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Synthesis of Thiophenecarboxamides, Thieno[3,4-*c*]pyridin-4(5*H*)-ones and Thieno[3,4-*d*]pyrimidin-4(3*H*)-ones and Preliminary Evaluation as Inhibitors of Poly(ADP-ribose)polymerase (PARP)

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Abstract—Inhibitors of poly(ADP-ribose)polymerase (PARP) inhibit repair of damaged DNA and thus potentiate radiotherapy and chemotherapy of cancer. Treatment of 3-cyanothiophene with potassium nitrate and concentrated sulphuric acid gave 5-nitrothiophene-3-carboxamide. 4-Nitrothiophene-2-carboxamide and 5-nitrothiophene-2-carboxamide were formed similarly from 2-cyanothiophene. Reduction with tin(II) chloride gave the corresponding aminothiophenecarboxamide salts which were isolated via their *N*-Cbz derivatives. Lithiation of 3,4-dibromothiophene at −116 °C and quenching with alkyl chloroformates gave 4-bromothiophene-3-carboxylates, which were hydrolysed to 4-bromothiophene-3-carboxylic acid. Hurtley reactions with the enolates of pentane-2,4-dione and of 1-phenylbutane-1,3-dione, followed by acyl cleavage, led to 4-(2-oxopropyl)thiophene-3-carboxylic acid and 4-phenacylthiophene-3-carboxylic acid, respectively. Condensation with ammonia in acetic acid gave 6-methyl- and 6-phenyl-thieno[3,4-*c*]pyridin-4-ones, which were selectively nitrated at the 1- and 7-positions or were dinitrated. Ethyl 4-acetamido- and 4-benzamido-thiophene-3-carboxylates were cyclised to 2-methyl- and 2-phenyl-thieno[3,4-*d*][1,3]oxazin-4-ones, respectively. Ring-opening with ammonia and recyclisation led to 2-substituted thieno[3,4-*d*]pyrimidin-4-ones. The aminothiophenecarboxamides are analogues of 3-aminobenzamide, a selective inhibitor of poly(ADP-ribose)polymerase (PARP); the thienopyridinones and the thienopyrimidinones are analogues of isoquinolin-1-ones and quinazolin-4-ones, respectively, which inhibit this enzyme. In preliminary assays, several thienopyridinones and thienopyrimidinones showed potent inhibitory activity against PARP.

Introduction

Two of the main obstacles that stand in the path of effective treatment of cancer are the presence of hypoxic cells in solid tumours, which are relatively resistant both to radiotherapy and to chemotherapy,[1,2] and repair of tumour cell DNA that has been damaged by radiation or electrophilic drugs.[3] Many studies have shown that poly(ADP-ribose)polymerase (PARP) regulates repair of DNA damage[4–7] and there is strong evidence that inhibitors of PARP can act as radiosensitising[8–12] and chemosensitising[13–18] drugs. PARP is abundant in most cell nuclei and ca. 90% of the PARP in cells is bound to chromatin. It comprises a 113 KDa protein with three domains: a 46 KDa DNA-binding domain, a 22 KDa automodification domain and a 54 KDa catalytic domain but acts catalytically as a homodimer. It binds to DNA strand-breaks through the two zinc fingers of the DNA-binding domain and must be bound DNA to have enzymatic activity.[19,20] It catalyses the transfer of ADP-ribose units from its substrate NAD^+ **1** to several protein acceptors. Most of these are nuclear proteins involved in chromatin architecture and DNA metabolism (heteromodification) but the main protein to be poly(ADP-ribosyl)ated is PARP itself (automodification).[21,22]

Most of the known inhibitors of PARP mimic the nicotinamide of the substrate **1**. The first reported enzyme-selective inhibitor[23] was 3-aminobenzamide **2** ($IC_{50} = 33\ \mu M$). Optimum activity in the benzamide series is achieved[23–25] with an electron-donating group at the 3-position but with no substituent in the 2-, 4-, 5-, or 6-positions, although recent work[26] in this laboratory has shown that some apparently electron-withdrawing substituents are acceptable in the 3-position. Recognition[25] of the importance of the conformation of the benzamide pharmacophore led to development of 5-substituted isoquinolin-1-ones[25,27] and 3,4-dihydroisoquinolin-1-ones[25] **3** and of 2,8-disubstituted quinazolin-4-ones[27–29] **4**, which are some 10- to 50-fold more potent as inhibitors (**3** $R^1 = OH$, $R^2 = H$, $X = CH_2$: $IC_{50} = 0.14\ \mu M$; **4** $R^1 = OH$, $R^2 = Me$, $X = N$: $IC_{50} = 0.44\ \mu M$). In **3** and **4**, the heterocyclic ring constrains the conformation of

Key words: Poly(ADP-ribose)polymerase; DNA repair; thieno[3,4-*c*]pyridin-4(5*H*)-one; thieno[3,4-*d*]pyrimidin-4(3*H*)one; Hurtley reaction.
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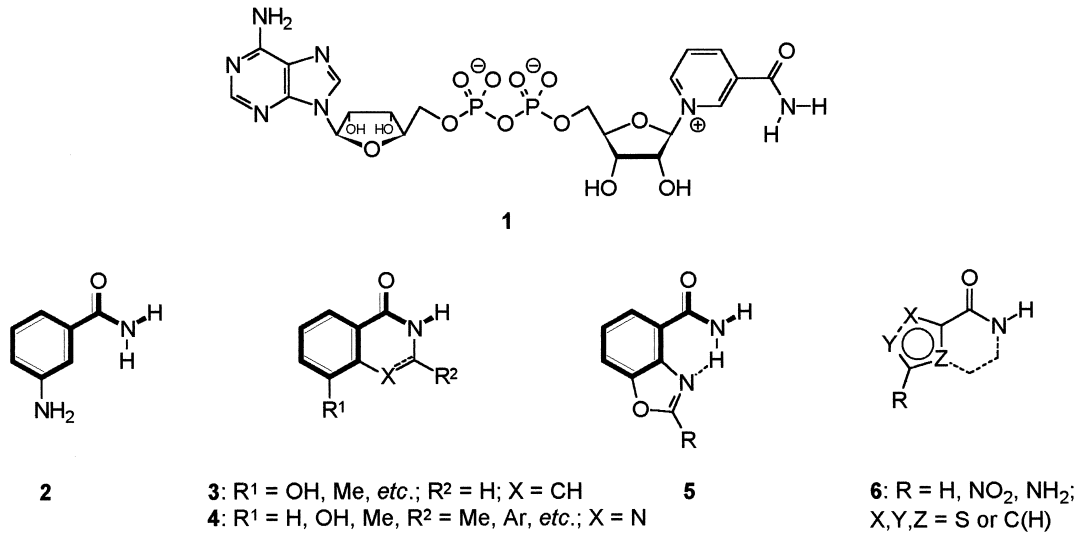


Figure 1. Structures of NAD^+ **1** and known inhibitors of PARP **2–5**, and general structure of proposed inhibitors **6**.

