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

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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.

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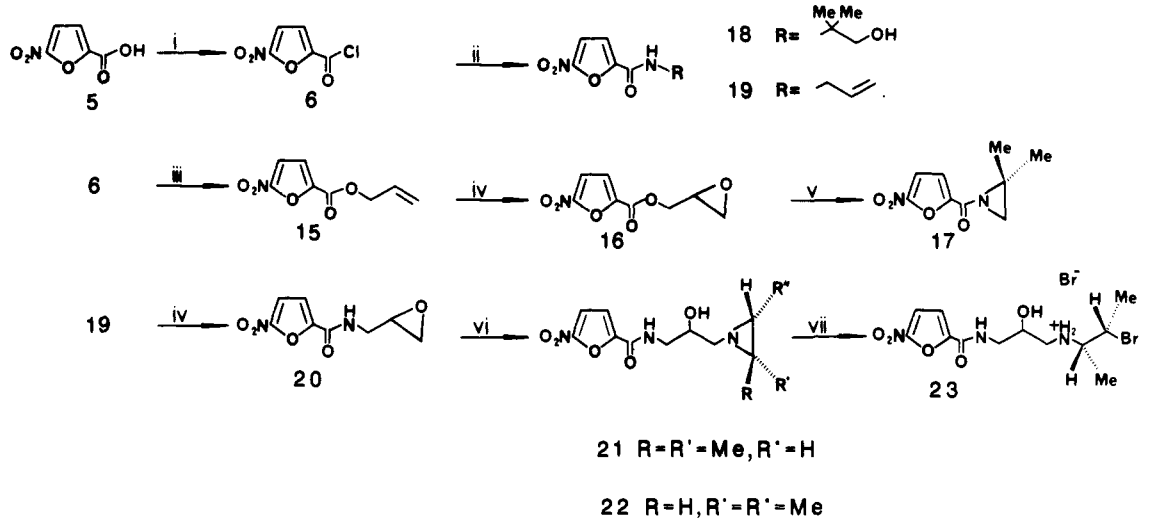
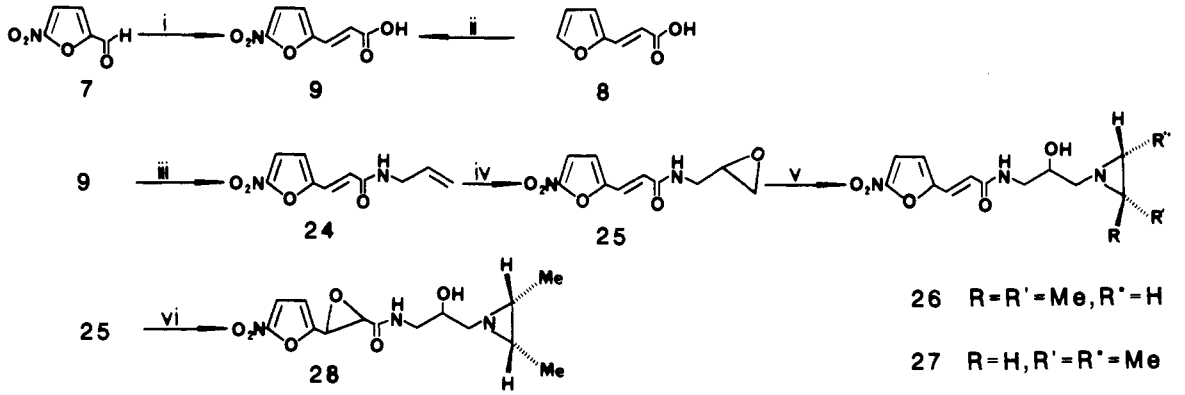
**Scheme I**[a]



[a] Reagents: (i) $SOCl_2$/DMF; (ii) $RNH_2/Et_3N/CH_2Cl_2$; (iii) allylalcohol/$Et_3N/CH_2Cl_2$; (iv) MCPBA/$CH_2Cl_2$; (v) 2,2-dimethylaziridine/THF; (vi) *cis*-2,3-dimethylaziridine/THF; (vii) HBr (aq).

**Scheme II**[a]



[a] Reagents: (i) $Ac_2O$/NaOAc; (ii) $Ac_2O/HNO_3$; (iii) allylamine/$Et_3N/CH_2Cl_2$; (iv) MCPBA/$CH_2Cl_2$; (v) 2,2-dimethylaziridine/THF; (vi) *cis*-2,3-dimethylaziridine/THF/MCPBA/$Et_3N$.

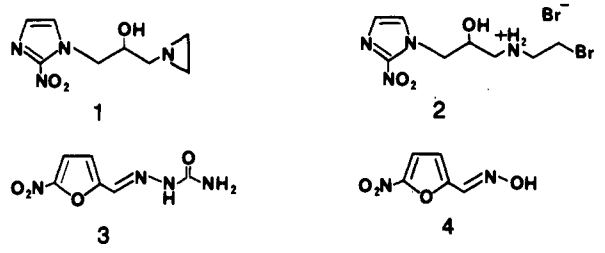


**Figure 1.**

## Chemistry

The common starting material for the series of 5-nitrofuran-2-carboxamides **15–23** (of which, **15** has been reported previously[11]) was 5-nitro-2-furoyl chloride, prepared from commercially available 5-nitro-2-furoic acid (**5**) by treatment with refluxing thionyl chloride in the presence of a catalytic amount of dimethylformamide (DMF). Subsequent amides or esters were then prepared directly from this intermediate with the appropriate alcohol or amine (Scheme I).

