Fisher reagent grade. All nonaqueous reactions were carried out under an inert atmosphere unless otherwise noted.

[1*S*-[1*R,2*R**,4*R**(1*R**,2*R**)]]-*N*-[[5-(Dimethylamino)-1-naphthylenyl]sulfonyl]-1-phenylalanyl-*N*-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-*N*^{im}-[(4-methylphenyl)sulfonyl]-L-histidinamide.** To a well-stirred solution of Boc-His(Tos)-LVA-Ile-Amp[7] (270 mg, 0.32 mmol) in dichloromethane (5 mL) was added trifluoroacetic acid (5 mL). The reaction mixture was stirred at room temperature for 0.5 h, the solvents were removed in vacuo, and the residue was redissolved in dichloromethane. The solution was washed with saturated sodium bicarbonate solution, water, and brine, dried over anhydrous sodium sulfate, and concentrated. The solid was dissolved in dry dimethylformamide (5 mL), dansyl-Phe-OSu[9] (247 mg, 0.50 mmol) and triethylamine (101 mg, 1.0 mmol) were added, and the reaction mixture was stirred at room temperature for 72 h. The dimethylformamide was removed in vacuo, and the residue was dissolved in dichloromethane and poured into brine. The aqueous phase was extracted with 5% methanol/dichloromethane, and the extracts were combined, dried, (anhydrous sodium sulfate), and concentrated. The residue was chromatographed on silica gel (elution with 5% methanol/dichloromethane) to yield dansyl-Phe-His(Tos)-LVA-Ile-Amp (172 mg, 48%) as an amorphous light green solid: IR (mull, cm^{-1}) 3291, 1648, 1619, 1545, 1173, 1093; MS (FAB) *m/z* 1106 [M + H]$^+$; UV (MeOH, nm) 236 (sh, 23 630), 252 (sh, 19 260), 259 (sh, 17 500), 266 (sh, 12 8700, 338 (4120).

[1*S*-[1*R,2*R**,4*R**(1*R**,2*R**)]]-*N*-[[5-(Dimethylamino)-1-naphthylenyl]sulfonyl]-1-phenylalanyl-*N*-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-L-histidinamide.** A solution of dansyl-Phe-His(Tos)-LVA-Ile-Amp (42 mg, 0.037 mmol) and 1-hydroxybenzotriazole (15 mg, 0.11 mmol) in methanol (2 mL) was stirred at room temperature for 24 h. The reaction mixture was concentrated and the residue was chromatographed on silica gel (elution with 5% methanol/dichloromethane followed by 5% methanol (saturated with gaseous ammonia)/dichloromethane) to provide dansyl-Phe-His-LVA-Ile-Amp[14] (28 mg, 80%) as an amorphous solid: IR (mull, cm^{-1}) 3288, 1640, 1571, 1545, 1145, 1061; MS (FAB) *m/z* 952 [M + H]$^+$; UV (MeOH, nm) 255 (15 950), 260 (sh, 15 370), 266 (sh, 11 560), 338 (4080); exact mass calcd for $C_{51}H_{69}N_9O_7S$ 952.5119, found 952.5119.

[1*S*-[1*R,2*R**,4*R**(1*R**,2*R**)]]-*N*-[[5-(Dimethylamino)-1-naphthylenyl]sulfonyl]-1-phenylalanyl-*N*-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-L-histidinamide 2-Hydroxy-1,2,3-propanetricarboxylate (1:3).** Dansyl-Phe-His-LVA-Ile-Amp (0.055 g, 0.058 mmol) was dissolved in methanol (2 mL) and citric acid (0.037 g, 0.176 mmol) was added. Upon complete solution, the methanol was removed in vacuo and the residue was dissolved in water (5 mL). The salt (88 mg, 95%) was obtained as a fluffy white solid after lyophilization.

Dissociation Constants. The dissociation constants, K_d's, of the renin inhibitors were determined by the fluorescence displacement assay,[8] with the following modifications: **3** (23 nM, in duplicate) was added to recombinant human renin (33 nM) in 0.01 M Tris, pH 7.4, 5 mM β-octyl glucoside, at 37 °C and incubated for 1.5 h. The fluorescence intensities were recorded prior to (F_o) and after (F_{max}) the addition of dansylated inhibitor. Unlabeled inhibitors (6.6 and 13 nM) were then added, and the incubation was continued an additional 2.5 h, after which the final fluorescence intensity, F_x, was recorded. The fluorescence intensities were normalized to a volume of 2.5 mL.

Inhibition Constants.[8] Recombinant human renin (0.010 77 or 0.0586 μM) was preincubated at 37 °C for 2 h with, for example, **2** (0–0.27 μM) or **3** (0–1.5 μM), respectively. Twenty-five microliters of the renin/inhibitor preincubation mixture was added to 100 μL of RSP, 856 and 1344 μM, respectively in 0.01 M phosphate, 10 mM *n*-octyl β-D-glucopyranoside, pH 6.5, to begin hydrolysis. Cleavage of RSP by renin was followed by quantitation of the products by HPLC. The data was plotted and fitted to an integrated form of the Michaelis–Menton equation (see text).

(14) Amino acid analysis: Ile, 0.994; His, 1.002. The sulfonamide linkage of dansyl-Phe was not hydrolyzed under standard conditions.

Synthesis of a Series of Nitrothiophenes with Basic or Electrophilic Substituents and Evaluation as Radiosensitizers and as Bioreductively Activated Cytotoxins

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

Medical Research Council Radiobiology Unit, Chilton, Didcot, Oxfordshire OX11 0RD, U.K. *Received November 14, 1990*

A series of 2- and 3-nitrothiophene-5-carboxamides bearing *N*-(ω-aminoalkyl) side chains has been prepared by treatment of the thiophenecarbonyl chloride with the appropriate (protected) ω-aminoalkylamine. Analogous *N*-(oxiranylmethyl)nitrothiophene-5-carboxamides have been synthesized by epoxidation of the corresponding *N*-allylamide. Compounds in both classes were evaluated in vitro both as radiosensitizers of hypoxic mammalian cells and as selective bioreductively activated cytotoxins. The most potent radiosensitizers were those agents with strong tertiary amine bases or oxiranes in the side chain. Studies in vivo showed that 2-methyl-*N*-[2-(dimethylamino)ethyl]-3-nitrothiophene-5-carboxamide caused slight radiosensitization of the KHT sarcoma in mice given 0.34 mmol kg^{-1}. However, administration of this and related tertiary amines at higher doses was precluded by systemic toxicity.

The relative resistance of cells in hypoxic regions of solid tumors to killing by ionizing radiation remains an important reason for failure of local control of cancer by radiotherapy since molecular oxygen is required as an electron acceptor for the manifestation of damage to DNA. Electron-affinic nitroheterocycles can, however, act as mimics of molecular oxygen in this process and thus can be effective as radiosensitizers of hypoxic cells.[1,2] Indeed, a correlation between the one-electron reduction potential (E^1_7) of such compounds and their efficiency as sensitizers of hypoxic cells in vitro to ionizing radiation has been reported.[3,4]

* Author to whom correspondence should be addressed at School of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY, U.K.

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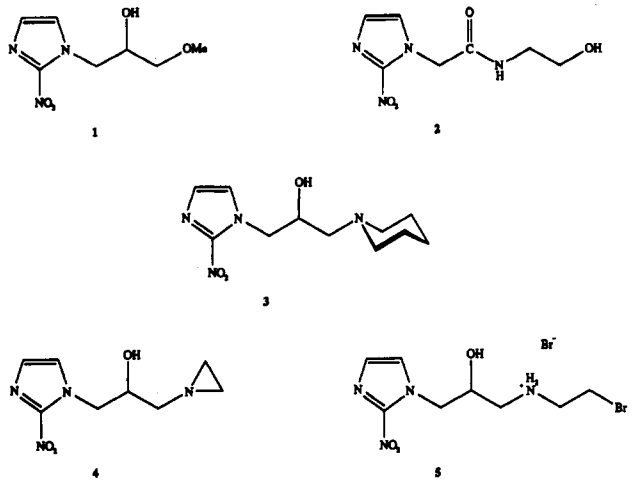
0022-2623/91/1834-2112$02.50/0

Chart I. Structures of 2-Nitroimidazole Radiosensitizers Misonidazole (1), Etanidazole (2), Pimonidazole (3), RSU 1069 (4), and RB 6145 (5)



The first such electron-affinic radiosensitizer to receive extensive clinical study was misonidazole (1, Chart I); however, owing to neurotoxicity, it was found[5] not to be possible to administer a sufficient dose of this compound to achieve radiosensitization of tumors. The second generation of agents to be the subjects of clinical trials were also 1-substituted-2-nitroimidazoles. Etanidazole (SR 2058, 2)[6,7] was designed to avoid the neurotoxicity by being highly polar and thus less able to cross the "blood–brain barrier". Pimonidazole (Ro 03-8799; 3),[8,9] which bears a basic tertiary amine, was designed to be selectively taken up into acidic regions of tumor tissue. RSU 1069 (4),[10-12] a "dual-function" agent with electrophilic and electron-affinic groups, is more potent as a radiosensitizer in experimental systems but elicited dose-limiting gastrointestinal toxicity in the clinic. RB 6145 (5),[13,14] a prodrug of 4, has recently been developed in an attempt to alleviate this problem.