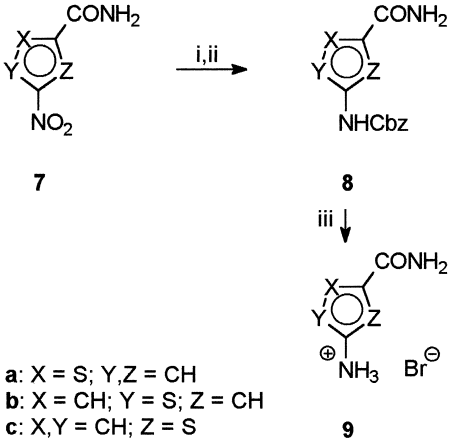
the benzamide with the N–H bond *trans* to the carbonyl-arene bond. An interesting new approach to maintaining this required conformation was developed by Griffin et al.[29] who used a hydrogen-bond in the benzoxazolecarboxamides **5** (**5** R = Ph: $IC_{50} = 2.1\,\mu M$). A large study[27] of a variety of cyclic amides and related compounds confirmed the need for the *trans*-arylamide structural feature; large planar lactams such as 4-amino-1,8-naphthalimide ($IC_{50} = 0.18\,\mu M$) and phenanthridin-6(5*H*)-one ($IC_{50} = 0.30\,\mu M$) were particularly potent as inhibitors of PARP. In structures **2–5**, the consensus pharmacophore is shown in bold (Fig. 1). Heterocycle-carboxamides have been claimed not to have good inhibitory activity towards PARP; for example, thiophene-3-carboxamide has been claimed[30] to have $IC_{50} = 5\,mM$. In this paper, we present syntheses of thiophenecarboxamides, thieno[3,4-*c*]pyridin-4(5*H*)-ones and thieno[4,5-*c*]pyrimidin-7-ones. These can be considered as heterocyclic analogues **6** of benzamides, isoquinolin-1-ones and quinazolin-4-ones and test the effect on inhibitory activity of replacing the benzene ring with thiophene. However, it should be noted that the inhibitory activities of 3-substituted and 4-substituted isoquinolin-1-ones (corresponding to the 6-substituted and 7-substituted thieno[3,4-*c*]pyridin-4-ones) against PARP are unknown.

Chemical synthesis: thiophenecarboxamides

Since the 3-substituted benzamide inhibitors of PARP have a *meta* relationship between the functional groups, sets of thiophenecarboxamides with this relationship were chosen for synthesis and biochemical evaluation. To test the requirement for electron-withdrawing or electron-donating substituents, all three possible '*meta*' nitrothiophenecarboxamides and aminothiophenecarboxamides were proposed (4-substituted-thiophene-2-carboxamides **7a** and **9a**, 5-substituted-thiophene-3-carboxamides **7b** and **9b**, and 5-substituted-thiophene-2-carboxamides **7c** and **9c**; Scheme 1). The nitrothiophenecarboxamides **7a–c** are available in high yield from our previously reported one-pot nitration and hydrolysis[31] of the corresponding cyanothiophenes. Selective reduction of the nitro groups was achieved with tin(II) chloride. Acidic conditions were necessary during the reductions to ensure that the aminothiophenes were maintained in the protonated state for stability. Direct isolation of the highly polar aminothiophenecarboxamide salts from the reaction mixtures was not possible, so they were converted in modest yields to their neutral *N*-(benzyloxycarbonyl) (Cbz) derivatives **8a–c**. Deprotection was achieved with hydrogen bromide, giving the three required aminothiophenecarboxamide regioisomers as their hydrobromide salts **9a–c**.

Chemical synthesis: thieno[3,4-*c*]pyridin-4(5*H*)-ones and thieno[3,4-*d*]pyrimidin-4(3*H*)-ones