Compounds **24–28** were prepared from the common starting material 5-nitro-2-furylacrylic acid (**9**), prepared by Perkin condensation from 5-nitro-2-furaldehyde (**7**) with acetic anhydride/sodium acetate or directly by nitration of 2-furylacrylic acid (**8**). Corresponding propenamides (of which, **24** has been reported previously as an antibacterial agent[12]) were then prepared from the acyl chloride (Scheme II).

Compounds **29** and **30** were prepared by nitration of 2-methyl-3-furoic acid (**11**) according to the procedure of Gilman et al.,[13] followed by coupling to allylamine by a mixed anhydride method. This coupling was used owing to the instability of 2-methyl-5-nitrofuran-3-carboxylic acid (**12**) in the presence of thionyl chloride. The 3-nitro analogue **31** was prepared from 2-methyl-5-furoic acid by nitration of the methyl ester (**13**) according to the method of Rinkes,[14] followed by direct coupling of the hydrolyzed ester (**14**) with allylamine (Scheme III).

Oxiranes **16**, **20**, **25**, and **30** were generally synthesized from the allyl amide or ester by treatment with 3-chloroperbenzoic acid (MCPBA). With the exception of **30**, which was broken down to unidentified products in refluxing aziridines under a variety of conditions, treatment of these oxiranes with the appropriate aziridines in boiling THF gave the N-substituted aziridines (**21**, **22**, **26**, and **27**,

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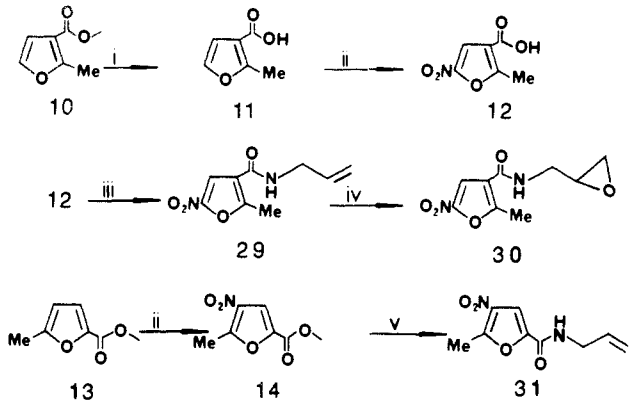
**Scheme III**[a]



[a] Reagents: (i) $NaOH/H_2O/EtOH$; (ii) $Ac_2O/HNO_3$; (iii) ethyl chloroformate/allylamine/$Et_3N/CH_2Cl_2$; (iv) $MCPBA/CH_2Cl_2$; (v) NaOH/EtOH then allylamine/DCC/THF.

Schemes I and II). Treatment of the oxiranes with unsubstituted aziridine was unsuccessful due to the instability of the products. Treatment of the ester **16** with 2,2-dimethylaziridine gave only the *N*-acyl aziridine **17** by reaction at the carbonyl center of the molecule rather than at the oxirane (Scheme I). 2,2-Dimethylaziridine and stereochemically pure *cis*-2,3-dimethylaziridine were prepared from the appropriately substituted 2-aminoethanols by O-sulfation and elimination with potassium hydroxide, according to the methods of Dickey et al.[15] The potentially bifunctionally alkylating analogue **28** was prepared by reaction of **25** with *cis*-2,3-dimethylaziridine in the presence of MCPBA and triethylamine ($Et_3N$). Compound **18**, with a nonalkylating hydroxyethyl substituent was prepared from the corresponding acyl chloride and the appropriate amine as described. The ring-opened analogue **23** was prepared by treatment of **21** with HBr by methods similar to those we have described elsewhere for a series of 2-nitroimidazoles.[8]

## Results and Discussion

The 5-nitrofurans investigated show high radiosensitizing activity in vitro with $C_{1.6}$ values of as low as 8 $\mu$M for compounds **25** and **26**, 5-nitro-2-furanpropenamides with oxiranyl and 2,2-dimethylaziridinyl substituents, respectively (Table I). Generally $C_{1.6}$ values of 10–50 $\mu$M were found for this series of nitrofurans. These radiosensitizing efficiencies compare favorably with that of compound **3** and all compounds are more efficient radiosensitizers than the nitroimidazoles such as misonidazole and RSU-1069 (**1**),[4,5] as would be expected for such highly electron affinic compounds. The less oxidizing 3-nitro analogue, **31**, is considerably less effective as a radiosensitizer. With the exception of compounds **25** and **26**, which were considerably more potent sensitizers than their nonalkylating analogues, there appears to be little benefit in terms of radiosensitizing efficiency when bifunctional character is introduced in this series of compounds. This is in contrast to the trends observed with nitroimidazoles.

The 5-nitrofurans in this study showed some selective activity in the assay used for direct bioreductive cytotoxicity. The highest ratios of air to nitrogen toxicity ($C_{50}$(air)/$C_{50}(N_2)$) are displayed by those compounds having aziridinyl or (bromoethyl)amino substituents, but these ratios are lower than those observed in our previous studies with nitroimidazoles. For example, compounds bearing the 2,2-dimethylaziridine substituent (**21** and **26**) are 5–10 times less effective as hypoxia-selective cytotoxins than is the corresponding nitroimidazole analogue $\alpha$-((2,2-dimethyl-1-aziridinyl)methyl)-2-nitro-1*H*-imidazole-1-ethanol (RSU-1150).[7] The most potent compound showing direct hypoxia-selective cytotoxic properties is the (2-bromo-1,1-dimethylethyl)amino compound **23**, with a differential toxicity ratio of 6. This is a comparable differential to that found for the known nitrofuran, nitrofurazone **3**, but the oxic and hypoxic toxicities are 10 times higher. With the exception of **25**, differential cytotoxicity was not shown by the corresponding oxiranes.

