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Studies on the reductively triggered release of heterocyclic and steroid drugs from 5-nitrothien-2-ylmethyl prodrugs

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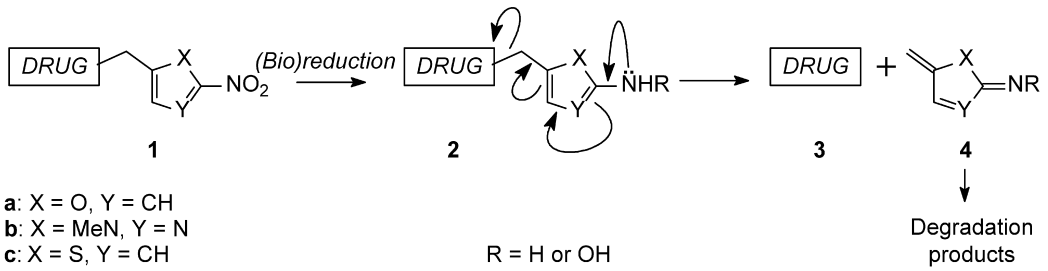
Abstract—Hypoxia (inadequate concentrations of dioxygen in tissues) is present in several disease states, including cancer and rheumatoid arthritis. Prodrug systems, which after bioreduction, selectively release active drugs in these tissues may be important in therapy. The 5-nitrothien-2-ylmethyl ester of aspirin was synthesised by treatment of 5-nitrothiophene-2-methanol with 2-acetoxybenzoyl chloride, whereas that of prednisolone hemisuccinate was prepared by reaction of prednisolone with 5-nitrothien-2-ylmethyl pentafluorophenyl butanedioate. In chemical model systems, both of these ester-linked potential prodrugs suffered hydrolysis, rather than reductively triggered release of the corresponding drug. 1-(5-Nitrothien-2-ylmethoxy)isoquinolines released the corresponding isoquinolin-1-ones (potent inhibitors of poly(ADP-ribose)polymerase) rapidly upon reduction of the nitro group with sodium borohydride/palladium, showing that these may be useful as reductively triggered prodrugs. In approaches to N-linked potential prodrugs, isoquinolin-1-one and nifedipine (a 1,4-dihydropyridine Ca^{2+} channel antagonist) were alkylated at nitrogen by 2-chloromethylthiophene but the corresponding 5-nitrothien-2-ylmethyl derivatives were synthetically inaccessible.

1. Introduction

In many solid tumours, the vasculature is poorly developed and structured and, in most tumours, there are regions with acute or chronic hypoxia.[1,2] In these hypoxic tissues, viable cells are relatively resistant to radiotherapy and to many chemotherapeutic strategies.[1,2] Chronic hypoxia is also present in rheumatoid arthritis and other disease states.[3,4] Prodrugs for selective delivery of drugs to tumours have attracted much interest, with particular effort being expended on development of bioreductively activated cytotoxins.[5–8] It is only relatively recently that attention has been focussed on exploiting the physiological difference in concentration of O_2 between normal and hypoxic tumour tissue by design of biologically inactive prodrug systems which, upon selective bioreduction in hypoxic tissue, would release known therapeutic drugs only in that tissue. This would improve greatly the selectivity of biodistribution of such agents. Denny has described[9] such prodrugs as comprising Trigger, Linker and Effector units. Previously,[10–12] we have reported potential general reductively activated prodrug systems with redox-sensitive Triggers for delivery of isoquinolinones, amines and diols. Others have investigated indolequinones in this way.[13,14] The proposed mechanism for reductively triggered drug release from the nitroheterocyclylmethyl prodrugs is shown in Scheme 1. In this mechanism, reduction of the nitro group in the nitrofurans **1a** or nitroimidazoles **1b** to the corresponding



Scheme 1. Proposed mechanism of reported reductively triggered release of drugs from nitrofuranylmethyl prodrugs **1a** and from nitroimidazolylmethyl prodrugs **1b**.

Keywords: chronic hypoxia; prodrug; isoquinolin-l-one; nitrothiophene.

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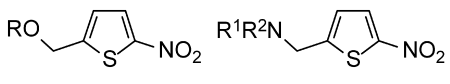
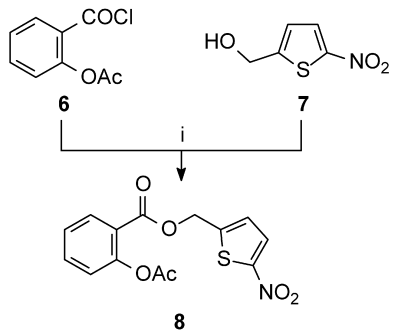


5a **5b**

Figure 1. General structures of O-linked and N-linked nitrothienylmethyl prodrugs in this study.