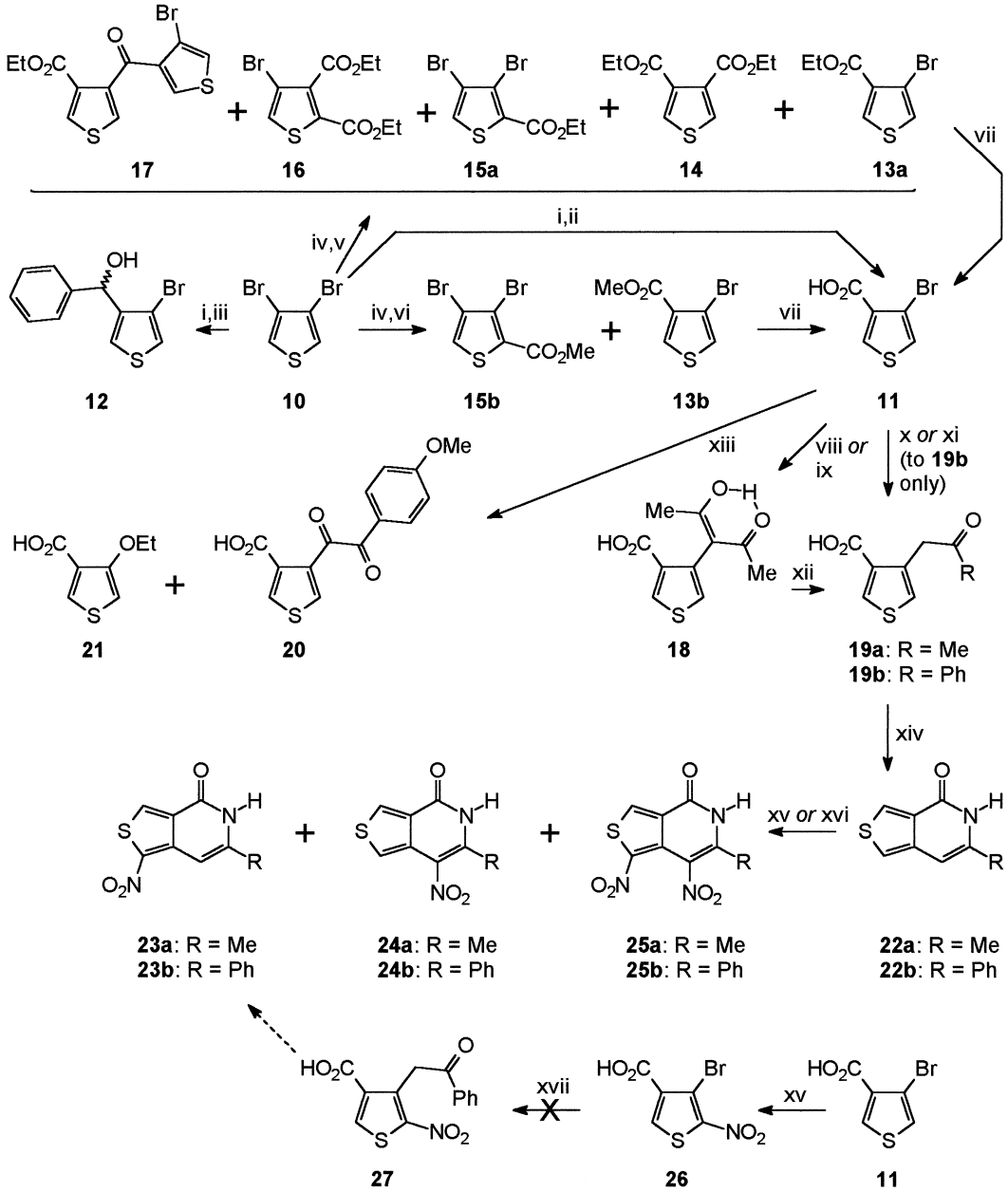
Thiophenes with carbon substituents in both β-positions but unsubstituted at both α-positions are reported relatively infrequently in the literature and are usually only accessible with difficulty, owing to the greater reactivity of the α-positions with electrophiles. Our synthetic



Scheme 1. Synthesis of '*meta*' aminothiophenecarboxamide hydrobromides **9a–c**. *Reagents*: (i) $SnCl_2$, concd aq HCl; (ii) CbzCl, aq NaOH; (iii) HBr, HOAc.

approach to the thieno[3,4-*c*]pyridin-4-one skeleton broadly followed the route taken by Ames and Ribiero,[32] in this route, the critical step in assembling the carbon framework is a Hurtley coupling[33,34] of a β-diketone enolate with 4-bromothiophene-3-carboxylic acid **11**.

Lithium–halogen exchange of one bromine in **10** with butyllithium in diethyl ether at −78 °C, followed by rapid quench with solid carbon dioxide gave **11** in very poor yield (12%) (Scheme 2), in contrast to the 78% reported by Lawesson[35] for this process. This yield could not be increased by modification of the reaction conditions. Since failure of this carboxylation may be due to competing proton abstraction at the more acidic 2-position of the thiophene,[36] the lithiation procedure was checked by trapping the putative 4-bromo-3-lithiothiophene with a more reactive carbonyl electrophile. Lithiation with butyllithium in diethyl ether at −78 °C and quench with benzaldehyde gave the secondary alcohol **12** in 63% yield, confirming that lithiation had taken place at the 3-position by transmetallation, as predicted, but that the nature and reactivity of the trapping electrophile was important. Instability of the lithiothiophene would be expected to be a greater problem at higher temperatures and with less reactive



Scheme 2. Reactions of 3,4-dibromothiophene **10** with butyllithium and electrophiles, Hurtley reactions of 4-bromothiophene-3-carboxylic acid **11** and synthetic routes to thieno[3,4-*c*]pyridin-4-ones **22a,b–25a,b**. *Reagents and conditions*: (i) BuLi, Et_2O, −78 °C; (ii) CO_2; (iii) PhCHO, Et_2O; (iv) BuLi, Et_2O, −116 °C; (v) EtO_2CCl, Et_2O; (vi) MeO_2CCl, Et_2O; (vii) NaOH, aq EtOH, Δ; (viii) $(MeCO)_2CH_2$, $KOBu^t$, Cu, Bu^tOH, Δ; (ix) $(MeCO)_2CH_2$, NaH, CuBr, PhMe, Δ; (x) $PhCOCH_2COMe$, NaOEt, Cu, EtOH, Δ; (xi) $PhCOCH_2COMe$, NaH, CuBr, PhMe, Δ; (xii) aq NH_3; (xiii) 4-$MeOC_6H_4COCH_2COMe$, NaOEt, Cu, EtOH, Δ; (xiv) NH_4OAc, HOAc, Δ; (xv) 90% HNO_3, Ac_2O, various temperatures (see text); (xvi) KNO_3, CF_3CO_2H, −15 °C; (xvii) various Hurtley reaction conditions.

electrophiles. Lithiation of **10** with butyl lithium at −116 °C, followed by quench after 2.5 min with ethyl chloroformate (a reactive carboxy electrophile equivalent), gave the required ester **13a** in 41% optimised yield. Changes in reaction time, temperature, nature of the lithiating agent (e.g. *t*-butyllithium, methyllithium, phenyllithium) led to lower yields of **13a** but more formation of by-products. The principal by-products are shown in Scheme 2. The diester **14** clearly arises from dilithiation by double halogen–metal exchange. Abstraction of the relatively acidic thiophene α-proton[36] from **10** and from **13a** by butyllithium or by a lithiothiophene intermediate would give an appropriate nucleophile for reaction with ethyl chloroformate to give the trisubstituted thiophenes **15a** and **16**, respectively. Regiocontrol of the proton abstraction from **13a** would be controlled by coordination to the adjacent ester function, leading to the thiophene-2,3-dicarboxylate **16**, rather than the isomeric diethyl 3-bromothiophene-2,4-dicarboxylate (which was not isolated). Finally, the bis-thiophene **17** can arise from two reaction pathways: (i) reaction of ethyl 4-lithiothiophene-3-carboxylate (intermediate in the formation of **14**) with **13a**; (ii) reaction of 4-bromo-3-lithiothiophene with **14**. Both pathways confirm the reactivity of lithiothiophenes with electrophilic esters. A similar lithiation of **10** with butyllithium, followed by quench with methyl chloroformate, afforded methyl 4-bromothiophene-3-carboxylate **13b** in moderate yield, together with methyl 3,4-dibromothiophene-2-carboxylate **15b**. However, the anion failed to react with an analogous electrophile, di-*t*-butyl dicarbonate, to give the *t*-butyl ester. The required carboxylic acid **11** was then formed by base-catalysed hydrolysis of **13a,b**.