The toxicities of these compounds towards oxic cells are at least 1 order of magnitude greater than are the toxicities of similar nitroimidazoles, a property which could account for the poor differentials observed. High oxic toxicities could be related to high one-electron reduction potentials,[2] coupled with alkylating moieties. However, a number of compounds (**15**, **18**, **19**, and **24**) have higher toxicities than **3** despite comparable electron-affinities and similarly do not possess alkylating moieties. It therefore appears that other factors are contributing towards toxicity in this series of furans. One-electron reduction potentials ($E_7^1$) measured at pH 7 for a selection of the 5-nitrofuran-2-carboxamides and propenamides using a pulse-radiolytic method were found to be between −210 and −270 mV, ie; 100–150 mV more positive than the nitroimidazoles (Table I). There is some variation in $E_7^1$ with changes in the side chain at the 2-position. The compounds bearing aziridinyl groups are more electron affinic than their oxiranyl analogues, but sensitizing efficiency was unaffected by these changes. In view of the high oxic toxicity and poor hypoxia selectivity that appears to be related to highly positive $E_7^1$, the re-

**Table I.** Physicochemical and in Vitro Biological Data of Nitrofurans and Selected Nitroimidazoles

| compound | $-E_7^1$, mV | radiosensitization:[a] $C_{1.6}$, mM | $C_{50}$,[b] mM air | $C_{50}$,[b] mM $N_2$ | d tox[c] |
|---|---|---|---|---|---|
| misonidazole | 389[d] | 0.3[d] | 45.0 | 4.0 | 11.3 |
| **1** | 389[e] | 0.1[f] | 0.3 | 0.0045 | 66.6 |
| **2** | nd[m] | 0.1[g] | 2.3[h] | 0.09[h] | 25.6[h] |
| **3** | 257[i] | 0.05[j] | 1.7 | 0.2 | 8.5 |
| **15** | 211[k] | 0.05 | 0.1 | 0.025 | 4.0 |
| **16** | 268[k] | 0.025 | 0.2 | 0.075 | 2.7 |
| **17** | nd | 0.1 | 0.18 | 0.18 | 1.0 |
| **18** | 220[k] | 0.05 | 0.25 | 0.12 | 2.1 |
| **19** | nd | 0.05 | 0.08 | 0.045 | 1.8 |
| **20** | 241[k] | 0.02 | 0.05 | 0.05 | 1.0 |
| **21** | nd | 0.04 | 0.15 | 0.075 | 2.0 |
| **22** | 219[k] | 0.05 | 0.06 | 0.02 | 3.0 |
| **23** | nd | 0.03 | 0.14 | 0.025 | 5.6 |
| **24** | nd | 0.03 | 0.06 | 0.025 | 2.4 |
| **25** | 252[k] | 0.008 | 0.03 | 0.01 | 3.0 |
| **26** | nd | 0.008 | 0.045 | 0.01 | 4.5 |
| **27** | 231[k] | 0.035 | 0.07 | 0.04 | 1.8 |
| **28** | 335[k] | 0.025 | 0.012 | 0.012 | 1.0 |
| **29** | 325[k] | 0.075 | 0.35 | 0.8 | 0.4 |
| **30** | 327[k] | 0.04 | 0.1 | 0.1 | 1.0 |
| **31** | >600[k] | nd[l] | 0.015 | 0.03 | 0.5 |

[a] $C_{1.6}$ = molar concentration of the compound required to give a sensitizer enhancement ratio (ER) of 1.6. Hence the lower the value of $C_{1.6}$ the greater is the sensitizing efficiency. [b] $C_{50}$ (Air or $N_2$) = concentration required to reduce optical density by 50% in the MTT assay (proportional to cell killing).[20] [c] d tox = differential toxicity; $C_{50}$ (air)/$C_{50}$ ($N_2$) (mean of at least three experiments). [d] Data taken from ref 3. [e] Data taken from ref 21. [f] Data taken from ref 7. [g] Data taken from ref 8. [h] These values are concentrations required to reduce cell survival to 1%.[8] [i] Data taken from ref 22. [j] Data taken from ref 2. [k] Referred to the normal hydrogen electrode (NHE) using benzyl viologen as redox couple; $[BV^{2+}/BV^{\bullet+}] = -354$ mV. [l] Not determined due to toxicity. ER = 1.4 at 0.02 mM. [m] nd = not determined.

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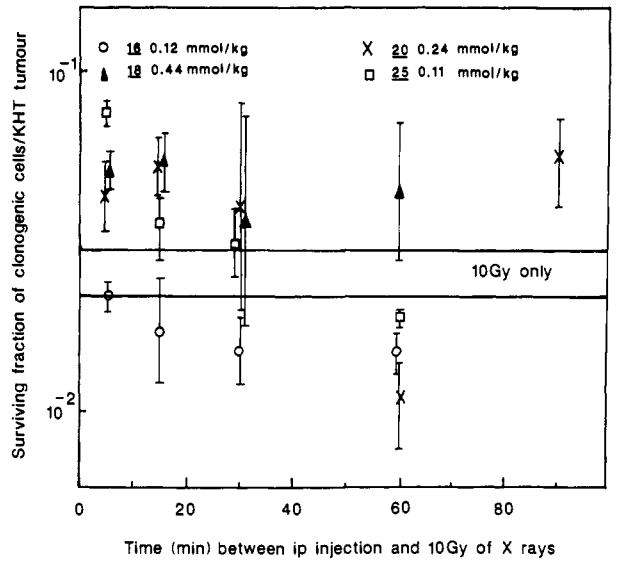