Many of the 1-substituted-2-nitroimidazoles are also selectively toxic toward hypoxic cells.[15] The basis of this selective cytotoxicity is that the 2-nitroimidazole is bioreduced in these cells to an electrophile. The nature of this electrophile is still the subject of investigation, although the corresponding 2-nitrosoimidazoles and 2-(hydroxylamino)imidazoles have been implicated.[16-18] For example, 1 has been reported[19] to be 11 times more toxic toward V79 cells incubated under nitrogen than toward those incubated under air. Aziridinyl compound 4 is even more potent in this differential toxicity, the corresponding ratio[19] being 67. This potentiation is due to conversion from a monofunctional to a bifunctional electrophile upon bioreduction, which is capable[20] of cross-linking and cleaving the strands of DNA.

Recently, we have reported the synthesis and biological activity of two other series of nitroheterocycles (nitrotriazoles[21] and 2-nitrofuran-5-carboxamides[19]) augmented with basic and/or electrophilic (alkylating) side chains. In this paper, the extension of this program of drug development through the synthesis and biological evaluation of a series of 2-nitrothiophene-5-carboxamides and 2-methyl-3-nitrothiophene-5-carboxamides with electrophilic and with basic side chains is presented.

Chemical Synthesis

For the preparation of the series of nitrothiophene-2-carboxamides with the nitro group in the β-position of the heterocyclic ring, it was necessary to direct the nitration by blocking with an alkyl group the remaining α-position of a thiophene-2-carboxylic acid synthon. To this end, 2-methylthiophene-5-carboxylic acid (6) was prepared from 2-methylthiophene by Friedel–Crafts acetylation and subsequent haloform reaction, generally according to the method of Hartough.[22] Nitration with nitric acid/acetic anhydride at low temperature then gave the central intermediate 7 (Scheme I).

For the preparation of nitroheterocycles with basic amino groups in the side chains, it was necessary to couple a range of (protected) ω-aminoalkylamines with the appropriate nitroheterocyclecarboxylic acids. Formation of acid chloride 8 and subsequent treatment with the corresponding amines furnished ω-(tertiary)aminoalkylamides 9–13 in moderate to good yields after separation from highly colored byproducts and conversion to the hydrochloride salts (Scheme I). The deep colorations may be due to a small amount of nucleophilic ring opening of the nitrothiophene analogous to those reported by Dell'Erba et al.[23,24] ω-(Primary)alkylamino amide 19 was synthesized from N_α-BOC-lysine (14) in five steps. Protection of the ϵ-amine as the FMOC derivative was achieved with fluoren-9-ylmethyl chloroformate, giving 15, and the carboxylic acid was blocked as the methyl ester to give the

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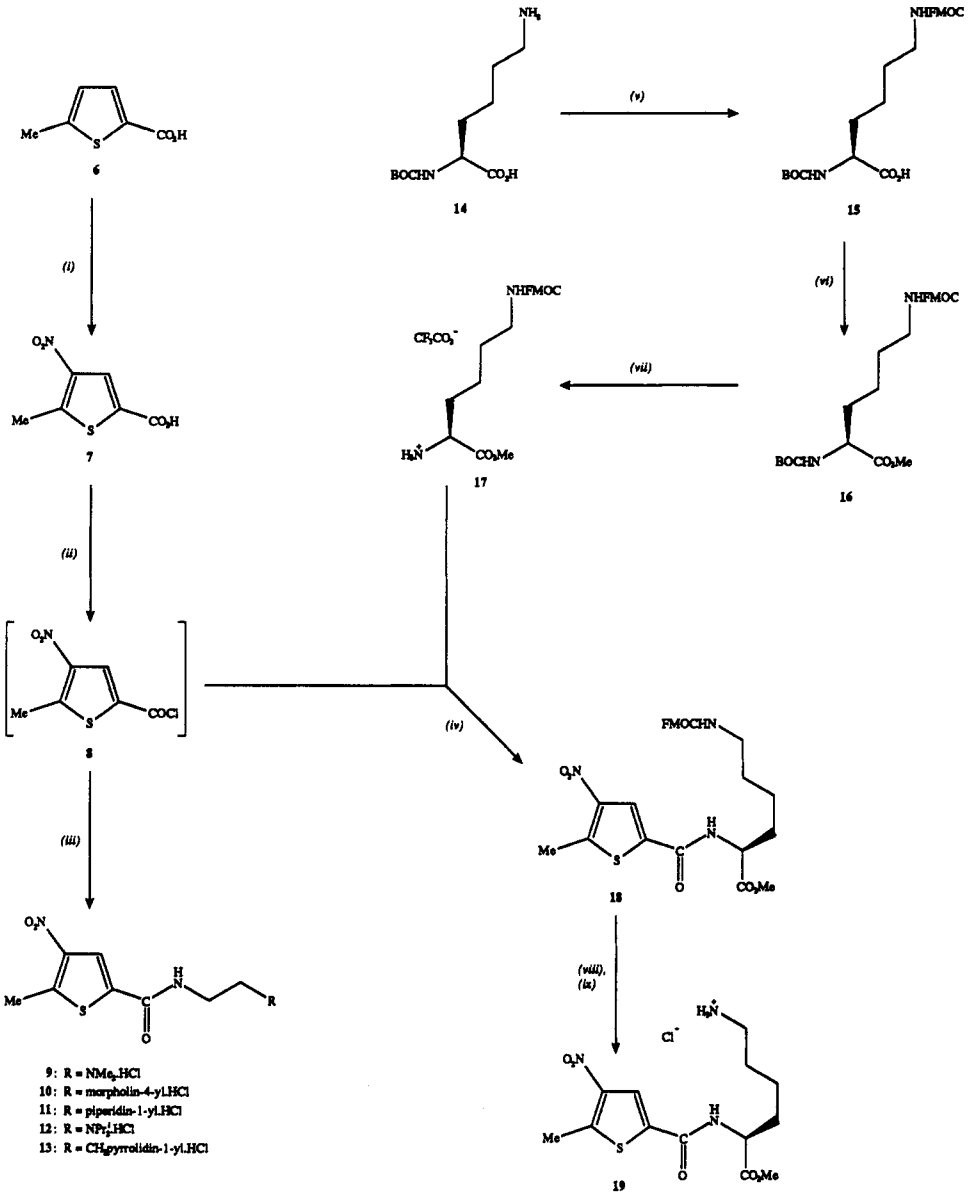
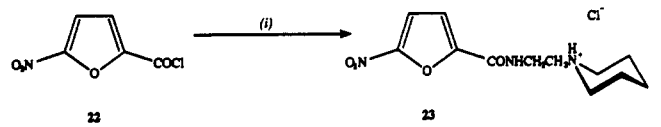
Scheme I.[a] Synthesis of 3-Nitrothiophenes 9–13 and 19 with Basic Side Chains



[a] **Reagents:** (i) HNO_3/Ac_2O; (ii) $SOCl_2$; (iii) $RCH_2CH_2NH_2/Et_2O$; (iv) Na_2CO_3/CH_2Cl_2; (v) $FMOCCl/NaHCO_3$/dioxane/H_2O; (vi) CH_2N_2/Et_2O; (vii) CF_3CO_2H; (viii) piperidine; (ix) HCl. BOC = *tert*-butoxycarbonyl. FMOC = (fluoren-9-ylmethoxy)carbonyl.

Scheme II.[a] Synthesis of Basic Nitrofuran 23

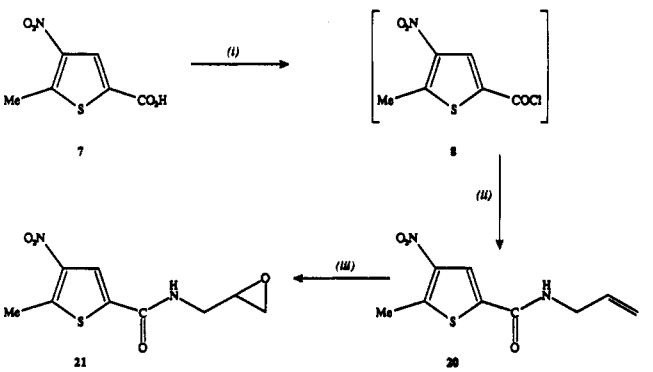


[a] **Reagents:** (i) 1-(2-aminoethyl)piperidine/Et_3N/CH_2Cl_2.

fully orthogonally protected amino acid **16**. Deprotection of the α-amino group with trifluoroacetic acid rapidly afforded the salt of amine **17**, which was coupled with **8** under mildly basic conditions, giving **18**. The required ω-aminoalkylamide **19** was then obtained by facile deprotection of the terminal amine effected by the secondary amine piperidine. In a similar manner to the general coupling, 2-nitrofuran-5-carbonyl chloride (**22**), prepared as described previously,[19] was treated with *N*-(2-aminoethyl)piperidine to yield the required nitrofuran with a basic side chain (**23**) (Scheme II).

