The crystals were separated by filtration, washed with ether, and dried to give 9.3 g (62%) of **16b** as colorless crystals: mp 161–164 °C; ^{1}H NMR (DMSO-d_6) δ 2.65 [t, 2, $-CH_2C(NH)-$], 3.53 [t, 2, $CH_2CHC(NH)$], 3.63 (t, 2, OCH_2CH_2), 4.6 (t, 2, OCH_2), and 8.6–9.4 (br s, NH). Anal. ($C_6H_{12}ClN_3O_2$): C, H, Cl, N.

***S*-[2-[(2'-Amidinoethyl)amino]ethyl]phosphorothioic Acid (18b).** To a stirred solution of $Li_3SPO_3 \cdot 4.7H_2O$ (8.05 g, 0.037 mol) in water (175 mL) was added **17b** (14 g, 0.039 mol). After dissolution, DMF (87.5 mL) was added, and the mixture was stirred for 2 h. Most of the product had precipitated during that time. The mixture was refrigerated overnight, filtered, and washed first with DMF (350 mL) and then with ether (2 L). After the mixture was dried in vacuo overnight, 8 g (86%) of **18c** was obtained as a colorless solid: mp 177 °C dec; ^{1}H NMR (D_2O) δ 2.95–3.05 [m, 4, $-CH_2CH_2C(N=)-$], 3.4–3.49 (m, 4, $-SCH_2CH_2NH-$); ^{31}P NMR (D_2O) δ 16.11 (J = t, 11 Hz). Anal. ($C_5H_{14}N_3O_3PS \cdot 0.5H_2O$): C, H, N, P, S.

***N*-[2-(4,5-Dihydroimidazolyl)ethyl]oxazolidinone (19).** A mixture of 25.8 g (0.15 mol) of *N*-[2-(methoxyimino)ethyl]oxazolidinone **15** and 9 g (0.15 mol) of ethylenediamine in 50 mL of absolute ethanol was refluxed for 30 min. After cooling to room temperature, the mixture was treated with ether until crystals separated and then was refrigerated for 3 days. The colorless crystals were filtered and dried to give 19.9 g (73%) of **19**: mp 118–123 °C; ^{1}H NMR ($CDCl_3$) δ 2.54 [t, 2, $CH_2C(=N)$], 3.57 (s, 4, $=NCH_2CH_2—NH$), 3.62 (m, 4, CH_2NCH_2), 4.32 (m, 2, OCH_2), 4.57 (b, NH). Anal. ($C_8H_{13}N_3O_2$): C, H, N.

***S*-[2-[[2'-(4,5-Dihydroimidazolyl)ethyl]amino]ethyl] Lithium Hydrogen Phosphorothioate (21).** To a stirred solution of 16.8 g (0.044 mol) of **20** was added 8 g (0.039 mol) of $Li_3SPO_3 \cdot 4.1H_2O$ in 195 mL of water. The mixture was cooled in an ice bath, and DMF (97.5 mL) was added. The ice bath was removed, and stirring was continued for 30 min. Dicyclohexylamine (30 mL) was added, and the resulting mixture was stirred for another 30 min. The mixture was filtered, and the filtrate was added dropwise over a period of 20 min to 1.17 L of well-stirred DMF. After 20 min of stirring, the precipitate was filtered, washed first with DMF (700 mL) and then with ether (1.8 L), and dried in vacuo overnight to afford 9 g (73%) of **21** as a white powder: mp >150 °C dec; ^{1}H NMR (D_2O) δ 2.65–3.04 (m, 8 H), 3.83 (s, 4 H, $=NCH_2CH_2NH$); ^{31}P NMR (D_2O) δ 16.23 (t, J = 11 Hz). Anal. ($C_7H_{15}LiN_3O_3PS \cdot H_2O$): C, H, N, P, S.

Acknowledgment. We express our gratitude to Dr. Robert R. Engle and H. A. Musallam for many helpful suggestions and discussions during the course of this work. This work was supported by the U.S. Army Medical Research and Development Command under Research Contract DAMD-17-84-C-4002 and DAMD-17-85-C-5156. This paper has been designated as Contribution No. 1864 to the

(26) Lynn, J. W. U.S. Patent 2,975,187, March 14, 1961; *Chem. Abstr.* **1961**, *55*, 16568.