**Figure 2.** Evaluation of nitrofurans as radiosensitizers in vivo.

duction potential was lowered to below -300 mV by the synthesis of analogues bearing the functionalized side chain at the 3-position and a methyl group at the 2-position (compounds **29** and **30**). These analogues were less toxic than the corresponding 2-carboxamides but showed no improvement in differential toxicity. Compound **28**, in which the conjugation between the furan ring and propenamide side chain (present in compounds **24–27**) was removed by epoxidation, had a greatly lowered $E_7^1$ of -335 mV. This analogue, however, showed a slight increase in both aerobic and anaerobic toxicity over **24–27**, probably due to the presence of two potential alkylating moieties and showed no hypoxia selectivity. The only 3-nitro analogue synthesized, **31**, showed no differential cytotoxicity towards hypoxic cells and in fact displayed slightly higher oxic toxicity. The $E_7^1$ was measured for this compound and found to be <-600 mV. Further 3-nitro analogues of this type were therefore not investigated in view of probable similarly low electron affinities.

Despite promising in vitro radiosensitization results with this series of nitrofurans, and some improvement over nitrofurazone(**3**), they were ineffective as radiosensitizers of murine KHT tumors in vivo (Figure 2), and no greater killing of cells could be achieved than was apparent with radiation alone. Indeed, slight radioprotection appeared to occur 5–15 min after administration of **18**, **20**, and **25**, possibly as a consequence of unknown physiological effects of these agents. Only **16** showed a consistent but small radiosensitizing effect. Together with possible poor distribution and tumor uptake, the ineffectiveness of the compounds as radiosensitizers in vivo could be a consequence of rapid metabolism and/or interactions of the compounds with noncritical targets in the animals, as has been postulated previously with some other nitrofurans.[16,17] This could involve, for example, the reaction of metabolized nitrofurans with cellular thiol groups, although in culture medium we have not found these compounds to be reactive towards the thiols glutathione and *N*-acetylcysteine.

In conclusion, these nitrofurans show little promise as mixed-function radiosensitizers of clinical potential unless the high oxic toxicity can be overcome and significant radiosensitizing activity in vivo can be demonstrated. It is pertinent to note, however, that the doses of the nitrofurans which could be administered to mice were relatively low compared to doses used for similar nitroimidazoles, even with those compounds which were not limited by poor solubility. The relatively high toxicity of these compounds toward oxic cells in vitro may lead to dose-limiting toxicity towards normal tissues in vivo. The design of nitrofurans and other nitroheteroarenes with lower (more negative) $E_7^1$ values may reduce oxic toxicity and could lead to greater hypoxia selectivity. This approach is currently being investigated in this laboratory.

## Experimental Section

**Chemical Synthesis.** NMR Spectra were obtained at 60 MHz with a Jeol PMX60SI spectrometer with $SiMe_4$ as internal standard. Mass spectra were carried out on a Finnigan 4500 instrument in the electron-impact mode. Melting points are corrected. $CH_2Cl_2$ was purified by passing through basic alumina, drying over $CaCl_2$, and distilling prior to use. Tetrahydrofuran (THF) was distilled from $CaH_2$; other solvents were unpurified commercial grades unless stated otherwise. Commercially available 3-chloroperbenzoic acid (MCPBA) was further purified by washing with phosphate buffer (pH 7) and drying the residue at reduced pressure in the presence of $P_2O_5$. Elemental analyses were determined by Elemental Microanalysis Ltd., Okehampton, UK. Solutions in organic solvents were dried by treatment with anhydrous $Na_2SO_4$ and filtration. Solvents were removed by evaporation under reduced pressure. All compounds were racemic. The one-electron reduction potentials ($E_7^1$) of selected compounds (Table I) were determined by using pulse-radiolysis techniques as previously described.[18]

**Prop-2-enyl 5-Nitrofuran-2-carboxylate (15).** 5-Nitro-2-furoyl chloride (0.5 g, 2.85 mmol) in $CH_2Cl_2$ (3 mL) was added to allyl alcohol (1.4 g, 24 mmol) and $Et_3N$ (3 mL) in $CH_2Cl_2$ (5 mL) at 0 °C under $N_2$. The solution was stirred at 0 °C for 1 h, poured onto ice (50 g), and extracted with $CH_2Cl_2$ (50 mL). The organic layer was washed with 5% aqueous $NaHCO_3$ and $H_2O$ and dried. The solvent was evaporated, and the residue was recrystallized from EtOH to give **15** (0.35 g, 62%) as pale yellow prisms: mp 71–71.5 °C; NMR ($CDCl_3$) $\delta$ 4.8 (d, $J$ = 6 Hz, 2 H, allylic $CH_2$), 5.2–6.2 (m, 3 H, olefinic-H), 7.25 (s, 2 H, furan-H). Anal. ($C_8H_7NO_5$) C, H, N.

**Oxiranylmethyl 5-Nitrofuran-2-carboxylate (16).** Dried 3-chloroperbenzoic acid (7.3 g, 42 mmol) in $CH_2Cl_2$ (75 mL) was added to **15** (3.5 g, 17.7 mmol) in $CH_2Cl_2$ (30 mL). The solution was heated under gentle reflux for 1.5 h before being washed with 10% aqueous $Na_2SO_3$ (100 mL), 5% aqueous $NaHCO_3$ (twice), $H_2O$, and saturated aqueous NaCl. The organic layer was then dried, and the solvent was evaporated. The residue was recrystallized from EtOH to give **16** (2.3 g, 61%) as a colorless solid: mp 98–99 °C; NMR ($CDCl_3$) $\delta$ 2.7 (dd, $J$ = 4.5 and 3 Hz, 1 H) and 2.9 (t, $J$ = 4.5 Hz, 1 H), oxiranyl 3-$CH_2$, 3.25 (m, 1 H, oxiranyl 2-H), 4.2 (dd, $J$ = 12 and 6 Hz, 1 H), and 4.7 (dd, $J$ = 12 and 3 Hz, 1 H), $CO_2CH_2$, 7.25 (s, 2 H, furan-H). Anal. ($C_8H_7NO_6$) C, H, N.