Ames and Ribeiro[32] noted that the Hurtley reaction of **11** in boiling ethanol with the enolate of pentane-2,4-dione with copper powder as catalyst produced a mixture of the dione **18** and the acyl-cleavage product **19a**, but that the deacetylation could be avoided by performing the reaction with potassium *t*-butoxide in *t*-butyl alcohol, (i.e. in the absence of exogenous nucleophiles). In our laboratory, this reaction gave **18** in 76% yield and deacetylation with aqueous ammonia formed the required monoketone **19a**. Copper(I) ions have been reported[36,37] to be the actual catalytic species in the Hurtley reaction. Thus **18** was formed in good yield when copper(I) bromide was used as catalyst when the reaction of **11** with the sodium enolate of pentane-2,4-dione was performed in hot toluene. In the phenyl series, **11** failed to react with the potassium enolate of 1-phenylbutane-1,3-dione and copper powder in hot *t*-butanol. However, the original sodium ethoxide/copper powder/ethanol conditions effected the Hurtley condensation and the retro-Claisen cleavage to provide the phenacylthiophenecarboxylic acid **19b** directly, albeit in low yield (22%) (Scheme 2). 4-Ethoxythiophene-3-carboxylic acid **21** was isolated as a by-product in this process; this presumably arises from a direct S_NAr reaction of ethoxide ion with **11**. Products of cleavage of the β-diketone by ethoxide ion were also observed. Use of the sodium hydride/copper(I) bromide/hot toluene conditions raised the yield of **19b** to an acceptable 40%. It is interesting to note that the cleavage of the acetyl group from the intermediate β-diketone has taken place without an exogenous nucleophile; presumably excess enolate serves as the required nucleophile. In contrast to these straightforward Hurtley reactions, treatment of the enolate of 1-(4-methoxyphenyl)butane-1,3-dione with **11** in ethanol in the presence of copper powder afforded the unexpected α-diketone **20**, in addition to traces of **21**. Formation of **20** requires a formal increase in oxidation state by two; the precise nature of the oxidant is unclear but one may speculate that copper(II) ions are involved either in oxidation of the intermediate β-diketone or of the (4-methoxyphenacyl)thiophenecarboxylic acid.

Cyclisation of **19a,b** with ammonium acetate in boiling acetic acid,[32] gave good yields of the target 6-substituted thieno[3,4-*c*]pyridin-4-ones **22a,b**. Selective introduction of a nitro substituent at the 1-position of **22a,b** (corresponding to the 3-position of benzamides and to the 5-position of the analogous isoquinolin-1-one PARP inhibitors) was more challenging. Reactions of thieno-[3,4-*c*]pyridin-4-ones with electrophiles are hitherto unreported. Treatment of the 6-methylthienopyridinone **22a** with acetyl nitrate for a short contact time (5 min) at ambient temperature gave a separable mixture of the products of nitration at the 1-position (**23a**) and at the 7-position (**24a**). Doubling the reaction time led to complete nitration at the 7-position of **23a** to form the 1,7-dinitro bicycle **25a**. However, the 7-nitrothienopyridinone was unaffected by this longer contact with the nitrating agent. Thus the presence of a nitro group at the 1-position does not deactivate the nucleophilicity of the 7-position but the presence of an electron-withdrawing group at the 7-position does deactivate the 1-position. Similar treatment of the 6-phenyl analogue **22b** with acetyl nitrate for 5 min gave the mononitrothienopyridinones **23b** and **24b**, whereas longer reaction times afforded **24b** and the dinitro compound **25b**. These and related observations of rapid nitration at the 7-position under modified conditions show that the reactivity of this position corresponds closely with that of the analogous 4-position of isoquinolin-1-ones.[38,39] We[39] and others[40] have previously noted that nitration of isoquinolin-1-ones can be diverted away from the 4-position by carrying out the reaction in strongly acidic media. For example, 2-(furan-2-ylmethyl)isoquinolin-1-one is nitrated exclusively at the furan 5-position with nitric acid in trifluoroacetic acid.[39] However, this tactic of directing nitration exclusively to the 5-membered heterocycle failed with **22a,b**, giving only the dinitro bicycles **25a,b**. Assignment of the regioisomeric identities of the mononitrothienopyridinones **23a,b** and **24a,b** was made through the coupling constants in the ^{1}H NMR spectra (Table 1). In the parent bicycles **22a,b**, no coupling is observed between the 7-H and either the 1-H or the 3-H, whereas 1-H and 3-H are mutually coupled with $^4J = 2.9$ Hz. This coupling constant is typical for W-coupling between the α-protons of thiophenes.[41] In the spectra of the 1-nitrothienopyridinones **23a,b**, no coupling was observed between the 3-H and the 7-H but the spectra of the 7-nitrothienopyridinones **24a,b** revealed a W-coupling $^4J = 3.3$ Hz between the