amino- or hydroxylamino-heterocycles **2a**,**b** triggers expulsion of the drug, owing to the availability of a pair of electrons, as shown. Using chemical model systems for the reduction, we have achieved rapid expulsion of 'drugs' from O-linked prodrugs, such as carbamates[10] and diols,[11] and from the N-linked prodrugs 2-(5-nitrofuran-2-ylmethyl)-isoquinolin-1-one[10] and 2-(1-methyl-2-nitroimidazol-5-yl-methyl)isoquinolin-1-one.[12] In these studies, the release of the drugs was studied qualitatively by thin layer chromatography (TLC) and, in one case, by high performance liquid chromatography (HPLC)[12] but release from this type of prodrug has not, as yet, been studied by NMR. The analogous nitrothiophenes **1c** have not hitherto been investigated. The redox potentials of 2-nitrofurans are relatively high (E_7^1=−325 mV for a 5-nitrofuran-3-carboxamide)[5] for this application; those of 2-nitroimidazoles are more appropriate for selective bioreduction in hypoxic tumour tissue (E_7^1=−389 mV for 1-alkyl-2-nitroimidazoles).[5] Nitrothiophenes are somewhat less electron-affinic, with 5-nitro-2-(oxiranylmethyl)thiophene having E_7^1=−481 mV.[15] A series of 5-nitrothiophene-2-carboxamides has shown unexpectedly powerful radiosensitising activity towards hypoxic tumour cells in culture.[15] Thus it was hypothesised that nitrothienylmethyl prodrugs of general type **1c** may be useful for reductively triggered release of drugs in therapy of cancer, rheumatoid arthritis and other diseases involving hypoxia.



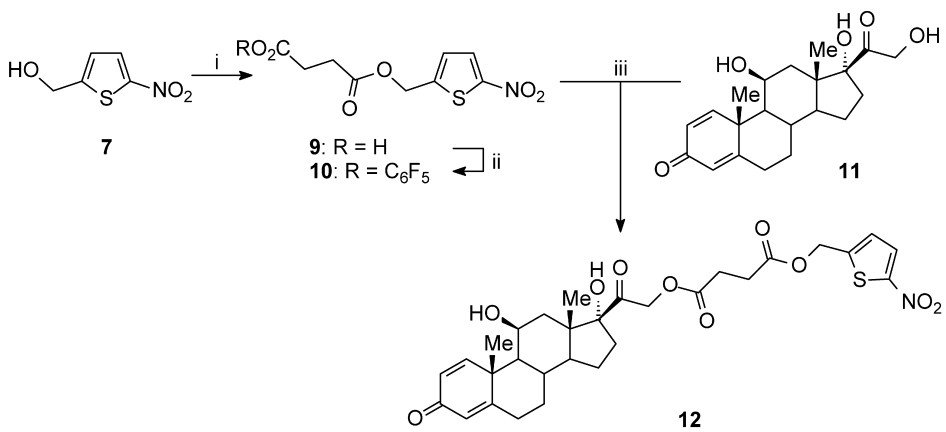
6 **7** **8**

Scheme 2. Synthesis of **8**, the nitrothienylmethyl ester of aspirin. *Reagents*: (i) Et_3N, CH_2Cl_2.

2. Results and discussion

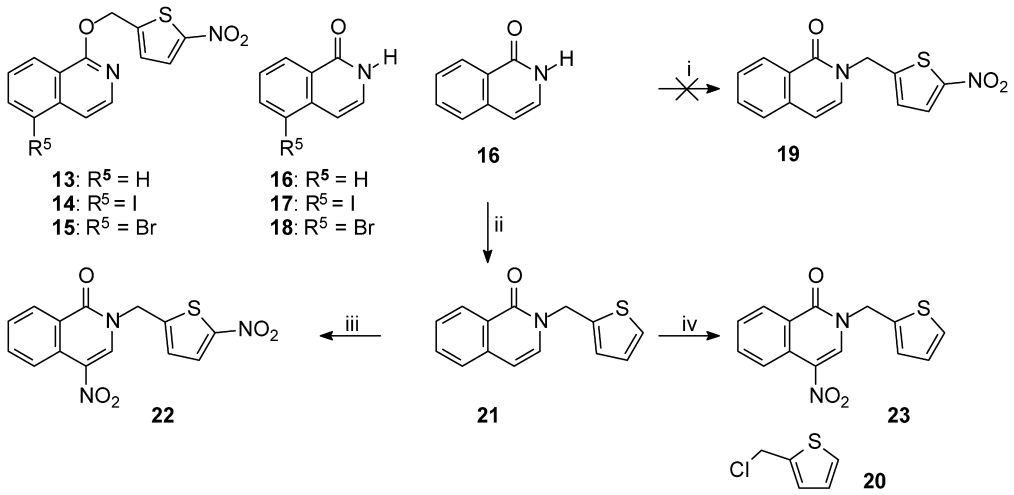
Five series of nitrothienylmethyl prodrugs were designed to test this hypothesis. These series included examples of type **5a** (Fig. 1), where the drug is linked through oxygen, and examples of type **5b**, where the linkage is through nitrogen. The drugs chosen for linkage to nitrothiophene were aspirin (used in the treatment of rheumatoid arthritis[16] and in the prophylaxis of cardiovascular disease), prednisolone (rheumatoid arthritis[17]), isoquinolin-1-ones (inhibitors of poly(ADP-ribose)polymerase (PARP),[18] with applications in cancer,[19] inflammation[20] and hypoxia-reperfusion injury[21]) and nifedipine (a Ca^{2+}-channel antagonist[22]). Clearly, the point of attachment of the nitrothienylmethyl unit to aspirin has to be through the oxygen of the carboxylic acid, making the ester **8** (Scheme 2) the synthetic target. Prednisolone **11** also carries only oxygen-based functionality to which the heterocyclic trigger could be attached. However, preliminary synthetic studies showed that the primary alcohol of **11** and its alkoxide did not react with halomethylthiophenes. Thus a linker had to be inserted between the steroid and the trigger. Prednisolone hemisuccinate, the monoester of **11** at the primary alcohol with butanedioic acid, is a known prodrug of **11** in its own right,[23] being cleaved efficiently to **11** in vivo by esterases. Thus the butanedioate diester **12** (Scheme 3) was designed as the reductively triggered prodrug for prednisolone; as with **8**, the group to be expelled after reductive triggering is a carboxylate.