**2,2-Dimethyl-1-(5-nitro-2-furoyl)aziridine (17).** Epoxide ester **16** (1.0 g, 5 mmol) was stirred at 20 °C with 2,2-dimethylaziridine (4.0 g, 56.3 mmol) for 5 h. Excess aziridine was then evaporated, and the residue was recrystallized from EtOH to give **17** (0.6 g, 57%) as a pale yellow solid: mp 104–106 °C dec; NMR ($CDCl_3$) $\delta$ 1.5 (s, 6 H, aziridine-$(CH_3)_2$), 2.45 (s, 2 H, aziridine $CH_2$), 7.2 (d, $J$ = 3.5 Hz, 1 H, furan-H), 7.3 (d, $J$ = 3.5 Hz, 1 H, furan-H). Anal. ($C_9H_{10}N_2O_4$) C, H, N.

***N*-(1,1-Dimethyl-2-hydroxyethyl)-5-nitrofuran-2-carboxamide (18).** 5-Nitro-2-furoyl chloride (1.5 g, 8.6 mmol) in $CH_2Cl_2$ (10 mL) was added to 2-amino-2-methylpropan-1-ol (10 mL, ca. 100 mmol) and $Et_3N$ (10 mL) in $CH_2Cl_2$ (30 mL). The mixture was stirred at 25 °C for 0.5 h and was then washed with $H_2O$ and dried. The solvent was evaporated, and the residue was recrystallized twice from $CH_2Cl_2$ to afford **18** (1.35 g, 69%) as a colorless solid: mp 110–112 °C; NMR ($CDCl_3$) $\delta$ 1.4 (s, 6 H, $(CH_3)_2$), 3.65

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(s, 2 H, $CH_2OH$), 6.5 (br, 1 H, NH), 7.2 (d, $J$ = 3.5 Hz, 1 H, furan-H), 7.3 (d, $J$ = 3.5 Hz, 1 H, furan-H). Anal. ($C_9H_{12}N_2O_5$) C, H, N.

**5-Nitro-*N*-(prop-2-enyl)furan-2-carboxamide (19).** 5-Nitro-2-furoyl chloride (5.0 g, 28.5 mmol) in $CH_2Cl_2$ (50 mL) was added dropwise to allylamine (12 mL, 276 mmol) and $Et_3N$ (30 mL) in $CH_2Cl_2$ (50 mL) at 0 °C under $N_2$, and the solution was stirred at 0 °C for a further 0.75 h. The mixture was washed with 5% aqueous $NaHCO_3$ and saturated aqueous NaCl and was dried. Evaporation of the solvent and recrystallization from EtOH gave **19** (3.3 g, 59%) **5** as pale yellow needles: mp 64–65.5 °C; NMR ($CDCl_3$) $\delta$ 4.1 (br t, $J$ = 6 Hz, 2 H, allylic $CH_2$), 5.2 (br d, $J$ = 13 Hz, 1 H) and 5.3 (br d, $J$ = 17 Hz, 1 H) olefinic $CH_2$, 5.6–6.2 (m, 1 H, allylic 2-H), 7.0 (br, 1 H, NH), 7.25 (d, $J$ = 3.5 Hz, furan-H), 7.35 (d, $J$ = 3.5 Hz, furan-H). Anal. ($C_8H_8N_2O_4$) C, H, N.

***N*-(Oxiranylmethyl)-5-nitrofuran-2-carboxamide (20).** Epoxidation of **19** was carried out as described for the preparation of **16**. The product was recrystallized from EtOH to afford **20** (1.14 g, 53%) as colorless prisms: mp 110–112 °C; NMR ($CDCl_3$) $\delta$ 2.6–4.1 (m, 5 H, oxirane-$CH_2$ and oxiranyl-H), 6.9 (br, 1 H, NH), 7.2 (d, $J$ = 3.5 Hz, furan-H), 7.3 (d, $J$ = 3.5 Hz, furan-H). Anal. ($C_8H_8N_2O_5$) C, H, N.

***N*-(3-(2,2-Dimethyl-1-aziridinyl)-2-hydroxypropyl)-5-nitrofuran-2-carboxamide (21).** Epoxide **20** (0.2 g, 0.94 mmol) was heated under reflux with 2,2-dimethylaziridine (0.2 g, 2.8 mmol) and $Et_3N$ (0.03 mL) in THF (3 mL) for 5 h. The solvent was then evaporated, and the residue was purified by chromatography (silica gel (75 g); EtOH/$Et_3N$ (99:1, v/v)). Recrystallization from EtOH/$Et_3N$ (99:1) afforded **21** (0.07 g, 26%) as a pale yellow solid: mp 94–95 °C; NMR ($CDCl_3$) $\delta$ 1.2–1.3 (m, 7 H, aziridine-$(CH_3)_2$ and aziridine-H), 1.85 (d, $J$ = 3 Hz, 1 H, aziridine-H), 2.3–2.6 (m, 2 H, $CHCH_2$ aziridine), 3.6–3.9 (m, 4 H, $NHCH_2CHOH$), 7.20 (d, $J$ = 3.5 Hz, furan-H), 7.25 (d, $J$ = 3.5 Hz, furan-H), 7.8 (br, 1 H, NH). Anal. ($C_{12}H_{17}N_3O_5$) C, H, N.