Synthesis of nitrothiophenecarboxamides with electrophilic oxirane side chains was achieved through the corresponding *N*-allylamides, as shown in Schemes III and

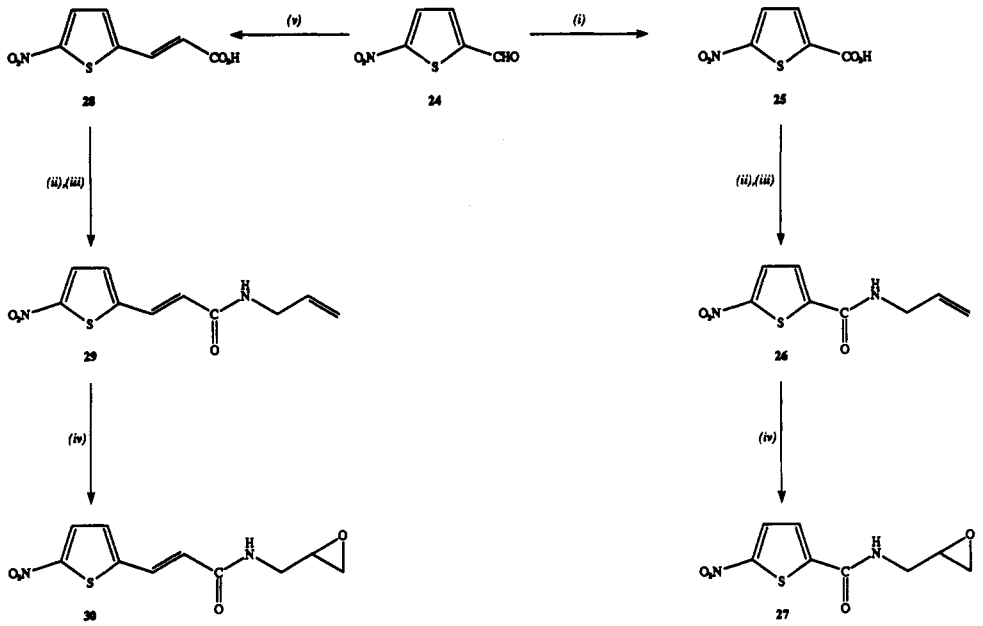
Scheme III.[a] Synthesis of 3-Nitrothiophene 21 with an Electrophilic Side Chain



[a] **Reagents:** (i) $SOCl_2$, (ii) allylamine/Et_2O, (ii) 3-chloroperoxybenzoic acid/CH_2Cl_2.

IV. Reaction of acid chloride **8** with allylamine gave 3-nitro amide **20** in excellent yield, without the attendant discoloration noted during the syntheses of the free bases

Scheme IV.[a] Synthesis of *N*-(Oxiranylmethyl)-2-nitrothiophenamides **27** and **30**

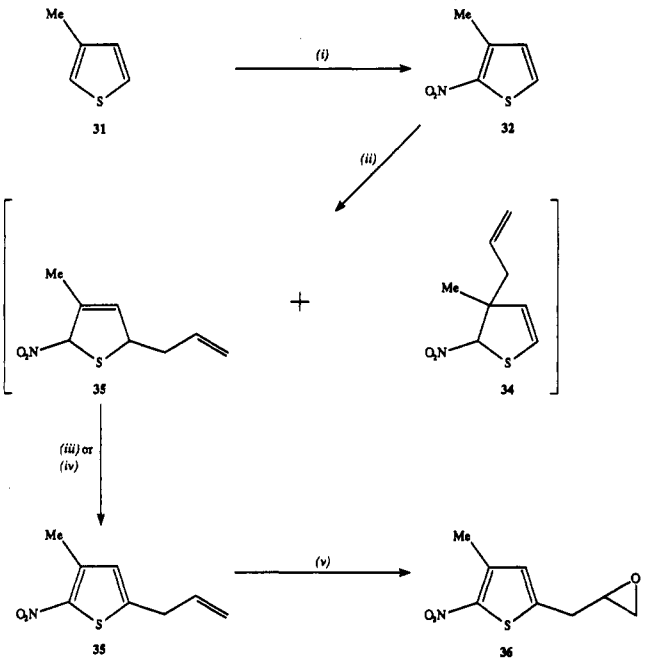


[a] Reagents: (i) $AgNO_3/H_2O$; (ii) $SOCl_2$; (iii) allylamine/CH_2Cl_2; (iv) 3-chloroperoxybenzoic acid/CH_2Cl_2; (v) malonic acid/pyridine.

of the tertiary amine amides **9–13**. Epoxidation was effected using 3-chloroperoxybenzoic acid, giving oxiranylmethylamide **23** (Scheme III). In the 2-nitro series, the commercially available 2-nitrothiophene-5-carboxaldehyde **24** was oxidized smoothly to the corresponding carboxylic acid **25** with silver(I) nitrate (Scheme IV). Treatment of **24** with potassium permanganate under a variety of conditions gave **25** in only trace amounts, in contrast to the reported[25] synthesis of **25** from 5-methyl-2-nitrothiophene with this reagent. Reaction of the corresponding acid chloride with allylamine and subsequent epoxidation with the same peroxyacid yielded allylamide **26** and oxiranylmethylamide **27**, respectively. Nitrothiophenepropenoic acid **28** was formed in moderate yield by Doebner condensation of aldehyde **24** with malonic acid in hot pyridine in a modification of the procedure of Tirouflet et al.[26] Again, the acid chloride reacted smoothly with allylamine (forming **29**). As expected, epoxidation under acidic conditions occurred only at the relatively electron-rich terminal olefin, affording the *N*-oxiranylmethyl unsaturated amide **30**.

The coupling of an appropriately functionalized three-carbon side chain to a nitrothiophene could be accomplished via conjugate addition of a Grignard reagent to an appropriate substrate and oxidative rearomatization of the intermediate addition product. Ballini et al.[27] have recently reported on the addition of a variety of alkylmagnesium halides to unsubstituted 2-nitrothiophene, noting that the products of 2,3- and 2,5-addition were both formed in each case and were separated only with great difficulty, if at all. The yields were reported to be modest. On the basis of this information, we predicted that addition of allylmagnesium bromide to 3-methyl-2-nitrothiophene (**32**) may be governed, at least in part, by steric factors

Scheme V.[a] Conjugate 2,3-Addition of a Grignard Reagent to Nitrothiophene **32**



[a] Reagents: (i) HNO_3/Ac_2O; (ii) allylmagnesium bromide/THF; (iii) 5,6-dichloro-2,3-dicyanobenzoquinone/THF; (iv) $BF_3{\cdot}Et_2O$/THF; (v) 3-chloroperoxybenzoic acid/CH_2Cl_2. THF = tetrahydrofuran.

tending to favor formation of the product (**33**) of 2,5-addition, rather than isomer **34**. Furthermore, isolation of the required 2,3,5-trisubstituted thiophene **35** was facilitated by the inability of **34** to be oxidatively rearomatized with dichlorodicyanobenzoquinone (DDQ), owing to the presence of a quarternary carbon atom at C-3 (Scheme V). The ^1H NMR spectrum of the crude product mixture showed the presence of dihydrothiophene **34** and aromatic thiophene **35**. The latter compound was isolated easily from this mixture by chromatography in modest overall yield. Interestingly, the oxidative rearomatization was effected with equal efficiency by air in the presence of catalytic Lewis acid (boron trifluoride diethyl etherate),

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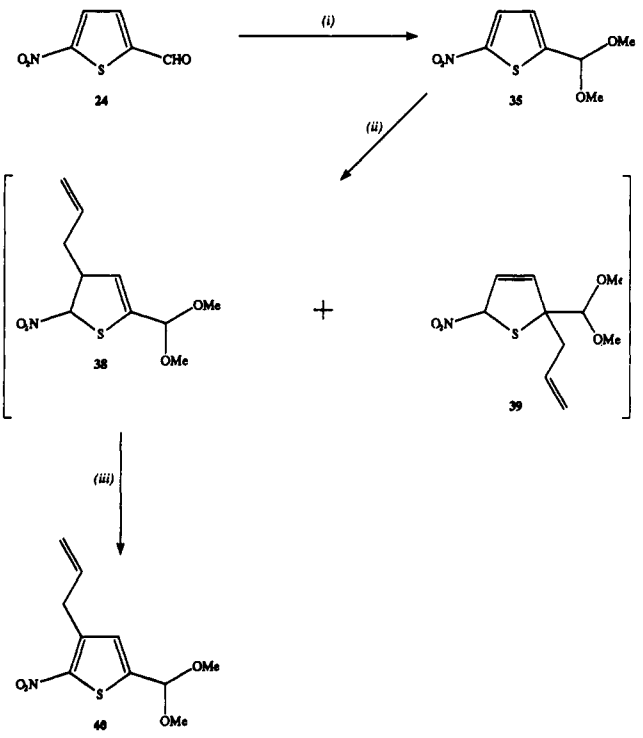
Scheme VI.[a] Conjugate 2,5-Addition of a Grignard Reagent to Nitrothiophene **36**