The inhibitory activity of (5-substituted) isoquinolin-1-ones depends critically on the arylamide motif in which the N–H is held *syn* to the carbonyl oxygen. Masking of this pharmacophore can be achieved either by alkylation at oxygen (giving 1-alkoxyisoquinolines) or at nitrogen (giving 2-alkyisoquinolin-1-ones). Thus both 1-(5-nitrothienylmethyl)isoquinolines (e.g. **13**–**15**, Scheme 4) and 2-(5-nitrothienylmethyl)isoquinolin-1-ones (e.g. **19**, Scheme 4) were sought as potential prodrugs. Finally, attachment of the nitrothienylmethyl unit to the heterocyclic nitrogen of the



7 **9**: R = H, **10**: R = C_6F_5 **11** **12**

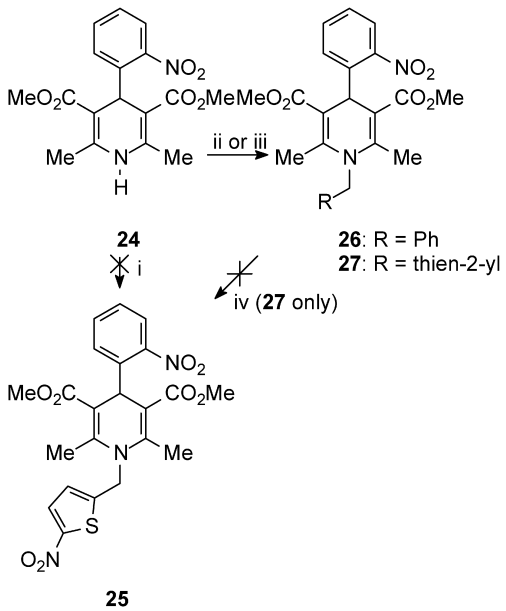
Scheme 3. Synthesis of **12**, the nitrothienylmethyl prodrug of prednisolone and prednisolone hemisuccinate. *Reagents and conditions*: (i) succinic anhydride, DMAP, pyridine, 50°C, 5 h; (ii) C_6F_5OH, dicyclohexylcarbodiimide, EtOAc, 4°C, 16 h; (iii) prednisolone, DMAP, DMF, 20°C, 2 days.



Scheme 4. Structures of O-linked prodrug nitrothienylmethoxyisoquinolines **13**–**15** and of PARP inhibitors **16**–**18** and attempted synthesis of a corresponding N-linked nitrothienylmethylisoquinolinone **19**. *Reagents and conditions*: (i) various nitrating conditions; (ii) 20, $LiN(SiMe_3)_2$, NaI, THF, DMF, 20°C, 2 days; (iii) HNO_3, CF_3CO_2H, −10°C→20°C, 16 h; (iv) HNO_3, Ac_2O, AcOH, −10°C, 40 min.

1,4-dihydropyridine nifedipine in **25** (Scheme 5) was predicted to mask its pharmacological activity.

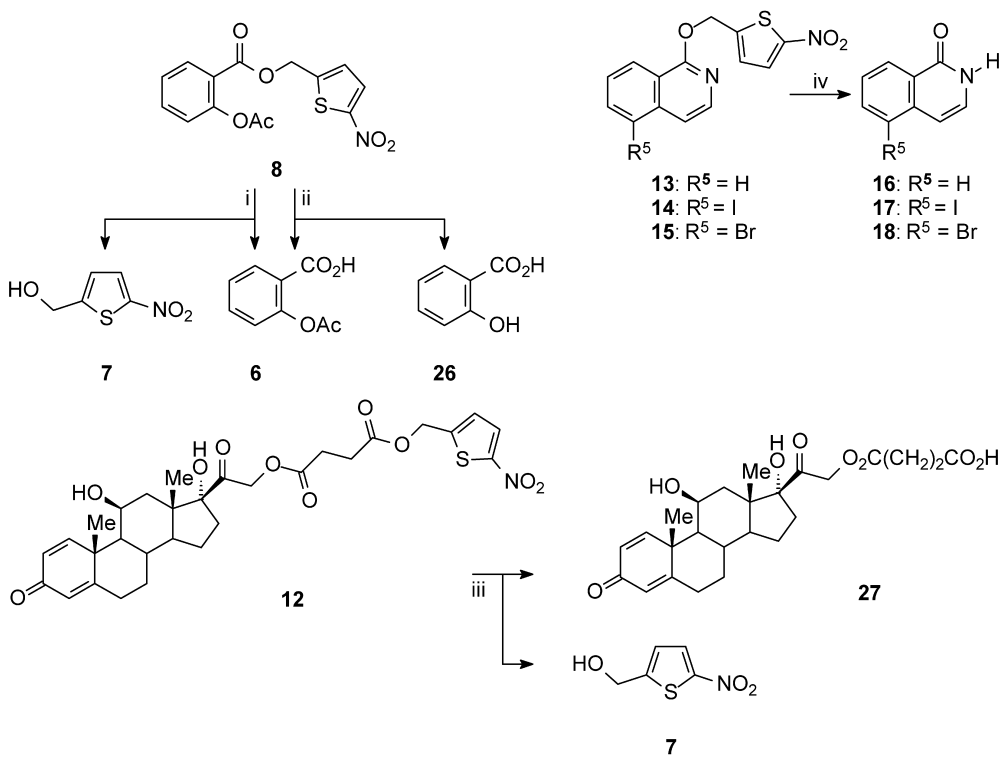
Synthesis of the nitrothienylmethyl ester **8** of aspirin (Scheme 2) was achieved in moderate yield, as expected, by careful treatment of the corresponding acid chloride **6** with 5-nitrothiophene-2-methanol **7**. For the other ester-linked prodrug **12** (Scheme 3), initial unsuccessful attempts were made to couple commercially available prednisolone hemisuccinate with the nitrothienylmethanol **7**. The synthesis was achieved by joining the Trigger and Linker units first, then attaching the drug. Treatment of alcohol **7** with succinic anhydride under basic conditions afforded the monoester **9** in good yield. The remaining carboxylic acid