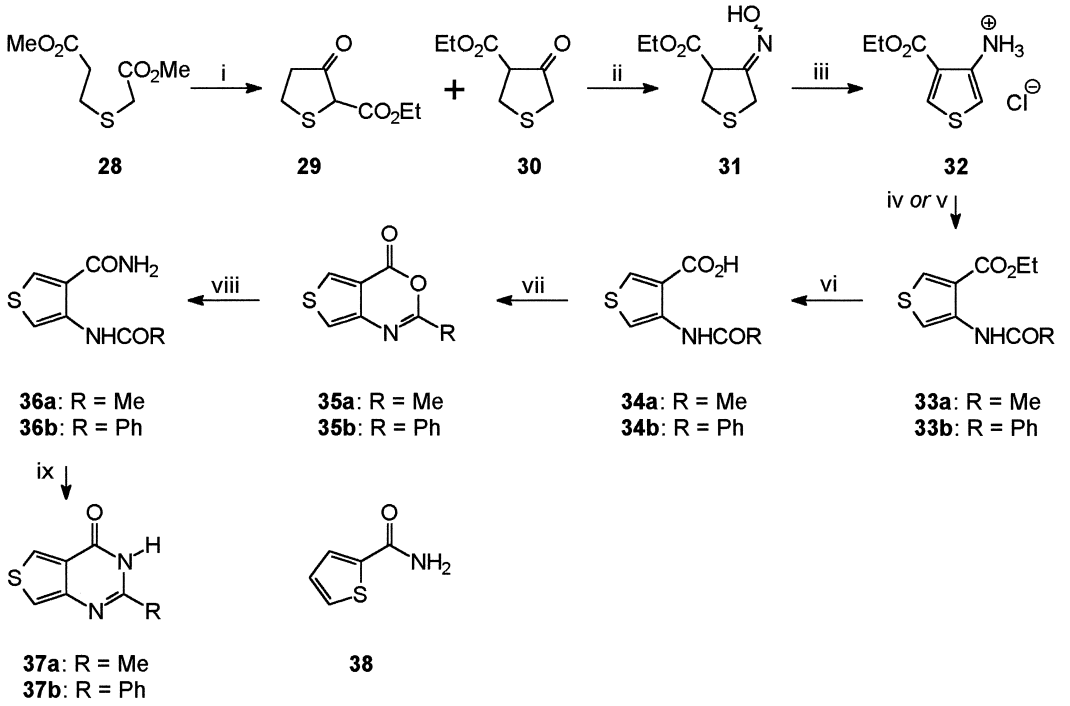
remaining 1-H and 3-H of the bicycle. Trends in chemical shifts were also noted. Introduction of a nitro group in the thiophene ring (1-position) caused pronounced downfield shifts of the resonance of 3-H (Δδ 0.38–0.57 ppm) and on 7-H (Δδ 0.58 and 0.73 ppm). Nitration in the pyridinone ring (7-position) had less effect on 3-H (Δδ 0.08–0.30 ppm) but a strong deshielding influence on 1-H (Δδ 0.73 and 0.74 ppm). Most notable were the effects on the resonances of the 6-substituents. As expected, introduction of a single nitro group at the 1-position had only a small deshielding effect on the 6-methyl group (Δδ 0.17 ppm) whereas the effect on the 6-Me of a single nitro group at the 7-position was more profound (Δδ 0.54 ppm). However, replacement of the 1-H of **24a** with nitro, giving **25a**, shielded the protons of the 6-Me (Δδ −0.21 ppm), suggesting that the steric interaction between the two nitro groups has caused one or both of them to twist out of the plane of the bicycle and out of conjugation. Similar effects are observed when the 6-substituent is phenyl. The *ortho* protons of this group resonate downfield of the *meta* and *para* protons in the 7-unsubstituted thienopyridinones **22b** and **23b**, indicating some conjugation with the π-system of the bicycle. In contrast, the presence of a 7-nitro group moves the resonances of these ortho protons upfield to be co-incident with those of the *meta* and *para* protons, showing that the 7-phenyl group is twisted out of conjugation with the heterocyclic π-system in **24b** and in **25b**.

In view of the stringent control of conditions necessary for synthesis of the modest yields of the 1-nitrothienopyridinones **23a,b**, incorporation of the required nitro group prior to the Hurtley reaction appeared to be an alternative route. 4-Bromothiophene-3-carboxylic acid **11** was selectively nitrated in good yield at the more nucleophilic 5-position. However, the bromonitrothiophenecarboxylic acid **26** failed to participate in any of the variants of the Hurtley reaction, despite the predicted activation of the electrophilicity of the thiophene caused by the nitro group.[42]

The syntheses of the 2-methyl- and 2-phenyl-thieno[3,4-*d*]pyrimidin-4-ones **37a,b** required a thiophene with carbon and nitrogen substituents at the 3- and 4-positions, respectively. 4-Aminothiophene-3-carboxylates, such as

Table 1. ^{1}H NMR data for the thienopyridin-4-ones

Compound	1-H	3-H	7-H	6-Me	6-Ph	NH
22a	7.48 (d, J = 2.9 Hz)	8.35 (d, J = 2.9 Hz)	6.16 (s)	2.10 (s)	—	10.56 (br)
22b	7.42 (d, J 2.9 = Hz)	8.40 (d, J = 2.9 Hz)	6.68 (s)	—	7.48 (3 H, m), 7.61 (2H, dd, J = 8.1, 1.7 Hz)	8.95 (br)
23a	—	8.87 (s)	6.89 (s)	2.27 (s)	—	11.53 (br)
23b	—	8.97 (s)	7.26 (s)	—	7.56 (3 H, m), 7.80 (2H, dd, J = 8.4, 2.0 Hz)	11.80 (br)
24a	8.21 (d, J = 3.3 Hz)	8.65 (d, J = 3.3 Hz)	—	2.64 (s)	—	11.73 (br)
24b	8.16 (d, J = 3.3 Hz)	8.67 (d, J = 3.3 Hz)	—	—	7.48 (s)	11.72 (br)
25a	—	8.99 (s)	—	2.43 (s)	—	12.23 (br)
25b	—	9.05 (s)	—	—	7.53 (s)	12.29 (br)



Scheme 3. Synthesis of 2-substituted thieno[3,4-*d*]pyrimidin-4-ones **37a,b** and structure of thiophene-2-carboxamide **38**. *Reagents and conditions*: (i) NaOEt, EtOH, Δ; (ii) $NH_2OH.HCl$, $BaCO_3$, MeOH, Δ; (iii) HCl, Et_2O; (iv) AcCl, Et_3N, 4-(dimethylamino)pyridine, $CHCl_3$; (v) PhCOCl, Et_3N, 4-(dimethylamino)pyridine, $CHCl_3$; (vi) NaOH, EtOH, Δ; (vii) Ac_2O, Δ; (viii) NH_3, 1,4-dioxan; (ix) NaOH, H_2O, Δ.