Army Drug Development program.

Registry No. 4a, 127914-02-7; 4b, 127914-17-4; 4c, 127914-18-5; 5, 886-64-6; 6, 107-95-9; 7, 127914-03-8; 8a, 127914-04-9; 8b, 127914-19-6; 8c, 127943-56-0; 9a, 127914-05-0; 9b, 127914-20-9; 9c, 127914-21-0; 10a, 127914-06-1; 10b, 127914-22-1; 10c, 127914-23-2; 11a, 127914-07-2; 11b, 127914-29-8; 12a, 127914-08-3; 12b, 127914-24-3; 13a, 127914-09-4; 13b, 127914-25-4; 14, 65194-10-7; 15, 127914-10-7; 15·HCl, 127914-26-5; 16a, 127914-11-8; 17a, 127914-12-9; 18a, 127914-13-0; 18b, 127914-27-6; 19, 127914-14-1; 20, 127914-15-2; 21, 127914-16-3; $Cl(CH_2)_2OCOCl$, 627-11-2; $Cl(CH_2)_2OCONH(CH_2)_2COCl$, 127914-28-7; $MeNH_2$, 74-89-5; $H_2N(CH_2)_2NH_2$, 107-15-3; 2-oxazolidinone, 497-25-6; acrylonitrile, 107-13-1.

Synthesis and Evaluation of Novel Electrophilic Nitrofuran Carboxamides and Carboxylates as Radiosensitizers and Bioreductively Activated Cytotoxins

Matthew A. Naylor,* Miriam A. Stephens, Shirley Cole, Michael D. Threadgill,† Ian J. Stratford, Peter O'Neill, E. Martin Fielden, and Gerald E. Adams

Medical Research Council Radiobiology Unit, Chilton, Didcot, Oxon OX11 ORD, U.K. *Received January 22, 1990*

A series of 5-nitrofuran-2- and 3-carboxamides bearing alkylating side-chains has been synthesized and tested for their ability to radiosensitize selectively hypoxic Chinese hamster cells (V79) to the lethal effects of ionizing radiation and also for their ability to act directly and selectively as cytotoxic drugs on hypoxic V79 cells. The compounds were extremely efficient radiosensitizers of such cells in vitro and were more efficient than known nitroimidazoles of similar type. Their efficiencies as radiosensitizers correlated with their high electron affinity (E_7^1) as measured by pulse-radiolysis. However the compounds showed little radiosensitizing activity towards KHT sarcomas in C3H mice. The compounds in this series of nitrofurans were generally more toxic towards hypoxic cells than towards oxic cells in vitro but were less effective upon the basis of a differential effect than were similar nitroimidazoles reported previously.

Nitroheterocyclic compounds can act as radiosensitizers of hypoxic cells and as bioreductively activated cytotoxins.[1] Radiosensitization is a fast, free-radical process, and a correlation has been observed between the one-electron reduction potentials (E_7^1) of a large number of chemically diverse nitroheterocycles and their ability to act as radiosensitizers of hypoxic cells.[2,3] Nitro compounds can be reductively metabolized to form highly potent cytotoxins. Since bioreductive activation occurs more readily in hypoxic tissue, there is a sound basis for a high degree of specificity in poorly oxygenated solid tumors.