Scheme 5. Attempted synthesis of N-(nitrothienylmethyl)nifedipine **25**. *Reagents and conditions*: (i) 2-chloromethyl-5-nitrothiophene or 5-nitrothien-2-ylmethyl 4-methylbenzenesulfonate, various conditions; (ii) $LiN(SiMe_3)_2$, $PhCH_2Cl$, NaI, THF, DMF, 20°C, 2 days; (iii) $LiN(SiMe_3)_2$, 20, NaI, THF, DMF, 20°C; (iv) various nitrating conditions.

was converted virtually quantitatively to its pentafluorophenyl active ester **10**; this ester reacted selectively with the most sterically unencumbered (primary) alcohol of prednisolone **11** to give the candidate prodrug **12**.

Whereas deprotonation of isoquinolin-1-ones and treatment with haloalkanes usually gives products of alkylation at nitrogen,[10,12,24] we have recently reported that application of the Mitsunobu reaction gives either alkylation at nitrogen or at oxygen,[25] depending on the precise conditions and on the nature of the reactants. Starting with isoquinolin-1-one **16** (a relatively weak inhibitor of PARP)[18] and its 5-iodo- and 5-bromo analogues **17** and **18** (more potent inhibitors of the enzyme),[18] treatment with 5-nitrothiophene-2-methanol **7** under Mitsunobu conditions gave[25] the required O-linked candidate prodrugs **13**–**15** (Scheme 4), respectively. However, synthetic approaches to the N-linked analogue **19** were more challenging. Whereas the anion derived from **16** reacts with 5-nitrofuran-2-ylmethyl tosylate to give the N-(2-nitrofuran-2-ylmethyl)isoquinolinone in modest yield, this reaction failed with 2-chloromethyl-5-nitrothiophene.[26] An alternative strategy was then investigated, in which the thienylmethyl unit is attached first and is subsequently nitrated. Deprotonation of isoquinolin-1-one **16** with lithium bis(trimethylsilyl)amide and reaction of the anion with 2-chloromethylthiophene **20**[27] in the presence of catalytic sodium iodide gave 2-(thien-2-ylmethyl)isoquinolinone **21**, as reported previously.[25] Nitration of **21** with nitric acid in trifluoroacetic acid under usual conditions for nitration of thiophenes gave the dinitro compound **22** in reasonable yield as the only isolable product. Several other nitrating systems gave the same product but in lower yields. This outcome parallels the dinitration[10] of 2-(furan-2-ylmethyl)isoquinolin-1-one under all but the mildest conditions. Rationalising that milder conditions may give selective nitration at the thiophene and mindful of the precedent[10] that 2-(furan-2-ylmethyl)isoquinolin-1-one can be selectively nitrated at the furan, **21** was treated briefly with acetyl nitrate (formed *in situ* from nitric acid and acetic anhydride) at −10°C. However, mononitration took place at the isoquinolinone 4-position solely, giving **23**; thus the isoquinolinone is more nucleophilic than the thiophene and



Scheme 6. Outcomes of chemical model reductive release experiments. *Reagents*: (i) Zn, NH_4Cl, MeOH; (ii) $NaBH_4$, Pd/C, aq. Pr^{i}OH, 40°C; (iii) $SnCl_2$, MeOH; (iv) $NaBH_4$, Pd/C, aq. Pr^{i}OH, 20°C.

the desired selective reaction is unlikely to be possible, in contrast to that of the analogous furan.

Scheme 5 shows the attempted synthetic approaches to the nitrothienylmethyl derivative **25** of nifedipine **24**. Nifedipine **24** is a 1,4-dihydropyridine in which the ring nitrogen is a two-fold 'vinylogous amide' and thus is of low nucleophilicity. Indeed, N-alkylations of analogous 1,4-dihydropyridines have only been achieved either using reactive Mannich reagents[28] or oxiranes[29] or, after deprotonation, using activated alkyl halides[30,31] or simple electrophiles.[32,33] In this study, nifedipine **24** was deprotonated with lithium hexamethyldisilazide and the anion was treated with a model electrophile, benzyl chloride; the N-benzyl derivative **26** was obtained in 40% yield. However, this alkylation could not be transferred to the desired attachment of the nitrothienylmethyl unit directly using 2-chloromethyl-5-nitrothiophene.[26] Alkylation of the nifedipine-derived anion was achieved with chloromethylthiophene **20**[27] in poor yield but attempted selective nitration of the thienylmethyl-nifedipine at thiophene gave only products of oxidative degradation of the dihydropyridine.