32, are intermediates of this type and are accessible from the oxime **31**. Woodward and Eastman[43] and Duus[44] have reported that the direction of Dieckmann cyclisation of the dimethyl ester **28**[45] may be controlled by choice of reaction conditions. When the cyclisation is undertaken at 0 °C using sodium methoxide as a base in methanol, methyl 4-oxotetrahydrothiophene-3-carboxylate is the major product, whereas the sole product in boiling toluene is the isomeric methyl 3-oxotetrahydrothiophene-2-carboxylate. This dual potentiality of the sulphide diester has been interpreted[43] to be a result of anion stabilisation by sulphur with the 2-carboxylate being the thermodynamic product. In our laboratory, neither of these sets of conditions (or, indeed several other sets of bases, solvents and temperatures) provided a satisfactory yield of the 4-oxotetrahydrothiophene-3-carboxylate. However, treatment of **28** with sodium ethoxide in boiling ethanol gave a chromatographically separable mixture of the ethyl esters **29** (10%) and **30** (14%). Proton NMR analysis of these β-ketoesters showed that the enol tautomer predominates at equilibrium in chloroform in the latter but not in the former. In analogy with the reported reactivity[46] of the methyl ester, **30** was converted to a mixture of geometrical isomers of its oxime **31** and then, by acid-catalysed dehydration, to the aminothiophene salt **32**. The free amine of **32**, formed by treatment with sodium hydroxide, was trapped by acetyl chloride and by benzoyl chloride in situ to give the stable amides **33a** and **33b**, respectively. These resisted all attempts to effect direct cyclisation to the thienopyrimidinones **37a,b** using ammonia-based reagents. Thus it was necessary to proceed stepwise and form the primary carboxamides **36a,b** before the final cyclisation. The carboxylic acids **34a,b** were formed in high yield by hydrolysis of **33a,b** under basic conditions. Acetic anhydride then effected cyclisation to the thieno[3,4-*d*][1,3]oxazin-4-ones **35a,b**. These bicycles are analogous to cyclic anhydrides and reacted rapidly with ammonia to give the primary amides **36a,b**. Cyclisation under strongly basic conditions formed the target 2-substituted thieno[3,4-*d*]pyrimidin-4-ones **37a,b** in high yields.

Biological Evaluation and Discussion

As a preliminary screen, the inhibitory effects of 3-substituted benzamides and 5-substituted isoquinolin-1-ones on PARP were examined in vitro at ca. 10 μM concentration of the candidate inhibitors. The enzyme was extracted from nuclei isolated from L929 cells (mouse areolar and adipose tissue cells). PARP activity was estimated by the incorporation of radioactivity from [^{3}H]adenosine-NAD$^+$ into acid-insoluble macromolecular material, according to the method published previously.[23] The acid-precipitated radioactivity of the test incubations was expressed as a percentage of the control to which the solvent buffer alone was added. The results are given in Table 2. Thiophene-3-carboxamide has variously been described as a very weak inhibitor of PARP[30] or as inactive.[13] Our preliminary results show that the isomeric thiophene-2-carboxamide **38** displays good inhibition of the enzyme with potency

Table 2. Results of preliminary studies of inhibition of PARP activity by thiophenecarboxamides, thieno[3,4-*c*]pyridin-4(5*H*)-ones and thieno[3,4-*d*]pyrimidin-4(3*H*)-ones

Compound	Inhibition of PARP activity at ca. 10 μM
Monocyclic thiophenecarboxamides	
7a	52% @ 8.9 μM
7b	46% @ 9.3 μM
7c	63% @ 9.3 μM
9a	76% @ 10.8 μM
9b	66% @ 10.1 μM
9c	82% @ 10.3 μM
38	73% @ 11.8 μM
Bicyclic thienopyridinones and thienopyrimidinones	
22a	90% @ 9.7 μM
22b	93% @ 9.9 μM
23a	54% @ 10.0 μM
23b	86% @ 9.9 μM
24a	89% @ 9.5 μM
24b	47% @ 9.5 μM
37a	81% @ 10.8 μM
37b	42% @ 8.8 μM

of the same order of magnitude as the direct benzene ring analogue, benzamide.[27] Similar inhibitory activity was observed for the three nitrothiophenecarboxamides **7a–c** with the '*meta*' relationship and for the corresponding aminothiophenecarboxamides **9a–c**, confirming that the heterocyclic thiophene ring can indeed substitute for the benzene ring in simple PARP inhibitors without significant loss in potency. However, 3-nitrobenzamide is ca. fivefold less potent that 3-aminobenzamide,[23,27] whereas little difference is evident between the inhibitory activities of **7a–c** and **9a–c**, suggesting that the deleterious effect of an electron-withdrawing group observed for benzamides[23,27] and isoquinolinones[25] may not apply to the heterocyclic carboxamides. All the bicyclic thienopyridinones **22a,b, 23a,b** and **24a,b** and thienopyrimidinones **37a,b** inhibited PARP activity at ca. 10 μM. 5-Substituted isoquinolin-1-ones are among the most potent inhibitors of PARP.[25] The inhibitory activities of 3-substituted isoquinolin-1-ones are unknown but tolerance of a substituent in the corresponding 2-position of quinazolin-4-ones has been noted.[27,29] Among the thienopyridinones tested, there is no clear relationship between the presence and location of a nitro substituent, the nature of the 6-substituent (Me or Ph) and inhibitory activity. For example, introduction of the 1-nitro group appears to diminish activity in the 6-methyl series (**23a** versus **22a**), whereas there is no deleterious effect of this substitution in the 6-phenyl series (**23b** versus **22b**). The converse effect is observed for the 7-nitro compounds (**24a** versus **22a** and **24b** versus **22b**). These apparent anomalies may be connected with the conformational differences indicated by the ^{1}H NMR spectra (vide supra), although firm conclusions of this type cannot be drawn from these preliminary biochemical data. It is noteworthy that 5-nitroisoquinolin-1-one has[25] similar inhibitory potency ($IC_{50} = 3.2\,\mu M$) to that of the unsubstituted parent ($IC_{50} = 6.2\,\mu M$). The 2-methylthienopyrimidinone **37a**

showed good PARP inhibitory activity; this compound is the thieno analogue of the known inhibitor 2-methylquinazolin-4-one ($IC_{50} = 1.1\ \mu M$).[28]

Conclusion

Series of compounds related to known inhibitors of PARP have been synthesised. These compounds all have thiophenecarboxamides or analogous thienopyridinones, and thienopyrimidinones in place of the benzamide, isoquinolin-1-one and quinazolin-4-one structural elements previously thought to be essential for inhibition of this enzyme. In a preliminary assay, most of the compounds evaluated showed good inhibitory activity. This demonstrates for the first time that thiophene can replace the benzene ring in these inhibitors without great loss of activity, in contrast to an earlier report.[30] Refinement of the structural features of the thienopyridin-1-ones and thienopyrimidin-4-ones for inhibition of PARP may provide inhibitors with sufficient potency to merit their use in increasing the efficiency of radiotherapy and chemotherapy of cancer.