[a] Reagents: (i) MeOH/HCl; (ii) allylmagnesium bromide/THF; (iii) 5,6-dichloro-2,3-dicyanobenzoquinone/THF. THF = tetrahydrofuran.

obviating the need to remove the organic byproduct dichlorodicyanohydroquinone. Epoxidation with peroxyacid furnished oxiranylmethylnitrothiophene **36**. The usefulness of addition of appropriate Grignard reagents into substituted 2-nitrothiophenes was demonstrated in the synthesis of compound **40** (Scheme VI), which requires selective addition in the 2,3-mode, rather than in the 2,5-mode as above. Protection of the aldehyde function of **24** was carried out by formation of dimethyl acetal **37**. In this substrate, the dimethoxymethyl group should have a large steric requirement and direct addition toward the 2,3-mode. Addition of allylmagnesium bromide to **37** gave a mixture of dihydrothiophenes **38** and **39**. Of these, only **38** could be rearomatized with DDQ, giving **40** as the only thiophene product, albeit in poor yield. Attempted epoxidation of **40** failed owing to acid-catalyzed degradation of the acetal function.

Although strict analogues of lead compound **4**, bearing an aziridinyl group, were sought, treatment of oxiranes **21**, **27**, **30**, and **36** with aziridine gave only complex, highly colored mixtures. Similarly, reaction of these oxiranes with substituted aziridines 2,2-dimethylaziridine and cis-2,3-dimethylaziridine, which gave more stable adducts in the nitrofuran series,[19] also failed to furnish identifiable, stable products.

The one-electron reduction potentials (E^1_7) were measured, according to the method previously described by us,[11] for compounds **9–11**, **13**, **23**, **26**, **27**, **30**, and **36**. Amides of 2-methyl-3-nitrothiophene-5-carboxylic acid (**7**) are some 200–250 mV less oxidizing than those of 2-nitrothiophene-5-carboxylic acid (**25**); this trend is similar to that observed for related nitrofurans.[19] Interestingly, Breccia et al.[30] have recently reported values of redox potential for 5-nitrothiophenes, which are in broad agreement with those reported in this study. However, the value reported[30] for a close analogue of **7** (a 4-nitrothiophene) is at variance with those determined here for a range of 4-nitrothiophenes.

Table I. Radiosensitization by, and Redox Potentials of, Nitrothiophenes **9–13**, **19**, **21**, **23**, **26**, **27**, **30**, **36**, and **40** and Nitrofuran **23** in Vitro (Data for nitroimidazoles **1**, **4**, and **5** are shown for comparison.)

compd	$C_{1.6}$,[a] mM	ER max[b] (concn, mM)	E^1_7,[c] mV
1	0.6[d]	2.6 (10)[e]	−389[e]
4	0.1[f]	3.1 (0.5)[d]	−389[g]
5	0.1[d]	3.1 (0.5)[d]	
9	0.1	2.0 (0.5)	−499
10	1.0	1.6 (1.0)	−537
11	0.07	3.2 (1.0)	−537
12	0.07	2.2 (0.2)	
13	0.03	2.5 (0.5)	−519
19	0.05	1.6 (0.05)	
21	0.2	1.6 (0.2)	
23	0.01	1.9 (0.05)	−242
26	0.08	1.6 (0.08)	−272
27	0.05	1.9 (0.1)	−271
30	0.05	1.6 (0.05)[h]	−274
36	0.06	2.7 (0.1)	−481
40		1.3 (0.005)	

[a] Concentration of compound required to give enhancement ratio = 1.6. [b] Enhancement ratio at maximum concentrations of compounds (in parentheses). This maximum concentration was the highest concentration which did not cause significant cytotoxicity to the cells. [c] Referred to the normal hydrogen electrode (NHE), using methyl viologen, benzyl viologen, and triquat as redox couples ($E^1_7[V^{2+}/V^{\cdot+}]$ = −448, −370, and −549 mV, respectively).[28] [d] Data taken from ref 13. [e] Data taken from ref 4. [f] Data taken from ref 29. [g] Data taken from ref 11. [h] Concentration limited by solubility.

Biological Evaluation in Vitro

Nitrothiophenes **9–13**, **19**, **21**, **26**, **27**, **30**, **36**, and **40** and nitrofuran **23** were assayed for their ability to sensitize hypoxic V79 cells in vitro to γ-radiation, as described previously.[13,19] The results are expressed in Table I as ER max, the maximum factor (enhancement ratio) by which the cells were sensitized to radiation when compared to control cells without added nitroheterocycles, and $C_{1.6}$, the concentration of nitroheterocycle required to give an enhancement ratio of 1.6.

The 2-methyl-3-nitrothiophene-5-carboxamides with primary or tertiary amine bases in the side chains, compounds **9**, **11–13**, and **19**, all have values of $C_{1.6}$ in the range 0.03–0.1 mM. The exception is morpholinyl analogue **10**, which would be expected[31] to be markedly less basic than **9**, **11–13**, and **19**, owing to the presence of the heterocyclic oxygen; this compound gives $C_{1.6}$ = 1.0 mM. The structurally analogous *N*-oxiranylmethylcarboxamide **23**, with a potentially electrophilic rather than a basic side chain, is intermediate in potency.

For the strong tertiary amine bases **9** and **11–13**, significant toxicity toward the experimental cells was not observed at concentrations equal to $C_{1.6}$ and higher values of the enhancement ratio (up to 3.2 for **11**) were achieved at concentrations up to 1 order of magnitude higher than $C_{1.6}$. The remaining, less potent compounds, **10** and **21**, together with primary amine **19**, elicited significant killing of cells through toxicity at concentrations equal to $C_{1.6}$. Thus 2-methyl-3-nitrothiophene-5-carboxamides **9** and **11–13** are markedly more potent than is misonidazole (**1**)

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Table II. Selective Toxicity of Nitrothiophenes 10–12, 19, 21, 26, 27, and 30 and Nitrofuran 23 to Hypoxic V79 Cells in Vitro (Data for nitroimidazoles 1, 4, and 5 are shown for comparison.)

compd	C_{50}(AIR),[a] mM	$C_{50}(N_2)$,[a] mM	ratio[b]
1	45.0[c]	4.0[c]	11[c]
4	0.3[c]	0.0045[c]	67[c]
5	2.3[d]	0.09[d]	26[d]
10	2.5	2.5	1.0
11	1.8	0.8	2.3
12	0.5	0.25	2.0
19	0.35	0.35	1.0
21	0.35	0.4	0.9
23	0.05	0.016	3.0
26	0.30	0.11	2.5
27	0.15	0.15	1.0
30	0.08	0.08	1.0
36	0.065	0.045	1.3

[a] C_{50}(AIR) and $C_{50}(N_2)$ are the concentrations of compounds required to reduce the optical density by 50% compared to controls when the incubations in the MTT assay are performed under air or under nitrogen, respectively;[41] optical density is proportional to the number of viable cells present at the end of the period of incubation. [b] Ratio = C_{50}(AIR)/$C_{50}(N_2)$. [c] Data taken from ref 19. [d] These data are the concentrations required to reduce the survival of cells to 1% in a clonogenic assay and are taken from ref 13.

and, indeed, equal or greater in potency than is the leading 2-nitroimidazole with an electrophilic side chain, compound 4, despite having redox potentials (E^1_7) some 100 mV more negative (less oxidizing) than those of the 2-nitroimidazoles. These data are in apparent contrast to the relationship[3,4] between increasing radiosensitizing potency in vitro and more positive redox potential found for a wide range of nitro(hetero)aromatic agents. An apparent enhancement of radiosensitizing potency has, however, been reported[32-34] for electron-affinic compounds with strong organic bases in the side chain. This phenomenon has been ascribed[35-38] to enhanced uptake of these bases by cells in culture, leading to higher effective concentrations within the cell compared to those in the external medium. In addition, weak bases analogous to those described here are reported[39,40] to be able to concentrate adjacent to DNA through electrostatic interactions between the negatively charged DNA phosphate backbone and the positively charged conjugate acids of the amino groups on the sensitizers. This effect may well be operating for 9, 11–13, and 19 but not for the less basic morpholine 10. To investigate whether this method of increasing potency could be applied to a nitroheterocycle of more positive redox potential, 2-nitrofuran-5-carboxamide 23 was evaluated for radiosensitizing efficiency and

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Table III. Maximum Tolerated Ip Doses of Nitrothiophenes 9–11, 13 and 19 and Nitrofuran 23 in C3H/He Mice (Data for nitroimidazoles 1, 4, and 5 are shown for comparison.)

compd[a]	MTD (ip),[b] mmol kg^{-1}	compd[a]	MTD (ip),[b] mmol kg^{-1}
1	4.00[c]	11	<0.34
4	0.38[c]	13	0.24
5	1.00[c]	19	0.34
9	0.34	23	0.17
10	0.34		

[a] Compounds were dissolved in 0.1 M phosphate-buffered saline solution at pH 7.4 immediately before use and were injected in a total volume equivalent to 0.02 mL per g mouse body weight. [b] The maximum tolerated dose (MTD) is the highest single dose which did not produce severe or persistent clinical signs or death of the adult non-tumor-bearing mice within 24 h. [c] Data taken from ref 14.