We have previously reported three different chemical reductant systems to mimic bioreduction of heterocyclic nitro groups in hypoxic tissue.[10–12] In each of these, the conditions were designed not to permit hydrogenolysis of the 'benzylic' CH_2–O or CH_2–N bonds, which would not be biomimetic for the nitroreductases and the cytochrome P450 reductase enzymes. Sodium borohydride in aqueous methanol has long been used as a selective reductant for nitro groups[34] (although recent questions[35] have been raised about its complete selectivity, in that it also reduces alkynes) and this reagent has been used in aqueous propan-2-ol for biomimetic reductive triggering of release of nitroheterocyclic prodrugs.[10,12] Reductive debromination of 5-bromoisoquinolin-1-one **18** was also reported[12] with this reagent system. Tin(II) chloride causes reductively triggered release from 5-nitrofuran-2-ylmethyl[10] and 1-methyl-2-nitroimidazol-5-ylmethyl[12] prodrugs. Similarly, zinc powder/ammonium chloride in wet methanol reduces 2-nitroimidazoles to a mixture of the corresponding 2-amino- and 2-hydroxylamino-imidazoles.[12]

Initially, the Zn/NH_4Cl reduction system (Method A) was used to study its effects on aspirin nitrothienylmethyl ester **8**. HPLC analysis of the reaction mixture indicated rapid (<1 min) conversion of **8** to 5-nitrothien-2-ylmethanol **7** and aspirin **6**, indicating that hydrolytic cleavage of the ester bonds had taken place, without reduction of the nitro group. Treatment of the analogous prednisolone hemisuccinate nitrothienylmethyl ester **12** with tin(II) chloride in methanol (Method B) gave no reaction but gentle warming (40°C) again led to ester cleavage without reduction of the nitro function, as shown by HPLC analysis. Interestingly, the steroid product was prednisolone hemisuccinate **26** (Scheme 6), rather than prednisolone **11**. Thus, although both substrates **8** and **12** are diesters, only the nitrothienylmethyl ester was cleaved and without prior reduction of the nitro group. The mechanistic origin of this selectivity is unclear. It is interesting to note that Everett et al.[36] achieved only a very small yield of aspirin **6** upon radiolytic reduction of its prodrug ester with 1-methyl-2-nitroimidazole-5-methanol in aqueous buffer.

The nitrothienylmethyl prodrugs **13**–**15** of the isoquinolin-1-ones **16**–**18** were too insoluble in methanol for these

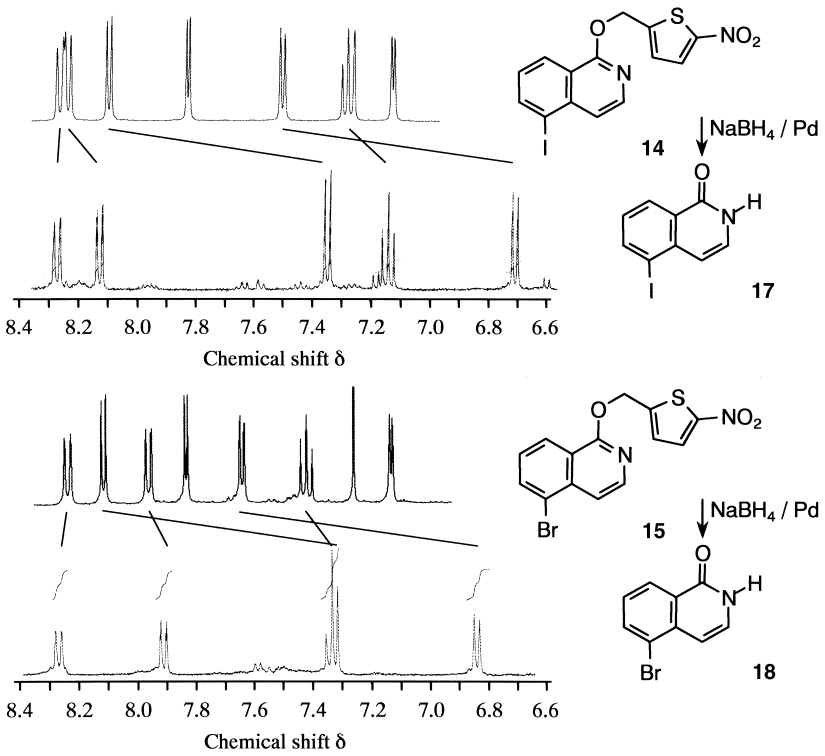


Figure 2. ^{1}H NMR spectra (400 MHz) of 5-halo-1-(5-nitrothien-2-ylmethoxy)isoquinolines **14** and **15** in CD_3OD before and after treatment with $NaBH_4$/Pd/C. Reduction of the nitro group causes expulsion of the 5-haloisoquinolinones **17** and **18**, respectively, while the aminothiophenes residues form aliphatic co-products. The major peaks in the spectra after treatment correspond to those of pure **17** and **18** in the same solvent.