***N*-(3-(*cis*-2,3-Dimethyl-1-aziridinyl)-2-hydroxypropyl)-5-nitrofuran-2-carboxamide (22).** Epoxide **20** was treated with *cis*-2,3-dimethylaziridine as for the preparation of **21** above to give **22** (0.32 g, 32%) as a pale yellow solid: mp 87–89 °C; NMR ($CDCl_3$) $\delta$ 1.2 (d, $J$ = 6 Hz, 6 H, 2 × aziridine-$CH_3$), 1.5 (m, 2 H, 2 × aziridine-H), 2.3 (dd, $J$ = 13 and 5 Hz, 1 H), and 2.45 (dd, $J$ = 13 and 5 Hz, 1 H) $CHCH_2$ aziridine, 3.6–4.0 (m, 4 H, $NHCH_2CHOH$), 7.15 (d, $J$ = 3.5 Hz, furan-H), 7.25 (d, $J$ = 3.5 Hz, furan-H), 7.7 (br, 1 H, NH); MS, $m/z$ 284 $(M + H)^+$, 266, 240, 139, 84 (100). Anal. ($C_{12}H_{17}N_3O_5$) C, H, N.

***N*-(3-((2-Bromo-1,1-dimethylethyl)amino)-2-hydroxypropyl)-5-nitrofuran-2-carboxamide Hydrobromide (23).** Aziridine **21** (0.1 g, 0.35 mmol) was treated with 48% aqueous HBr (2 mL) at 25 °C for 10 min, and the excess reagent was evaporated under reduced pressure at 40 °C. $H_2O$ (2 mL) was added and was evaporated; this operation was repeated. A small volume of EtOH was added to the residue, and the product was precipitated with $Et_2O$ and recrystallized from aqueous acetone to afford **23** (0.05 g, 40%) as a yellow solid: mp 115–117 °C; NMR ($CDCl_3$) $\delta$ 1.8 (s, 6 H, $(CH_3)_2$), 3.4 (m, 2 H, $CHCH_2^+NH_2$), 3.8–4.5 (m, 4 H, $NHCH_2CHOH$), 4.2 (s, 2 H, $CH_2Br$), 7.6 (s, 2 H, furan-H). Anal. ($C_{12}H_{19}N_3O_5Br_2 \cdot 0.5H_2O$) C, H, N.

**3-(5-Nitrofuran-2-yl)-*N*-(prop-2-enyl)propenamide (24).** 5-Nitro-2-furaldehyde (5.4 g, 40 mmol) and dry powdered NaOAc (2.0 g, 40 mmol) were added to acetic anhydride (10 mL), and the mixture heated was at 140–150 °C for 2.5 h. The dark solution was then cooled to 80 °C, and $H_2O$ (100 mL) was added, together with decolorizing charcoal, and the solution was boiled under reflux for 10 min. The hot solution was filtered and acidified with 2 M aqueous HCl. Cooling afforded 3-(5-nitrofuran-2-yl)propenoic acid (4.2 g, 57%) as a pale brown solid: mp 228–230 °C, (lit.[19] mp 235–236 °C). This material (1.83 g, 10 mmol) was boiled under reflux in $SOCl_2$ (3 mL) and dimethylformamide (0.1 mL) for 3.5 h. The $SOCl_2$ was evaporated, and the resulting acyl chloride, in $CH_2Cl_2$ (20 mL), was added dropwise under $N_2$ to allylamine (5 mL, ca. 70 mmol) and $Et_3N$ (12 mL) in $CH_2Cl_2$ (20 mL) at 0 °C. The solution was stirred at 0 °C for 1.5 h before being washed with $H_2O$, 5% aqueous $NaHCO_3$, and saturated aqueous NaCl and dried. The solvent was removed, and the residue was twice recrystallized from $CH_2Cl_2$ to give **24** (1.1 g, 50%) as pale yellow needles: mp 168–169 °C; NMR ($CDCl_3$) $\delta$ 4.1 (dt, $J$ = 7 and 1 Hz, 2 H, allylic $CH_2$), 5.20 (br d, $J$ = 13 Hz, 1 H), and 5.25 (br d, $J$ = 18 Hz, 1 H), and 5.6–6.2 (m, 1 H), $CH{=}CH_2$, 6.2 (br t, $J \approx 7$ Hz, 1 H, NH), 6.7 (d, $J$ = 16 Hz, 1 H, furan-CH=C*H*), 6.7 (d, $J$ = 3.5 Hz, 1 H, furan 3-H), 7.3 (d, $J$ = 3.5 Hz, 1 H, furan 4-H), 7.4 (d, $J$ = 16 Hz, 1 H, furan-C*H*=CH). Anal. ($C_{10}H_{10}N_2O_4$) C, H, N.

***N*-(Oxiranylmethyl)-3-(5-nitrofuran-2-yl)propenamide (25).** Epoxidation of **24** (1.0 g, 4.5 mmol) was carried out as described for compounds **16** and **20** but with an extended reaction time of 2.5 h and purification by column chromatography (silica gel; $CH_2Cl_2$) to afford, after recrystallization from EtOH, **25** (0.5 g, 47%) as yellow needles: mp 175–177 °C; H NMR ($CDCl_3$) $\delta$ 2.65 (dd, $J$ = 5 and 3 Hz, 1 H) and 2.85 (t, $J$ = 5 Hz, 1 H), and 3.2 (m, 1 H) 3 × oxirane-H, 3.3–4.1 (m, 2 H, $NCH_2$), 6.3 (br, 1 H, NH), 6.65 (d, $J$ = 16 Hz, 1 H, furan-CH=C*H*), 6.7 (d, $J$ = 3.5 Hz, 1 H, furan 3-H), 7.3 (d, $J$ = 3.5 Hz, 1 H, furan 4-H), 7.4 (d, $J$ = 16 Hz, 1 H, furan-C*H*=CH); MS, $m/z$ 239 $(M + H)^+$, 192, 166 (100), 134. Anal. ($C_{10}H_{10}N_2O_5$) C, H, N.