Table IV. Radiosensitization by Nitrothiophene 9 and Nitrofuran 23 of KHT Tumors in Vivo (Data for nitroimidazole 4 are shown for comparison.)

compd	optimum time,[a] min	dose,[b] mmol kg^{-1}	surviving fraction[c]
none			2.8×10^{-2} ($3.1–2.4 \times 10^{-2}$)
4	60	0.38	8.3×10^{-4} ($7.2–9.5 \times 10^{-4}$)
9[d]	30	0.34	5.0×10^{-3} ($2.9–8.5 \times 10^{-3}$)
23[d]	60	0.17	2.2×10^{-3} ($1.9–2.7 \times 10^{-3}$)

[a] Optimum time interval between injection of the compound and irradiation of the tumor. [b] Compounds were administered ip as in Table III, footnote *a*. [c] Surviving fraction of clonogenic cells (mean ± SE) after treatment with compound and X-rays (10 Gy). [d] Compounds 9 and 23 had no effect on the survival of clonogenic cells when irradiation was omitted.

was found to be 7-fold more potent than its analogue 11 in terms of $C_{1.6}$ (Table I). This furan has E^1_7 = −242 mV and, like other nitrofurancarboxamides,[19] is up to 270 mV more electron-affinic than the 2-methyl-3-nitrothiophene-5-carboxamides. Analogous 2-nitrofuran-5-carboxamides with potentially electrophilic or unreactive side chains have values of $C_{1.6}$ in the range 0.02–0.1 mM,[19] compared with $C_{1.6}$ = 0.01 mM for 23.

In contrast with the considerable potency of many of the compounds tested as radiosensitizers of hypoxic cells in vitro, no useful selective toxicity toward hypoxic V79 cells was shown by 3-nitrothiophenes 10–12, 19, and 21, nitrofuran 23, and 2-nitrothiophenes 26, 27, and 30 (Table II). Although the values of C_{50}(AIR), the concentration required to kill 50% of the aerobic cells under the conditions of the assay, range from 0.05 mM for the highly oxidizing nitrofuran 23 to 2.5 mM for nitrothiophene 10, little additional toxicity as a result of bioreduction is evident. Ratios of C_{50}(AIR)/$C_{50}(N_2)$ range from 0.9 to 3.0 with no apparent structure–activity relationship. Similar results have been reported by us[21] with a series of nitrotriazoles and the lack of differential toxicity toward hypoxic cells was attributed either to lack of bioreduction or to lack of chemical reactivity of the reduced products leading to lack of toxic effects. For comparison, even the monofunctional 2-nitroimidazole lead compound misonidazole (1) gives a corresponding ratio of 11, whereas "dual function" agents 4 and 5 show even higher selectivities in cytotoxicity toward hypoxic cells.

Biological Evaluation in Vivo

Selected compounds (nitrothiophenes 9–11, 13, and 19 and nitrofuran 23) were administered in escalating doses to mice by the ip route. The maximum single, nontoxic doses (MTD) were determined, initially to provide a basis for subsequent experiments on radiosensitization in vivo. The values of MTD are shown in Table III. The nitro-

thiophenes were generally not well-tolerated and at the MTD doses (0.24–0.34 mmol kg^{-1}) caused rapid respiration and bizarre stretching activity in the mice, which persisted from about 2 to 90 min after injection. At higher doses, immobility, tremors, and spasms were noted, and the mice did not recover. Nitrofuran **23** was also toxic, leading to convulsions and death after doses greater than 0.17 mmol kg^{-1}. Effects on the central nervous system are known to be caused by some tertiary amines. For example, valproic acid (2-propylbutanoic acid) is an anticonvulsant therapeutic drug[42] whereas the corresponding *N*-[2-(dimethylamino)ethyl]amide causes convulsions;[43] this amide has some structural analogy to **9** and **11–13**.

As the compounds had been shown to have little selective toxicity toward hypoxic cells in culture, they were not evaluated as bioreductively activated cytotoxins in vivo. The radiosensitizing potencies of **9** and **23** were investigated and are compared with the effect of **4** in Table IV. The compounds were administered at their MTD to mice bearing the subcutaneous KHT sarcoma at various times before local irradiation of the tumors with 240 KV X-rays (10 Gy). The tumors were excised after 24 h and the survival of tumor cells was determined in a clonogenic assay.[14] In this experimental system, **4** reduced the surviving fraction of cells after irradiation to a value which would be expected if all the tumor cells were aerobic;[14] i.e. sensitization was equivalent to that of oxygen. Compound **9** was much less effective, producing no radiosensitization when administered 15 or 60 min before irradiation and only a small reduction in surviving fraction when the highest possible dose was administered 30 min before the dose of X-rays. In contrast, the highly electron-affinic nitrofuran **23** does show some radiosensitizing properties in vivo. However, even when administered at its MTD, it is markedly less effective than is the lead compound **4** when given at an equitoxic dose. Other nitrothiophenes were excluded from evaluation as radiosensitizers in vivo as they elicited equal or greater toxicity (measured by MTD) than did **9**.

Conclusions

A series of nitrothiophenecarboxamides has been synthesized; those compounds (**9**, **11–13**, **19**) which have strong amine bases in the side chain are potent sensitizers of hypoxic cells to the effects of γ-radiation in vitro, as is nitrofuran **23**. Indeed, these compounds are markedly more potent as radiosensitizers in vitro than would be predicted[3,4] from their relatively negative one-electron redox potentials. However, systemic toxicity precludes the translation of these potencies into useful antitumor radiosensitization in vivo. Similarly, general toxicity prevents the demonstration of selective bioreductively activated cytotoxicity toward hypoxic V79 cells in vitro. Although the compounds were cytotoxic to hypoxic V79 cells at concentrations equal to or 1 order of magnitude lower than for misonidazole (**1**), despite much more negative values of E^1_7, strong nonselective (aerobic) toxicity was manifest also at similar concentrations.

It is concluded that despite the undoubted intrinsic potency of 2-methyl-3-nitrothiophene-5-carboxamides and related compounds as radiosensitizers of hypoxic cells, development of a clinically useful agent of this type will depend on the understanding of the mechanisms of general toxicity and design of suitable analogues to circumvent this problem.

Experimental Section

NMR spectra were obtained at 60 MHz with JEOL PMX60SI and at 270 MHz with JEOL GX270 spectrometers, using $SiMe_4$ as internal standard. A Philips PU9516 instrument furnished the IR spectra, which were obtained with Nujol mulls except where indicated. Mass spectra were obtained with a VG 7070 spectrometer in either the electron impact (EI) mode at 70 eV or in the chemical ionization mode (with 2-methylpropane). Melting points are uncorrected. Tetrahydrofuran (THF) was distilled from CaH_2. Brine refers to saturated aqueous NaCl. Elemental analyses were determined by Elemental Microanalysis Ltd., Okehampton, U.K. The one-electron reduction potentials (E^1_7) of selected compounds (Table I) were determined as previously described.[11] Solutions in organic solvents were dried by treatment with anhydrous Na_2SO_4 and filtration. Solvents were removed by evaporation under reduced pressure. Radial PLC refers to centrifugally accelerated preparative-layer chromatography using a Chromatotron (TC Research, Norwich, U.K.). All chromatography was performed with silica gel as stationary phase. 2-Methyl-3-nitrothiophene-5-carboxylic acid (**7**),[22,44] (*E*)-3-(3-nitrothiophen-5-yl)propenoic acid (**28**),[45] and 3-methyl-2-nitrothiophene (**32**)[46] were prepared by literature methods.