reductive systems (A and B) to be investigated. Addition of chloroform to the tin(II) chloride/methanol reaction mixture served to dissolve the substrates but no reductively-triggered release was observed. Thus the method previously used[10] for reductively triggered release of isoquinolin-1-one from 2-(5-nitrofuran-2-ylmethyl)isoquinolin-1-one was adapted for this study. Each of the nitrothienylmethyl prodrugs **13**–**15** was treated with sodium borohydride in the presence of palladium on carbon in aqueous isopropanol. TLC analysis indicated the disappearance of **13**–**15** and the presence of the isoquinolin-1-ones **16**–**18**, respectively, after 30 min (Scheme 6). Proton NMR analysis of the reaction mixtures reconstituted in tetradeuteriomethanol was carried out for each sample after 16 h reaction. Each spectrum showed complex peaks in the aliphatic region but the region δ 6.0–9.0 was clear. In each case, the spectra showed complete disappearance of the thiophene 3′,4′-protons. Aminothiophenes, the expected initial products, are unstable when lacking electron-withdrawing substituents and fragment by ring-opening to aliphatic materials. The sole peaks observable in the aromatic region corresponded to those of the released isoquinolin-1-ones. The part spectra for the studies on **14** and **15** are shown in Figure 2. In each case, marked differences were observed in the chemical shifts of the isoquinoline 3-H and 4-H before and after the treatment with $NaBH_4$/Pd, whereas those for the benzene ring protons at positions (5), 6, 7 and 8 were less affected. For example, in the case of the 5-iodo compound **14**, the 3-H signal moves upfield by 0.75 ppm upon reductively triggered release, the 4-H signal moves upfield by 0.81 ppm, the 6-H signal moves upfield by 0.11 ppm, the 7-H signal moves upfield by 0.13 ppm and the 8-H signal moves downfield by 0.02 ppm; the greater effect in the heterocyclic ring probably reflects the change from the 'alkoxypyridine' structure to the carbonyl 'pyridinone' tautomer.

3. Conclusions

These release experiments indicate that the nitrothienylmethyl esters are not good potential prodrugs, since they are prone to cleavage of the ester link between masking group and drug, without reductive triggering. In contrast, the 1-(5-nitrothien-2-ylmethoxy)isoquinolines were highly effective potential prodrugs of the potent PARP-inhibitory (5-substituted) isoquinolin-1-ones, in that the biologically active agents are released rapidly upon reduction of the nitrothiophenes. This expulsion of the isoquinolinone leaving group is irreversible, since the residual aminothiophene is chemically unstable and degrades to ring-opened fragments. The isomeric 2-(5-nitrothien-2-ylmethyl)isoquinolin-1-ones, in which the PARP-inhibitory pharmacophore (the *trans* arylamide) is also effectively masked, were not synthetically accessible and thus could not be compared for their efficiency of release of the 2-unsubstituted isoquinolin-1-ones. Such a difference in efficiency of release may exist,[37] since the precise nature of the leaving group is different (oxyanion vs nitrogen anion). Another potential prodrug, the nifedipine derivative **25** in which the leaving group would also be a nitrogen, was similarly synthetically inaccessible. This comparison of leaving groups in reductively activated prodrugs will require the synthesis of analogous isoquinolines carrying different redox-sensitive Triggers.

4. Experimental section

4.1. General

^1H and ^{13}C NMR spectra for characterisation of synthetic compounds were recorded using $CDCl_3$ as solvent. Melting points were determined using a Reichert-Jung Thermo Galen Koffler block and are uncorrected. Infra-red spectra were recorded as potassium bromide discs, except where noted. Mass spectra were obtained in the FAB positive ionisation mode, except where noted. Experiments were conducted at room temperature, unless otherwise stated. Solutions in organic solvents were dried with $MgSO_4$. Solvents were evaporated under reduced pressure. The brine was saturated. The stationary phase for flash column chromatography was silica gel. HPLC was performed using a semi-preparative Kromasil 10C18 column, a Jasco PU-986 preparative pump and Jasco UV-975 detector. MeOH was used as the eluant, with a flow rate of 5 mL min^{-1} the injection volume was 0.020 mL. 1-(5-Nitrothien-2-ylmethoxy)isoquinoline **13**, 5-iodo-1-(5-nitrothien-2-ylmethoxy)isoquinoline **14**, 5-bromo-1-(5-nitrothien-2-ylmethoxy)isoquinoline **15** and 2-(2-thienylmethyl)isoquinolin-1-one **21** were prepared as previously described by us.[25]

4.1.1. 5-Nitrothien-2-ylmethyl 2-acetoxybenzoate 8. 2-Acetoxybenzoyl chloride **6** (620 mg, 3.1 mmol) in CH_2Cl_2 (5 mL) was added to Et_3N (475 mg, 4.7 mmol) and **7** (500 mg, 3.1 mmol) in CH_2Cl_2 (5 mL) at 0°C. The mixture was stirred at 20°C for 1.5 h, then washed with aq. HCl (3 M), aq. $NaHCO_3$ and brine. Drying, evaporation and chromatography (hexane/EtOAc 1:1) gave **8** (360 mg, 35%) as a yellow oil: IR ν_{max} 1750, 1715, 1600, 1500 cm^{-1}; NMR δ_H 2.31 (3H, s, Me), 5.41 (2H, s, CH_2), 7.07 (1H, d, J=3.9 Hz, thiophene 3-H), 7.12 (1H, dd, J=8.2, 1.1 Hz, Ph 3-H), 7.33 (1H, dt, J=7.4, 1.1 Hz, Ph 5-H), 7.61 (1H, dt, J=7.4, 1.5 Hz, Ph 4-H), 7.83 (1H, d, J=3.9 Hz, thiophene 4-H), 8.04 (1H, dd, J=7.8, 1.5 Hz, Ph 6-H); δ_C 21.0, 60.8, 122.0, 123.8, 126.0, 126.7, 128.0, 131.6, 134.4, 145.4, 150.8, 163.4, 169.3; MS m/z 322.0385 (M+H) ($C_{14}H_{12}NO_6S$ requires 322.0385); Found: C, 52.28; H, 3.42; N, 4.35. $C_{14}H_{11}NO_6S$ requires C, 52.33; H, 3.42; N, 4.36%.