***N*-(3-(2,2-Dimethyl-1-aziridinyl)-2-hydroxypropyl)-3-(5-nitrofuran-2-yl)propenamide (26).** **25** (0.2 g, 0.84 mmol) was heated with 2,2-dimethylaziridine (0.2 g, 2.8 mmol) and $Et_3N$ (0.03 mL) in THF (3 mL) under reflux for 6 h. The solvent was then removed, and the residue was purified by centrifugally accelerated layer chromatography (silica gel; EtOH/$Et_3N$ (99:1 v/v)) to give **26** (0.15 g, 58%) as a pale yellow solid: mp 149–150 °C; NMR ($CDCl_3$) $\delta$ 1.3–1.5 (m, 7 H, $C(CH_3)_2$ and aziridine-H), 1.85 (d, $J$ = 3 Hz, 1 H, aziridine-H), 2.3–2.6 (m, 2 H, $CHCH_2$-aziridine), 3.8–4.2 (m, 4 H, $NHCH_2CHOH$), 6.65 (d, $J$ = 16 Hz, 1 H, furan-CH=C*H*), 6.7 (d, $J$ = 3.5 Hz, 1 H, furan 3-H), 7.3 (d, $J$ = 3.5 Hz, 1 H, furan 4-H), 7.4 (d, $J$ = 16 Hz, 1 H, furan-C*H*=CH); MS $m/z$ 310 $(M + H)^+$ (100), 85, 70, 56. Anal. ($C_{14}H_{19}N_3O_5 \cdot 0.33H_2O$) C, H, N.

***N*-(3-(*cis*-2,3-Dimethyl-1-aziridinyl)-2-hydroxypropyl)-3-(5-nitrofuran-2-yl)propenamide (27).** Epoxide **25** was treated with *cis*-2,3-dimethylaziridine as for the preparatin of **26** above to afford, after recrystallization from EtOH, **27** (0.15 g, 48%) as a yellow solid: mp 145–146 °C; NMR ($CDCl_3$) $\delta$ 1.2 (d, $J$ = 6 Hz, 6 H, 2 × aziridine-$CH_3$), 1.6 (m, 2 H, 2 × aziridine-H), 2.5 (m, 2 H, $CHCH_2$-aziridine), 3.1–3.8 (m, 4 H, $NHCH_2CHOH$), 6.65 (d, $J$ = 16 Hz, 1 H, furan-CH=C*H*), 6.7 (d, $J$ = 3.5 Hz, 1 H, furan 3-H), 7.3 (d, $J$ = 3.5 Hz, 1 H, furan 4-H), 7.4 (d, $J$ = 16 Hz, 1 H, furan-C*H*=CH) 7.5 (br, 1 H, NH). Anal. ($C_{14}H_{19}N_3O_5 \cdot 0.33H_2O$) C, H, N.

***N*-(3-(*cis*-2,3-Dimethyl-1-aziridinyl)-2-hydroxypropyl)-2,3-epoxy-3-(5-nitrofuran-2-yl)propenamide (28).** A crude sample of **25** (0.5 g, ca. 2.4 mmol) and 3-chloroperbenzoic acid (0.5 g, 2.9 mmol) was boiled under reflux with $Et_3N$ (0.24 mL) and *cis*-2,3-dimethylaziridine (0.4 mL, ca. 8 mmol) in THF (6 mL) for 6 h. The solvent and excess aziridine were then evaporated. The residue was subjected to column chromatography (silica gel; EtOAc/$Et_3N$ (99:1, v/v)) and recrystallization from EtOH to give **28** (0.25 g, 34%) as a yellow solid: mp 74–75 °C; NMR ($CDCl_3$) $\delta$ 1.2 (d, $J$ = 6 Hz, 6 H, 2 × aziridine-$CH_3$), 1.6 (m, 2 H, 2 × aziridine-H), 2.5–2.6 (m, 2 H, $CHCH_2$-aziridine), 2.7–4.0 (m, 6 H, $NHCH_2CHOH$ and 2 × oxirane-H), 6.5 (d, $J$ = 3.5 Hz, 1 H, furan 3-H), 7.05 (br, 1 H, NH), 7.25 (d, $J$ = 3.5 Hz, 1 H, furan 4-H). Anal. $C_{14}H_{19}N_3O_6$ C, H, N.

**2-Methyl-5-nitro-*N*-(prop-2-enyl)furan-3-carboxamide (29).** 2-Methyl-5-nitrofuran-3-carboxylic acid (prepared according to the methods of Gilman et al.[13]) (2.0 g, 11.7 mmol) was stirred for 1.5 h at 0 °C with ethyl chloroformate (1.26 g, 11.7 mmol) and $Et_3N$ (1 mL) in $CH_2Cl_2$ (20 mL). Allylamine (2.0 g, 35.1 mmol) was then added and stirring continued for 1 h at 0 °C. The solution was then poured onto ice (50 g), stirred for 0.5 h, and extracted with $Et_2O$ (2 × 50 mL). The combined organic extracts were washed with 10% aqueous $NaHCO_3$ (twice) and $H_2O$ and dried, and the solvent was removed. The residue was twice recrystallized from EtOH to give **29** (1.5 g, 61%) as colorless needles: mp 100–101 °C; NMR ($CDCl_3$) $\delta$ 2.8 (s, 3 H, furan-$CH_3$), 4.1 (br t, $J$ = 6.5 Hz, 2 H, allylic-$CH_2$), 5.05–6.3 (m, 3 H, $CH{=}CH_2$), 6.35 (br, 1 H, NH), 7.45 (s, 1 H, furan 4-H). Anal. ($C_9H_{10}N_2O_4$) C, H, N.