Both the bioreductive activity and radiosensitizing efficiency of 2-nitroimidazoles can be greatly increased by incorporating monofunctional alkylating groups into the molecule. One of the first examples of such a compound was α-(1-aziridinylmethyl)-2-nitro-1*H*-imidazole-1-ethanol (RSU-1069, 1),[4,5] a 2-nitroimidazole bearing an aziridine (Figure 1). This compound is about 5–10-fold more efficient than misonidazole (a related nitroimidazole not containing an alkylating group) as a radiation sensitizer of experimental tumors and shows considerably more cytotoxicity when reductively metabolized under hypoxic conditions. Preliminary clinical investigation of RSU-1069 has revealed gastrointestinal toxicity which restricts doses to levels not likely to produce significant radiosensitization.[6] Various analogues have been synthesized and evaluated in attempts to reduce toxicity towards normal tissues without a corresponding reduction in radiosensitization of tumors. Examples include compounds in which the aziridine group is deactivated by substitution.[7] Lower toxicity can also be achieved by using a prodrug of RSU-1069 such as the new and recently reported compound α-[[(2-bromoethyl)amino]methyl]-2-nitro-1*H*-imidazole-1-ethanol hydrobromide (RB-6145, 2).[8]

Increasing therapeutic benefit can also be achieved by increasing radiosensitizing potency. One possible way of obtaining this is to raise the redox potential of the nitroheterocycle. For example 5-nitrofurans are generally more electron affinic than 2-nitroimidazoles and both 5-nitro-2-furaldehyde semicarbazone (nitrofurazone, 3) and 5-nitro-2-furaldoxime (nifuroxime, 4) have been found to be very effective radiosensitizers of hypoxic cells in tissue culture systems.[9,10] We have investigated nitrofurans bearing alkylating side-chains, and with E_7^1 values of between −210 and −350 mV (ie; both higher and lower E_7^1 than the known nonalkylating sensitizer nitrofurazone, 3) by altering the patterns of substitution. Of particular importance is the position of the nitro group on the furan ring and possible conjugation with electron withdrawing substituents. Changes in the nature of the substituents likely to have an effect on electron affinity have also been investigated. This paper describes studies with the nitrofurans having the highest (most positive) electron affinities of those synthesized, namely the 5-nitrofuran carboxamides and propenamides bearing side chains of varying electrophilic reactivity.

† Present address: School of Pharmacy, University of Bath, Bath, BA2 7AY, U.K.

(1) Collected papers in *Biochem. Pharmacol.* **1986**, *35*, 1–122 (Eds. Alexander, P., Gielen, J., Sartorelli, A. C.).
(2) Adams, G. E.; Clarke, E. D.; Flockhart, I. R.; Jacobs, R. S.; Sehmi, D. S.; Stratford, I. J.; Wardman, P.; Watts, M. E.; Parrick, J.; Wallace, R. G.; Smithen, C. E. *Int. J. Radiat. Biol.* **1979**, *35*, 133.
(3) Adams, G. E.; Flockhart, I. R.; Smithen, C. E.; Stratford, I. J.; Wardman, P.; Watts, M. E. *Radiat. Res.* **1976**, *67*, 9.
(4) Adams, G. E.; Ahmed, I.; Sheldon, P. W.; Stratford, I. J. *Br. J. Cancer* **1984**, *49*, 571.
(5) Adams, G. E.; Ahmed, I.; Sheldon, P. W.; Stratford, I. J. *Int. J. Radiat. Oncol. Biol. Phys.* **1984**, *10*, 1653.
(6) Horwich, A.; Holliday, S. B.; Deacon, J. M.; Peckham, M. J. *Br. J. Radiol.* **1986**, *59*, 1238.
(7) Ahmed, I.; Jenkins, T. C.; Walling, J. M.; Stratford, I. J.; Sheldon, P. W.; Adams, G. E.; Fielden, E. M. *Int. J. Radiat. Oncol. Biol. Phys.* **1986**, *12*, 1079.
(8) Jenkins, T. C.; Naylor, M. A.; O'Neill, P.; Threadgill, M. D.; Cole, S.; Stratford, I. J.; Adams, G. E.; Fielden, E. M.; Suto, M. J.; Stier, M. A. *J. Med. Chem.*, in press.
(9) Chapman, J. D.; Reuvers, A. P.; Borsa, J.; Petkau, A.; McCalla, D. R. *Cancer Res.* **1972**, *32*, 2618.
(10) Sutherland, R. M.; Durand, R. E. *Int. J. Radiat. Biol.* **1972**, *22*, 613.

0022-2623/90/1833-2508$02.50/0