2-Methyl-*N*-[2-(dimethylamino)ethyl]-3-nitrothiophene-5-carboxamide Hydrochloride (9). Carboxylic acid **7** was treated with $SOCl_2$ and with 2-(dimethylamino)ethylamine as for the preparation of **12** below to afford **9** (43%) as off-white crystals: mp 156–157 °C; IR ν_{max} 3330, 1635, 1560, 1550 cm^{-1}; NMR (free base; $CDCl_3$) δ 2.30 (2, 6 H, $N(CH_3)_2$), 2.50 (t, J = 6 Hz, 2 H, Me_2NCH_2), 2.80 (s, 3 H, thiophene CH_3), 3.50 (q, J = 6 Hz, 2 H, $NHCH_2$), 7.05 (br, 1 H, NH), 7.90 (s, 1 H, thiophene H). Anal. C, H, N.

2-Methyl-*N*-(2-morpholin-4-ylethyl)-3-nitrothiophene-5-carboxamide Hydrochloride (10). Carboxylic acid **7** was treated with $SOCl_2$ and with 4-(2-aminoethyl)morpholine as for the preparation of **12** below to afford **10** (60%) as off-white crystals: mp 259–261 °C; IR ν_{max} 3200, 3110 (w), 2600–2450, 1635, 1540 cm^{-1}; NMR (D_2O) δ 2.80 (s, 3 H, CH_3), 3.4–4.2 (m, 12 H, morpholine 2,3,5,6-H + NCH_2CH_2N), 8.10 (s, 1 H, thiophene H); MS (EI) m/z 213 (M – C_4H_8NO), 170 (M – morpholine CH_2CH_2NH), 100 (100) (morpholine CH_2); MS (CI) m/z 300 (M + 1), 270, 100 (100) (morpholine CH_2). Anal. C, H, N.

2-Methyl-3-nitro-*N*-(2-piperidin-1-ylethyl)thiophene-5-carboxamide Hydrochloride (11). Carboxylic acid **7** was treated with $SOCl_2$ and with 1-(2-aminoethyl)piperidine as for the preparation of **12** below, except that the crude material was purified by radial PLC (Et_2O then $CHCl_3$/MeOH (9:1)) to furnish **11** (40%) as buff crystals: mp 237–238 °C; IR ν_{max} 3290, 3090 (w), 2700–2550, 1670, 1550 cm^{-1}; NMR (D_2O) δ 1.6–2.1 (m, 6 H, piperidine 3,4,5-H), 2.75 (s, 3 H, CH_3), 2.9–3.3 (m, 4 H, piperidine 2,6-H), 3.4–3.9 (m, 4 H, NCH_2CH_2N), 8.05 (s, 1 H, thiophene H); MS (EI) m/z 214 (M – piperidine CH_2), 98 (100) (piperidine CH_2); MS (CI) m/z 298 (M + H), 268 (M – NO), 98 (100) (piperidine CH_2).

***N*-[2-[*N*,*N*-Bis(1-methylethyl)amino]ethyl]-2-methyl-3-nitrothiophene-5-carboxamide Hydrochloride (12).** Carboxylic acid **7** (380 mg, 2 mmol) was boiled under reflux in $SOCl_2$ (3 mL) for 10 min and the excess reagent was evaporated. (2-Aminoethyl)bis(1-methylethyl)amine (0.75 mL) was added to the residue in Et_2O (20 mL). After 10 min, the suspension was poured into CH_2Cl_2, was washed twice with saturated aqueous $NaHCO_3$, with H_2O, and with brine, and was dried. The evaporation residue, in propan-2-ol (100 mL), was treated with aqueous HCl (10 M, 0.6 mL). Recrystallization of the evaporation residue from propan-2-ol gave **12** (420 mg, 59%) as off-white crystals: mp 216–218 °C dec; IR ν_{max} 3250, 2680, 1665, 1545 cm^{-1}; NMR (D_2O) δ 1.35 (d, J = 6 Hz, 12 H, 2 × $C(CH_3)_2$), 2.75 (s, 3 H, thiophene CH_3),

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3.05 (septet, J = 6 Hz, 2 H, 2 × CHMe$_2$), 3.3–3.8 (m, 4 H, NCH_2CH_2N), 8.10 (s, 1 H, thiophene H). Anal. C, H, N.

2-Methyl-3-nitro-*N*-(3-pyrrolidin-1-ylpropyl)thiophene-5-carboxamide Hydrochloride (13). Carboxylic acid **7** was treated with $SOCl_2$ and with 1-(3-aminopropyl)pyrrolidine as for the preparation of **12** above to afford **13** (31%) as off-white crystals: mp 214–215 °C dec; IR ν_{max} 3240, 2580, 2500, 1650, 1540 cm^{-1}; NMR (D_2O) δ 1.2–1.7 (m, 6 H, pyrrolidine 3,4-H + NCH_2CH_2CH_2N), 2.70 (s, 3 H, CH_3), 3.0–3.5 (m, 4 H, NCH_2CH_2CH_2N), 8.05 (s, 1 H, thiophene H). Anal. C, H, N.

N_α-(*tert*-Butoxycarbonyl)-N_ϵ-[(fluoren-9-ylmethoxy)carbonyl]lysine Methyl Ester (16). Fluoren-9-ylmethyl chloroformate (2.585 g, 10 mmol), in 1,4-dioxane (60 mL), was added to N_α-(*tert*-butoxycarbonyl)lysine (**14**) (2.46 g, 10 mmol) and $NaHCO_3$ (2.1 g, 25 mmol) in H_2O (60 mL) and the mixture was stirred for 16 h before being partitioned between CH_2Cl_2 (400 mL) and aqueous H_2SO_4 (1.0 M, 150 mL). The organic phase was washed with H_2O and was dried. The solvent was evaporated to give N_α-(*tert*-butoxycarbonyl)-N_ϵ-[(fluoren-9-ylmethoxy)carbonyl]lysine (**15**) (4.34 g, 93%) as a gum: NMR ($CDCl_3$) δ 1.45 (s + m, 15 H, C(CH_3)$_3$ + lysine β,γ,δ-H), 3.20 (br q, J = 6 Hz, 2 H, lysine ϵ-H), 4.0–4.5 (m, 4 H, fluroene 9-H + fluorene CH_2 + lysine α-H), 5.2–5.8 (br, 2 H, 2 × NH), 7.2–7.8 (m, 8 H, fluorene 1–8-H). This acid (2.17 g, 4.64 mmol) in Et_2O (100 mL) was treated with excess CH_2N_2 in Et_2O (30 mL) for 10 min until evolution of N_2 ceased. A solution of AcOH in Et_2O (10%) was then added until no more N_2 was evolved and the yellow color was discharged. The solvent was evaporated to give **16** (2.235 g, quant.) as a white solid: mp 103–105 °C; NMR ($CDCl_3$) δ 1.45 (s + m, 15 H, C(CH_3)$_3$ + lysine β,γ,δ-H), 3.20 (br q, J = 6 Hz, 2 H, lysine ϵ-H), 3.70 (s, 3 H, OCH_3), 4.1–4.6 (m, 4 H, fluorene 9-H + fluorene CH_2 + lysine α-H), 4.7–5.3 (br, 2 H, 2 × NH), 7.2–7.8 (m, 8 H, fluorene 1–8-H). Anal. C, H, N.

N_α-[(2-Methyl-3-nitrothiophene-5-yl)carbonyl]lysine Methyl Ester Hydrochloride (19). Protected amino acid **16** (1.45 g, 3 mmol) was treated with CF_3CO_2H (10 mL) for 20 min before the excess reagent was evaporated. The residue (N_ϵ-[(fluoren-9-ylmethoxy)carbonyl]lysine methyl ester **17**), in CH_2Cl_2 (100 mL), was washed with saturated aqueous $NaHCO_3$ and was dried. Anhydrous Na_2CO_3 (4 g) was added, followed by 2-methyl-3-nitrothiophene-5-carbonyl chloride (**8** as described above; 2 mmol). The mixture was stirred for 30 min, was washed with H_2O (twice), aqueous H_2SO_4 (0.5 M), and H_2O, and was dried. Purification of the evaporation residue by radial PLC ($CHCl_3$) gave N_ϵ-[(fluoren-9-ylmethoxy)carbonyl]-N_α-[(2-methyl-3-nitrothiophene-5-yl)carbonyl]lysine methyl ester (**18**) (670 mg, 60%) as a gum: NMR ($CDCl_3$) δ 1.2–2.0 (m, 6 H, lysine β,γ,δ-H), 2.70 (s, 3 H, thiophene CH_3), 3.15 (br q, J = 6 Hz, 2 H, lysine ϵ-H), 3.75 (s, 3 H, OCH_3), 4.1–4.55 (m, 4 H, fluorene 9-H + fluorene CH_2 + lysine N_α-H), 4.70 (br q, J = 6 Hz, 1 H, lysine α-H), 5.40 (t, J = 6 Hz, 1 H, lysine N_ϵ-H), 7.3–7.9 (m, 8 H, fluorene 1–8-H), 8.20 (s, 1 H, thiophene H). This material (670 mg, 1.2 mmol) in THF (20 mL) was treated with piperidine (5 mL) for 10 min. The evaporation residue was purified by radial PLC (silica gel, $CHCl_3$/MeOH (9:1)) and was treated with aqueous HCl (10 M, 0.2 mL) in propan-2-ol (20 mL). The solvent was evaporated to obtain **19** (160 mg, 36%) as a white solid: mp 83–84 °C; NMR (D_2O) δ 1.5–2.1 (m, 6 H, lysine β,γ,δ-H), 2.80 (s, 3 H, thiophene CH_3), 3.15 (br t, $J \approx 7$ Hz, 2 H, lysine ϵ-H), 3.85 (s, 3 H, OCH_3), 4.60 (m, 1 H, lysine α-H), 8.20 (s, 3 H, thiophene H).