Experimental

General methods

Solutions in organic solvents were dried with anhydrous $MgSO_4$. The chromatographic stationary phase was silica gel. IR Spectra were obtained of samples as KBr discs and NMR spectra were obtained of solutions in $(CD_3)_2SO$, except where noted. Positive ion mass spectra were recorded. Dilute H_2SO_4 refers to a 10% aqueous solution, dilute HCl refers to a 2 M aqueous solution. The brine was saturated. The DMF was dry.

4-(Phenylmethoxycarbonylamino)thiophene-2-carboxamide (8a). Tin(II) chloride (5.24 g, 23 mmol) was added during 10 min to 4-nitrothiophene-2-carboxamide (**7a**)[31] (1.00 g, 5.8 mmol) in aq HCl (9 M, 17 mL) at 40 °C. The mixture was stirred for 1 h at 40 °C. The solvent was evaporated. BnOCOCl (1.78 g, 10.5 mmol) was added to the residue, in water (20 mL), and aq NaOH (2 M) was added to bring the pH to 11. The mixture was stirred for 2 days before being extracted with EtOAc. The extract was washed (water). Drying, evaporation, and chromatography (hexane/EtOAc, 1/1) yielded **8a** (550 mg, 34%) as a white solid: mp 184–186 °C; IR 3400, 3200, 1710, 1650, 1620 cm^{-1}; ^1H NMR δ 5.15 (2H, s, CH_2), 7.39 (7H, m, Ph-H_5 + 5-H + amide NH), 7.65 (1H, d, J = 1.1 Hz, 3-H), 8.03 (1H, s, amide NH), 10.2 (1H, br, carbamate NH); MS (FAB) m/z 277.0650 (M + H) ($^{12}C_{13}H_{13}N_2O_3S$ requires 277.0647).

5-(Phenylmethoxycarbonylamino)thiophene-3-carboxamide (8b). 5-Nitrothiophene-3-carboxamide (**7b**)[31] (1.00 g, 5.8 mmol) was warmed with aq HCl (2 M, 17 mL) to 45 °C. Tin(II) chloride (5.24 g, 23 mmol) was added during 10–15 min, maintaining the temperature at 40–45 °C. The solution was stirred for 1 h at 45 °C. The precipitate was collected and was stirred with BnOCOCl (890 mg, 5.2 mmol) in water (10 mL); aq NaOH (2 M) was added to bring the pH to 11. The mixture was stirred vigorously for 2 days before being extracted with EtOAc. The extract was washed (water). Drying, evaporation, and chromatography (hexane/EtOAc, 4/1) furnished **8b** (340 mg, 36%) as a white solid: mp 168–170 °C; ^1H NMR δ 5.18 (2H, s, CH_2), 6.87 (1H, d, J = 1.2 Hz, 4-H), 7.12 (1H, brs, amide NH), 7.38 (5H, m, Ph-H_5), 7.55 (1H, d, J = 1.2 Hz, 2-H), 7.71 (1H, brs, amide NH), 10.89 (1H, br, carbamate NH); MS (FAB) m/z 277.0638 (M + H) ($^{12}C_{13}H_{13}N_2O_3S$ requires 277.0647).

5-(Phenylmethoxycarbonylamino)thiophene-2-carboxamide (8c). Tin (II) chloride (4.46 g, 20 mmol) was added to 5-nitrothiophene-2-carboxamide (**7c**)[31] (850 mg, 4.9 mmol) in aq HCl (9 M, 15 mL) at 40–45 °C during 15 min. The mixture was boiled under reflux for 48 h. To the evaporation residue, in water (20 mL), was added BnOCOCl (1.52 g, 8.9 mmol). Aqueous NaOH (2 M) was added until the solution reached pH 11. The mixture was stirred for 48 h and was extracted thrice with EtOAc. The extracts were washed (water). Drying, evaporation, and chromatography (hexane/EtOAc, 1/1) yielded **8c** (420 mg, 31%) as a white solid: mp 121–123 °C; ^1H NMR δ 5.20 (2H, s, $PhCH_2$), 6.51 (1H, d, J = 4.4 Hz, thiophene 4-H), 7.14 (1H, br, NH), 7.39 (5H, m, Ph-H_5), 7.48 (1H, d, J = 4.0 Hz, thiophene 3-H), 7.75 (1H, br, NH), 11.08 (1H, br, NH); m/z (CI) 227 (M + H), 91 (100) (Bn); MS (FAB) m/z 277.0668 (M + H) ($^{12}C_{13}H_{13}N_2O_3S$ requires 277.0647).

4-Aminothiophene-2-carboxamide hydrobromide (9a). The carbamate **8a** (50 mg, 180 μmol) was stirred with HBr in AcOH (30% w/v, 0.4 mL) for 2 h. Trituration (Et_2O (11 × 2 mL)) yielded **9a** (39 mg, 97%) as a white solid: mp 210–213 °C; ^1H NMR δ 4.49 (3H, br, NH_3^+), 7.61 (1H, br, NH), 7.73 (1H, s, 5-H), 7.76 (1H, s, 3-H), 8.22 (1H, br, NH); MS (FAB) m/z 143.0292 (M + H) ($^{12}C_5H_7N_2OS$ requires 143.0279).

5-Aminothiophene-3-carboxamide hydrobromide (9b). The carbamate **8b** was treated with HBr, as for the synthesis of **9a**, to yield **9b** (98%) as a white solid: mp 181–183 °C; δ_H 6.02 (3H, br, NH_3^+), 7.24 (1H, d, J = 1.0 Hz, 2-H), 7.91 (1H, d, J = 1.0 Hz, 4-H); MS (FAB) m/z 143.0287 (M + H) ($^{12}C_5H_7N_2OS$ requires 143.0279).