4.1.2. 4-(5-Nitrothien-2-ylmethoxy)-4-oxobutanoic acid 9. Compound **7** (1.00 g, 6.3 mmol) was stirred with succinic anhydride (630 mg, 6.3 mmol) and 4-dimethylaminopyridine (5 mg, 41 μmol) in pyridine (5 mL) for 8 h at 50°C. The evaporation residue, in EtOAc, was washed with water and was dried. Evaporation and chromatography (EtOAc) gave **9** (1.23 g, 76%) as a white solid: mp 93–95°C; (Found: C, 41.20; H, 3.45; N, 5.61%. $C_9H_{10}NO_6S$ requires: C, 41.69; H, 3.47; N, 5.40%): IR ν_{max} 1730, 1690, 1470 cm^{-1}; NMR δ_H 2.70 (4H, m, CH_2CH_2), 5.27 (2H, s, CH_2), 7.03 (1H, d, J=5.0 Hz, thiophene 3-H), 7.25 (1H, d, J=5.0 Hz, thiophene 4-H); δ_C 173.9, 171.9, 146.3, 128.3, 126.7, 60.4, 28.9; MS m/z 260.0230 (M+H) ($C_9H_{10}NO_6S$ requires 260.0228).

4.1.3. 5-Nitrothien-2-ylmethyl pentafluorophenyl butanedioate 10. Compound **9** (220 mg, 850 μmol) was stirred with pentafluorophenol (150 mg, 850 μmol) and dicyclohexylcarbodiimide (170 mg, 850 μmol) in EtOAc (11 mL) for 5 h and was allowed to stand at 4°C for 16 h. Filtration and evaporation gave **10** (350 mg, 98%) as a pale buff oil: IR ν_{max} 1770, 1730, 1470 cm^{-1}; NMR δ_H 2.84 (2H, t, J=6.2 Hz, CH_2CO), 3.04 (2H, t, J=6.2 Hz, $COCH_2$), 5.29 (2H, s, CH_2O), 7.04 (1H, d, J=4.2 Hz, thiophene 3-H), 7.81 (1H, d, J=4.2 Hz, thiophene 4-H); δ_C 28.2, 28.7, 60.8, 126.9, 128.1, 145.3, 168.1, 170.8; MS m/z 426.0078 (M+H) ($C_{15}H_9F_5NO_6S$ requires 426.0070).

4.1.4. (5-Nitrothien-2-yl)methyl prednisolone-21-yl butanedioate 12. Prednisolone **11** (180 mg, 520 μmol) was stirred with **10** (220 mg, 520 μmol) and 4-dimethylaminopyridine (5 mg) in dry DMF (10 mL) for 2 days. The evaporation residue, in CH_2Cl_2, was washed with aq. HCl (1 M) and water and was dried. Evaporation yielded **12** (233 mg, 75%) as a yellow–orange glass: IR ν_{max} 3450, 1740, 1650, 1600, 1470 cm^{-1}; NMR δ_H 0.95 (3H, s, prednisolone 18-H_3), 1.0–1.2 (5H, m, prednisolone 7,8,14-H_5), 1.45 (3H, s, prednisolone 19-H_3), 1.45–1.95 (5H, m, prednisolone 9,12, 15-H_5), 2.0–2.6 (4H, m, prednisolone 6,16-H_4), 2.80 (4H, m, $COCH_2CH_2CO$), 4.49 (1H, s, prednisolone 11-H), 4.90 (1H, d, J=17.5 Hz, prednisolone 21-H_2), 5.00 (1H, d, J=17.5 Hz, prednisolone 21-H), 5.28 (2H, s, OCH_2), 6.00 (1H, s, prednisolone 4-H), 6.27 (1H, d, J=10.5 Hz, prednisolone 1-H), 7.04 (1H, d, J=4.3 Hz, thiophene 3-H), 7.29 (1H, d, J=10.5 Hz, prednisolone 2-H), 7.81 (1H, d, J=4.3 Hz, thiophene 4-H); δ_C 17.0, 21.1, 23.9, 31.3, 32.0, 34.1, 34.6, 39.5, 47.0, 51.4, 55.4, 67.2, 68.3, 69.9, 89.5, 122.2, 125.0, 148.0, 157.0, 171.0, 172.0, 186.1, 206.1; MS m/z 602.2084 (M+H) ($C_{30}H_{36}NO_{10}S$ requires 602.2059).

4.1.5. 4-Nitro-2-[(5-nitro-2-thienyl)methyl]isoquinolin-1-one 22. Conc. HNO_3 (0.050 mL, 800 μmol) was added to **21** (50 mg, 207 μmol) in CF_3CO_2H (1.0 mL) at −10°C and the mixture was stirred at 20°C for 16 h. The pH of the mixture was adjusted to 5 with aq. NaOH (2 M) and the mixture was extracted with EtOAc. The extract was washed with water, aqueous $NaHCO_3$ and brine. Drying, evaporation and chromatography (EtOAc/hexane 1:1) afforded **22** (28 mg, 42%) as an off-white solid: mp 70–73°C; IR ν_{max} 1638, 1598, 1570, 1392 cm^{-1}; NMR δ_H 5.40 (2H, s, CH_2), 7.18 (1H, d, J=4.1 Hz, thiophene 3-H), 7.65 (1H, t, J=7.0 Hz, isoquinoline 7-H), 7.80 (1H, d, J=4.1 Hz, thiophene 4-H), 7.89 (1H, t, J=7.0 Hz, isoquinoline 6-H), 8.51 (1H, d, J=7.8 Hz, isoquinoline 5-H), 8.70 (2H, m, isoquinoline 3,8-H_2); δ_C 48.6, 123.8, 127.3, 128.2, 128.8, 130.1, 134.6, 135.4, 143.9, 160.9; MS m/z 332.0340 (M+H) ($C_{14}H_{10}O_5N_3S$ requires 332.0341).