***N*-(Oxiranylmethyl)-2-methyl-5-nitrofuran-3-carboxamide (30).** Oxirane **30** was prepared as described for compounds

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Table II. In Vivo Studies. Maximum Doses Administered to Mice

| compound | vol injected per 25 g of body wt, mL | max dose, mmol kg$^{-1}$ [c] | limiting factor |
|---|---|---|---|
| 16 | 1.0 | 0.12 (25 mg kg$^{-1}$) | solubility[a] |
| 18 | 0.5 | 0.44 (100 mg kg$^{-1}$) | toxicity[b] |
| 20 | 0.5 | 0.24 (50 mg kg$^{-1}$) | toxicity[b] |
| 25 | 1.0 | 0.11 (25 mg kg$^{-1}$) | solubility[a] |

[a] Injected as suspensions. [b] No acute, severe, or persistent physical or behavorial effects were apparent in C3H/He mice with any of the doses shown in the table. However, following injection of 18, piloerection, an increased respiration rate, and decreased locomotor activity were observed in the mice for approximately 1 h after injection. After higher doses of any of the drugs limited by toxicity severe tremors and convulsions occurred, from which the mice did not recover. [c] Injected ip.

16 and 20 from 0.5 g (2.5 mmol) of 29. Recrystallization from EtOH gave 30 (0.3 g, 53%) as colorless needles: mp 129–130 °C; NMR ($CDCl_3$) δ 2.8 (s, 3 H, furan-$CH_3$), 2.75–4.1 (m, 5 H, $NCH_2$ and 3 × oxirane-H), 6.5 (br, 1 H, NH), 7.45 (s, 1 H, furan 4-H). Anal. ($C_9H_{10}N_2O_5$) C, H, N.

**2-Methyl-3-nitro-*N*-(prop-2-enyl)furan-5-carboxamide (31).** 2-Methyl-3-nitrofuran-5-carboxylate (prepared by the method of Rinkes[14]) (0.25 g, 1.4 mmol) was stirred with allylamine (3 mL, 69 mmol) and dicyclohexylcarbodiimide (2.1 g, 10 mmol) in THF (7 mL) at 25 °C for 12 h. Excess amine was then evaporated after filtration and the residue was purified by chromatography (silica gel; EtOAc/$CHCl_3$ (1:1, v/v)) to give 31 (0.1 g, 35%) as pale yellow prisms: mp 55–56 °C; NMR ($(CD_3)_2SO$) δ 2.6 (s, 3 H, furan-$CH_3$), 4.0 (m, 2 H, allylic $CH_2$), 5.2–5.8 (m, 3 H, $CH{=}CH_2$), 7.6 (s, 1 H, furan 4-H), 8.5 (br t, $J$ = 7 Hz, 1 H, NH). Anal. ($C_9H_{10}N_2O_4$) C, H, N.

**Biological Methods.** The radiosensitization studies in vitro were carried out as described previously with use of Chinese hamster V79-379A cells.[8] The methods for determining selective toxicity to hypoxic V79-379A cells using the MTT assay are also described elsewhere.[20]

On the basis of results from experiments in vitro, compounds 16, 18, 20, and 25 were selected for evaluation in vivo in C3H/He mice. The compounds were injected as suspensions or solutions in phosphate-buffered saline (pH 7.3). TLC analyses were carried out to show that the compounds were unchanged at the time of injection. Initial studies were carried out to determine the maximum doses of compounds which could be administered (Table II). Subsequently, the maximum single doses of each compound which could be administered (according to toxicity or solubility) were injected at various times (5–90 min) before local irradiation of subcutaneous KHT sarcomas with a 10-Gy dose of X-rays. Tumors were excised 24 h later and clonogenic assays performed in vitro to determine the survival of tumor cells.[8]

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# Inhibition of Human Leukocyte Elastase. 1. Inhibition by C-7-Substituted Cephalosporin *tert*-Butyl Esters

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Time-dependent inhibitors of the enzyme human leukocyte elastase have been developed based on the cephem nucleus. A series of cephalosporin *tert*-butyl esters has been examined, and the activity of these compounds has been found to be very sensitive to C-7 substituents, with small, α-oriented, electron-withdrawing groups showing greatest activity. Additionally, the oxidation state of the sulfur atom has been found to play a role in potency, with sulfones showing considerably greater activity than the corresponding sulfides or β-sulfoxides. The α-sulfoxides were inactive.

The azurophilic granules of human polymorphonuclear leukocytes (PMN) contain a serine protease referred to as human leukocyte elastase (HLE EC 3.4.21.37) because of its ability to degrade elastin in addition to a number of other connective-tissue substrates.[1] The possible pathological consequences of the release of HLE from the PMN into the extracellular environment have been the subject of considerable research and speculation for the past 25 years.[2,3] Substantial effort has gone into the study of the interaction of HLE with the major naturally occurring protease inhibitor of plasma, α-1 protease inhibitor ($\alpha_1$-PI).[4] In particular, $\alpha_1$-PI is considered the primary

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