2-Methyl-3-nitro-*N*-prop-2-enylthiophene-5-carboxamide (20). Compound **7** was treated with $SOCl_2$ and with allylamine as for the synthesis of **12** above, except that the treatment with HCl was omitted, to give **20** (81%) as a pale buff solid: mp 84.5–86 °C; IR ν_{max} 3320, 1625, 1535 cm^{-1}; NMR ($CDCl_3$) δ 2.80 (s, 3 H, CH_3), 4.05 (tt, J = 6 and 1 Hz, 2 H, NCH_2), 5.15 (br d, J = 11 Hz, 1 H) and 5.25 (br d, J = 16 Hz, 1 H) (C=CH_2), 5.90 (ddt, J = 16, 11 and 6 Hz, 1 H, CH=CH_2), 6.6 (br, 1 H, NH), 7.90 (s, 1 H, thiophene H). Anal. C, H, N.

2-Methyl-3-nitro-*N*-(oxiranylmethyl)thiophene-5-carboxamide (21). Amide **20** (1.89 g, 8.36 mmol) was boiled under reflux with 3-chloroperoxybenzoic acid (50%, 6.48 g, 18.8 mmol) in CH_2Cl_2 (100 mL) for 8 h. The solution was washed three times with aqueous Na_2SO_3 (10%), twice with saturated aqueous $NaHCO_3$, with H_2O, and with brine and was dried. Column chromatography of the evaporation residue gave **21** (650 mg, 32%) as a pale buff solid: mp 92–93 °C; IR ν_{max} 3350, 3110 (w), 1625, 1535 cm^{-1}; NMR ($CDCl_3$/CD_3OD) δ 2.70 (dd, J = 4 and 2 Hz, 1 H) and 2.80 (m, 1 H) (oxirane 3-H_2), 2.80 (s, 3 H, thiophene CH_3), 3.2–3.9 (m, 3 H, NCH_2CH), 8.07 (s, 1 H, thiophene H); MS (EI) m/z 242.0314 (M) ($C_9H_{10}N_2O_4S$ requires 242.0361), 224, 211, 199, 170 (100) (M − oxiranylmethyl); MS (CI) m/z 243 (100) (M + H), 170.

2-Nitro-*N*-(2-piperidin-1-ylethyl)furan-5-carboxamide Hydrochloride (23). 2-Nitrofuran-5-carbonyl chloride[19] (**22**) (1.50 g, 10 mmol) in CH_2Cl_2 (15 mL) was added to *N*-(2-aminoethyl)piperidine (1.0 mL, 7.5 mmol) and Et_3N (7.5 mL) in CH_2Cl_2 (50 mL) at 0 °C. Stirring continued at this temperature for a further 30 min before the solution was washed twice with saturated aqueous $NaHCO_3$ and with H_2O and was dried. The evaporation residue, in THF (10 mL), was treated with ethereal HCl (1.0 M, 5.0 mL). The solid was collected and was washed with Et_2O to afford **23** (1.93 g, 85%) as a white solid: mp 256–258 °C dec; NMR (D_2O) δ 1.6–1.9 (m, 6 H, piperidine 3,4,5-H), 3.4–3.8 (m, 8 H, piperidine 2,6-H + NCH_2CH_2N), 7.20 (d, J = 3.5 Hz, 1 H, furan 4-H), 7.30 (d, J = 3.5 Hz, 1 H, furan 3-H). Anal. C, H, N.

2-Nitrothiophene-5-carboxylic Acid (25). 2-Nitrothiophene-5-carboxaldehyde (**24**) (3.14 g, 20 mmol) was stirred with $AgNO_3$ (6.8 g, 40 mmol) and NaOH (3.27 g, 82 mmol) in H_2O at 5 °C for 20 min. The solution was filtered, acidified by addition of aqueous HCl, and extracted three times with Et_2O. The combined extracts were washed with H_2O and with brine and were dried. Recrystallization of the evaporation residue from Et_2O/hexane furnished **25** (1.88 g, 54%) as white crystals: mp 155–157 °C (lit.[25] mp 157–158 °C); NMR ($CDCl_3$/$(CD_3)_2SO$ 2:1) δ 7.60 (d, J = 5 Hz, 1 H, thiophene 4-H), 7.80 (d, J = 5 Hz, 1 H, thiophene 3-H), 9.75 (br, 1 H, CO_2H).

2-Nitro-*N*-prop-2-enylthiophene-5-carboxamide (26). Carboxylic acid **25** (690 mg, 4 mmol) was boiled under reflux in $SOCl_2$ (3 mL) until no acid remained (TLC of sample quenched in MeOH). Excess reagent was removed by distillation and the residue was treated with allylamine (2 mL) in CH_2Cl_2 (20 mL) for 1 H. The solution was washed twice with saturated aqueous $NaHCO_3$, with H_2O, and with brine and was dried. Purification of the evaporation residue by radial PLC (CH_2Cl_2) and recrystallization from CH_2Cl_2/hexane afforded **26** (520 mg, 62%), as white crystals: mp 110–111 °C; IR ν_{max} 3310, 1630, 1555 cm^{-1}; NMR ($CDCl_3$/CD_3OD) δ 4.00 (dt, J = 6 and 1 Hz, 2 H, NCH_2), 5.15 (br d, J = 11 Hz, 1 H) and 5.25 (br d, J = 15 Hz, 1 H) (C=CH_2), 5.75 (ddt, J = 15, 11, and 6 Hz, 1 H, CH=CH_2), 7.45 (d, J = 4 Hz, 1 H, thiophene 4-H), 7.75 (d, J = 4 Hz, thiophene 3-H); MS (EI) m/z 212.0208 (M) ($C_8H_8N_2O_3S$ requires 212.0256, 197, 166, 156 (100) (M − C_3H_6N), 110; MS (CI) m/z 213 (100) (M + H).

2-Nitro-*N*-(oxiranylmethyl)thiophene-5-carboxamide (27). Amide **26** (1.16 g, 5.5 mmol) was boiled under reflux with 3-chloroperoxybenzoic acid (85%; 2.04 g, 10 mmol) in CH_2Cl_2 (50 mL) for 6 h. The solution was washed with aqueous Na_2SO_3 (10%) (twice), H_2O, saturated aqueous $NaHCO_3$ (twice), H_2O, and brine and was dried. The evaporation residue was purified by column chromatography (CH_2Cl_2/MeOH 19:1) then by radial PLC ($CHCl_3$) to furnish **27** (540 mg, 43%) as a white solid: mp 113–114 °C dec; NMR ($CDCl_3$/CD_3OD) δ 2.65 (dd, J = 4 and 2 Hz, 1 H) and 2.85 (t, J = 4 Hz, 1 H) (oxirane 3-H_2), 3.25 (m, 1 H, oxirane 2-H), 3.4–4.0 (m, 2 H, NCH_2) 7.55 (d, J = 4 Hz, 1 H, thiophene 4-H), 7.85 (d, J = 4 Hz, 1 H, thiophene 3-H); MS (EI) m/z 228.0197 (M) ($C_8H_8N_2O_4S$ requires 228.0205).