4.1.6. 4-Nitro-2-(2-thienylmethyl)isoquinolin-1-one 23. Compound **21** (50 mg, 207 μmol) was stirred with conc. HNO_3 (65 mL, 1.0 mmol) and Ac_2O (94 μL, 1.0 mmol) in AcOH (5 mL) at 10°C for 40 min. Water was added and the mixture was extracted with EtOAc. The extract was washed with aq. Na_2CO_3 (10%). Drying, evaporation and chromatography (EtOAc/hexane 1:1) yielded **23** (20 mg, 40%) as an off-white solid: mp 75–77°C; IR ν_{max} 1670, 1630, 1508 cm^{-1}; NMR δ_H 5.43 (2H, s, CH_2), 7.01 (1H, m, thiophene 4-H), 7.22 (1H, d, J=3.8 Hz, thiophene 3-H), 7.33 (1H, d, J=4.6 Hz, thiophene 5-H), 7.64 (1H, t, J=7.2 Hz, isoquinoline 7-H), 7.85 (1H, t, J=7.2 Hz, isoquinoline 6-H), 8.53 (1H, d, J=6.8 Hz, isoquinoline 5-H), 8.67

(2H, m, isoquinoline 3,8-H_2); δ_C 47.3, 123.5, 124.1, 125.7, 126.4, 127.0, 128.3, 128.6, 128.8, 134.1, 135.8, 136.0, 160.8; MS *m/z* 287.0488 (M+H) ($C_{14}H_{11}O_3N_2S$ requires 287.0490).

4.1.7. Dimethyl 2,6-dimethyl-4-(2-nitrophenyl)-1-phenylmethyl-1,4-dihydropyridine-3,5-dicarboxylate 26. Nifedipine **24** (1.35 g, 3.9 mmol) was stirred with $LiN(SiMe_3)_2$ (1.0 M in THF, 7.8 mL, 7.8 mmol) in dry DMF (10 mL) for 2 h under Ar. Chloromethylbenzene (500 mg, 3.9 mmol) was added, followed by NaI (5 mg). The mixture was stirred for 2 days. The evaporation residue, in EtOAc, was washed with water and brine. Drying, evaporation and chromatography (hexane/EtOAc 1:1) gave **26** (680 mg, 40%) as a yellow glass: NMR δ_H 2.4 (6H, s, 2×Me), 3.63 (6H, s, 2×OMe), 4.93 (2H, s, CH_2), 5.70 (1H, s, 4-H), 7.20–7.65 (9H, m, Ph-H_5+Ph′-H_4); MS *m/z* 437 (M+H).

4.1.8. Dimethyl 2,6-dimethyl-4-(2-nitrophenyl)-1-(2-thienylmethyl)-1,4-dihydropyridine-3,5-dicarboxylate 27. Nifedipine **24** was treated with $LiN(SiMe_3)_2$, 2-chloromethylthiophene **20**[27] and NaI in DMF, as for the synthesis of **26**, to give **27** (10%) as a pale orange glass: IR ν_{max} 1730, 1690, 1470 cm^{-1}; NMR δ_H 2.53 (6H, s, 2,6-Me_2), 3.61 (6H, s, 2×OMe), 5.06 (2H, s, CH_2), 5.64 (1H, s, pyridine 4-H), 7.00–7.65 (7H, m, thiophene-H_3+Ph-H_4); δ_C 16.9, 44.1, 50.9, 52.1, 126.3, 124.7, 126.3, 127.3, 128.2, 132.4, 136.2, 137.1, 140.9, 146.9, 149.7, 168.1; MS *m/z* (FAB -ve) 441.1118 (M−H) ($C_{22}H_{21}N_2O_6S$ requires 441.1120).

4.2. Release studies

Method A. Zn dust (0.5 mg) was added to **8** (0.5 mg) and NH_4Cl (0.5 mg) in MeOH (2.0 mL) and water (50 μL). The mixture was stirred. Aliquots (100 μL) were removed at regular time points, filtered and analysed by HPLC.

Method B. $SnCl_2$ (0.5 mg) was added to **12** (0.5 mg) in MeOH (2.0 mL). The mixture was stirred. Aliquots (100 μL) were removed at regular time points and analysed by HPLC. The mixture was warmed to 40°C; again aliquots (100 μL) were removed at regular time points and analysed by HPLC.

Method C. $NaBH_4$ (2.0 mg) was added to the nitrothienylmethoxyisoquinoline (**13**,**14** or **15**) (5.0 mg), palladium on carbon (10%, 5.0 mg) and H_2O (0.04 mL) in propan-2-ol (5 mL). TLC analysis after 30 min indicated that the starting materials had been consumed and that the corresponding isoquinolin-1-ones **16**–**18**, respectively, had been formed. The mixture was stirred for 16 h. The evaporation residue was dissolved in CD_3OD (0.6 mL) and submitted to ^1H NMR analysis.

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