(*E*)-3-(2-Nitrothiophene-5-yl)-*N*-(oxiranylmethyl)propenamide (30). Carboxylic acid **28** (500 mg, 2.5 mmol) was boiled under reflux in $SOCl_2$ (2.5 mL) for 15 min. Allylamine (1.0 mL) was added to the evaporation residue in CH_2Cl_2 (10 mL) at 0 °C and the mixture was stirred for 30 min. The solution was washed with saturated aqueous $NaHCO_3$ and with brine before being dried. The evaporation residue was recrystallized from CH_2Cl_2/hexane to give (*E*)-3-(2-nitrothiophen-5-yl)-*N*-(prop-2-enyl)propenamide (**29**) (520 mg, 87%) as an off-white solid: mp 153–154 °C; NMR ($(CD_3)_2SO$/CD_3OD) δ 4.05 (dt, J = 6 and 1 Hz, 2 H, NCH_2), 5.18 (br d, J = 11 Hz, 1 H) and 5.30 (br d, J = 16 Hz, 1 H) (C=CH_2), 5.75 (ddt, J = 16, 11 and 6 Hz, 1 H, CH=CH_2), 6.80 (d, J = 15 Hz, 1 H) and 7.70 (d, J = 15 Hz, 1 H) (thiophene CH=CH), 7.50 (d, J = 4 Hz, 1 H, thiophene 4-H), 8.15 (d, J = 4 Hz, 1 H, thiophene 3-H). This amide (460 mg, 1.93

mmol) was boiled under reflux with 3-chloroperoxybenzoic acid (85%; 1.44 g, 7 mmol) in CH_2Cl_2 (50 mL) for 8 h. The solution was washed with aqueous Na_2SO_3 (twice), saturated aqueous $NaHCO_3$ (twice), H_2O, and brine and was dried. The evaporation residue was purified by column chromatography ($CHCl_3$) to furnish **30** (240 mg, 33%) as a white solid: mp 120 °C dec; IR ν_{max} 3270, 2800–2550, 1650, 1620, 1560 cm^{-1}; NMR ($CDCl_3$/$(CD_3)_2SO$) δ 2.60 (dd, J = 4 and 2 Hz, 1 H) and 2.75 (t, J = 4 Hz, 1 H) (oxirane 3-H_2), 3.0–3.9 (m, 3 H, NCH_2CH), 6.65 (d, J = 16 Hz, 1 H) and 7.50 (d, J = 16 Hz, 1 H) (propenamide 2,3-H), 7.20 (d, J = 4 Hz, 1 H, thiophene 4-H), 7.85 (d, J = 4 Hz, 1 H, thiophene 3-H), 8.3 (br, 1 H, NH); MS (EI) m/z 254.0366 (M) ($C_{10}H_{10}N_2O_4S$ requires 254.0361).

3-Methyl-2-nitro-5-prop-2-enylthiophene (35). Method A. Allylmagnesium bromide (1.0 M in THF; 5 mL, 5 mmol) was added to **32** (710 mg, 5 mmol) in THF (20 mL) during 15 min at −50 °C. Stirring continued for a further 15 min at −50 °C. 5,6-Dichloro-2,3-dicyanobenzoquinone (1.7 g, 7.5 mmol) in THF (25 mL) was added and the solution was stirred at ambient temperature for 3 h before being poured into aqueous NH_4Cl and extracted three times with Et_2O. The combined extracts were dried. The evaporation residue was purified by column chromatography (EtOAc/hexane 1:4) and by radial PLC (EtOAc/hexane 1:5) to afford **35** (170 mg, 19%) as a colorless glass: NMR ($CDCl_3$) δ 2.55 (s, 3 H, CH_3), 3.50 (br d, J = 6 Hz, 2 H, thiophene CH_2), 5.0–6.2 (m, 3 H, $CH{=}CH_2$), 6.60 (br s, 1 H, thiophene 4-H).

Method B. Compound **32** (720 mg, 5 mmol) in THF (20 mL) was added during 30 min to allylmagnesium bromide in THF (1.0 M, 6 mL, 6 mmol) at −65 °C. After a further 30 min at this temperature, $BF_3{\cdot}Et_2O$ (1.0 mL) was added and the mixture was stirred at ambient temperature for 16 h before being poured onto aqueous NH_4Cl. The mixture was stirred for 1 h and was extracted four times with Et_2O. The combined extracts were dried. The evaporation residue was purified by column chromatography (CH_2Cl_2) to give **35** (220 mg, 24%) identical with the material described above. These materials were used for the preparation of **36** without further purification.

3-Methyl-2-nitro-5-(oxiranylmethyl)thiophene (36). Nitrothiophene **35** (250 mg, 1.4 mmol) was boiled under reflux with 3-chloroperoxybenzoic acid (55%; 2.52 g, 8 mmol) in CH_2Cl_2 (40 mL) for 12 h. The mixture was washed with saturated aqueous Na_2SO_3 (three times), saturated aqueous $NaHCO_3$ (twice), and brine before being dried. The evaporation residue was purified by radial PLC (CH_2Cl_2) to give **36** (110 mg, 41%) as a colorless gum: IR (liquid film) ν_{max} 1555 (w) cm^{-1}; NMR (270 MHz; $CDCl_3$) δ 2.58 (s, 3 H, CH_3), 2.60 (dd, J = 4.8 and 2.6 Hz, 1 H) and 2.87 (dd, J = 4.5 and 3.5 Hz, 1 H) (oxirane 3-H_2), 2.95 (dd, J = 15.8 and 6.0 Hz, 1 H) and 3.08 (dd, J = 15.8 and 4.0 Hz, 1 H) (thiophene CH_2), 3.19 (ddt, J = 6.0, 2.6, and 4.0 Hz, 1 H, oxirane 2-H), 6.76 (s, 1 H, thiophene 4-H); MS (EI) m/z 199.0297 (100) (M) ($C_8H_9NO_3S$ requires 199.0303), 182, 156, 126, 110.

5-(Dimethoxymethyl)-2-nitro-3-(prop-2-enyl)thiophene (40). 2-Nitrothiophene-5-carboxaldehyde (**24**) (314 mg, 2 mmol) was boiled under reflux with MeOH (5 mL) and ethereal HCl (1.0 M, 4 mL) for 45 min. The solvent and excess reagent were evaporated to give 5-(dimethoxymethyl)-2-nitrothiophene (**37**) (406 mg, quant.) as a colorless gum: NMR ($CDCl_3$) δ 3.40 (s, 6 H, 2 × OCH_3), 5.60 (s, 1 H, $CH(OMe)_2$), 7.00 (d, J = 4 Hz, 1 H, thiophene 4-H), 7.80 (d, J = 4 Hz, 1 H, thiophene 3-H). This acetal was treated with allylmagnesium bromide according to method A above to afford **40** (15%) as a colorless oil: IR (liquid film) ν_{max} 2950, 1500, 1330 cm^{-1}; NMR ($CDCl_3$) 3.35 (s, 6 H, 2 × OCH_3), 3.75 (br d, J = 6 Hz, 2 H, thiophene CH_2), 5.05 (br d, J = 16 Hz, 1 H) and 5.10 (br d, J = 11 Hz, 1 H) ($C{=}CH_2$), 5.50 (s, 1 H, $CH(OMe)_2$), 5.80 (ddd, J = 16, 11 and 6 Hz, 1 H, $CH{=}CH_2$), 6.85 (s, 1 H, thiophene 4-H).

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Relative Structure–Inhibition Analyses of the *N*-Benzoyl and *N*-(Phenylsulfonyl) Amino Acid Aldose Reductase Inhibitors

Jack DeRuiter,* R. Alan Davis, Vinay G. Wandrekar, and Charles A. Mayfield

Department of Pharmacal Sciences, Division of Medicinal Chemistry, School of Pharmacy, Auburn University, Auburn University, Alabama 36849-5503. *Received May 11, 1990*

A number of *N*-benzoyl amino acids were synthesized and tested to compare structure–inhibition relationships with the isosteric *N*-(phenylsulfonyl) amino acid (PS-amino acid) aldose reductase inhibitors. Inhibition analyses with these series reveals that their kinetic mechanisms of inhibition are similar, but that significant differences in structure–inhibition relationships exist. For example, while the PS-alanines and PS-2-phenylglycines produce enantioselective inhibition ($S > R$), no consistent pattern of enantioselectivity is observed with the isosteric *N*-benzoylalanines and 2-phenylglycines. Also, *N*-methyl and *N*-phenyl substitution in the PS-amino acid series does not substantially alter inhibitory activity, while similar substitutions in the *N*-benzoyl series (particularly *N*-phenyl) results in a significant increase in inhibitory activity. Proton NMR analysis of the *N*-benzoylsarcosines reveals that these compounds exist as a mixture of rotamers in solutions including the enzyme assay buffer and that the preferred conformer is one in which the carboxymethyl moiety is trans to the aromatic ring. Similar analyses with the *N*-benzoyl-*N*-phenylglycines demonstrate that these derivatives exist exclusively in the trans rotameric conformation in solution. No such N-substituent effects on conformation were observed in the PS-amino acid series. These results suggest that the differences in structure–inhibition trends between these structurally related series may result from the effect of substituents on preferred conformation.

Introduction

Aldose reductase (alditol: $NADP^+$ oxidoreductase; EC 1.1.1.21; ALR2), an enzyme of the polyol pathway, catalyzes the NADPH-dependent reduction of glucose to sorbitol in a variety of mammalian tissues. During hyperglycemia there is increased flux of glucose through the polyol pathway in tissues such as the lens, retina, nerve, and kidney, and this is associated with several biochemical changes including intracellular sorbitol accumulation and *myo*-inositol depletion. Over the past decade, a significant

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