5-Aminothiophene-2-carboxamide hydrobromide (9c). The carbamate **8c** was treated with HBr, as for the synthesis of **9a**, to afford **9c** (100%) as a white solid: mp 160–162 °C; ^1H NMR δ 6.22 (1H, d, J = 3.7 Hz, 4-H), 6.85 (3H, br, N + H_3), 7.41 (1H, d, J = 3.7 Hz, 3-H); MS (EI) m/z 142.0206 (M) ($^{12}C_5H_6N_2OS$ requires 142.0201).

4-Bromothiophene-3-carboxylic acid (11). Method A. BuLi (2.5 M in hexanes, 0.52 mL, 1.3 mmol) was added during 30 s to 3,4-dibromothiophene (**10**) (300 mg, 1.2 mmol) in dry Et_2O (10 mL) at −78 °C under N_2. The mixture was stirred for 2.5 min before being added to powdered solid CO_2 (20 g). Water (20 mL) was added and the mixture was extracted with Et_2O. The organic fractions were extracted with aq NaOH (2 M). The aqueous solution was acidified with dilute H_2SO_4 and was extracted with

Et_2O. The extract was dried. Evaporation yielded **11** (30 mg, 12%) as a pale-yellow solid: mp 152–155 °C (lit.[47] mp 156–158 °C) with properties as below.

4-Bromothiophene-3-carboxylic acid (11). Method B. The ethyl ester **13a** (1.69 g, 7.2 mmol) was boiled under reflux with NaOH (560 mg, 14 mmol) in EtOH (50 mL) and water (29 mL) for 30 min. The evaporation residue, in water (50 mL) was acidified with dilute HCl. The solid was collected, washed (water), and dried to yield **11** (1.40 g, 94%) as a white solid: mp 151–155 °C (lit.[47] mp 156–158 °C); IR 3100–2500, 1700 cm^{-1}; ^1H NMR δ 7.77 (1H, d, J = 3.7 Hz, 5-H), 8.37 (1H, d, J = 3.7 Hz, 2-H), 12.98 (1H, br, CO_2H); m/z (EI) 208/206 (M). Compound **11** was also synthesised in 90% yield by analogous treatment of **13b**.

4-Bromo-α-phenylthiophene-3-methanol (12). BuLi (2.5 M in hexanes, 0.52 mL, 1.3 mmol) was added during 30 s to 3,4-dibromothiophene (**10**) (300 mg, 1.2 mmol) in dry Et_2O (10 mL) at −78 °C under N_2. The mixture was stirred for 2.5 min and PhCHO (150 mg, 1.4 mmol) in dry Et_2O (10 mL) was added. The mixture was stirred for 30 min at 20 °C, before being washed (water). Drying, evaporation, and chromatography (hexane/EtOAc, 5/1) gave **12** (210 mg, 63%) as a yellow oil: $bp_{0.1}$ 100–105 °C (lit.[48] $bp_{0.1}$ 100 °C); ^1H NMR δ 5.91 (1H, s, C*H*OH), 7.22 (1H, d, J = 3.4 Hz, thiophene 5-H), 7.57 (7H, m, Ph-H_5 + thiophene 2-H + OH); MS (EI) m/z 269 (M), 105 (100).

Ethyl 4-bromothiophene-3-carboxylate (13a). BuLi (2.5 M in hexanes, 0.52 mL, 1.3 mmol) was added rapidly to 3,4-dibromothiophene (**10**) (300 mg, 1.2 mmol) in dry Et_2O (10 mL) at −116 °C under N_2. The mixture was stirred for 1.5 min at −116 °C. EtOCOCl (270 mg, 2.5 mmol) was added and the mixture was stirred for 5 min at 20 °C. The solution was washed (water (2×), brine). Drying, evaporation and chromatography (hexane/EtOAc, 5/1) yielded **13a** (120 mg, 41%) as a pale-yellow oil: IR (film) 1735 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.39 (3H, t, J = 7.0 Hz, Me), 4.35 (2H, q, J = 7.0 Hz, CH_2), 7.32 (1H, d, J = 3.7 Hz, 5-H), 8.12 (1H, d, J = 3.7 Hz, 2-H); ^{13}C NMR ($CDCl_3$) δ 15.35, 63.01, 110.82, 125.21, 131.37, 134.13, 161.32.; MS (EI) m/z 235.9351 (M) ($^{12}C_7H_7{}^{81}BrO_2S$ requires 235.9330), 233.9366 (M) ($^{12}C_7H_7{}^{79}BrO_2S$ requires 233.9350). Further elution gave **15a** (23 mg, 6%) as a pale-yellow oil: (lit.[49] mp 62–63 °C); ^1H NMR ($CDCl_3$) δ 1.30 (3H, t, J = 7.0 Hz, Me), 4.17 (2H, q, J = 7.0 Hz, CH_2), 7.31 (1H, s, 5-H); MS (EI) m/z 314 (M). Further elution gave **16** (20 mg, 5%) as a pale-yellow oil: ^1H NMR ($CDCl_3$) δ 1.35 (3H, t, J = 7.0 Hz, Me), 1.41 (3H, t, J = 7.0 Hz, Me), 4.35 (2H, q, J = 7.3 Hz, CH_2), 4.45 (2H, q, J = 7.0 Hz, CH_2), 7.45 (1H, s, 5-H); MS (FAB) m/z 308.9624 (M + H) ($^{12}C_{10}H_{12}{}^{81}BrO_4S$ requires 308.9619), 306.9643 (M + H) ($^{12}C_{10}H_{12}{}^{79}BrO_4S$ requires 306.9640). Further elution gave **14** (30 mg, 11%) as a pale-yellow liquid: (lit.[49] bp_{760} 136–142 °C); ^1H NMR ($CDCl_3$) δ 1.36 (6H, t, J = 7.0 Hz, 2×Me), 4.34 (4H, q, J = 7.0 Hz, 2×CH_2), 7.84 (2H, s, 2,5-H_2); MS (EI) m/z 228 (M). Further elution gave **17** (40 mg, 10%); ^1H NMR ($CDCl_3$) δ 1.16 (3H, t, J = 7.2 Hz, Me), 4.14 (2H, q, J = 7.2 Hz, CH_2), 7.35 (1H, d, J = 3.5 Hz, thiophene′ 5-H), 7.62 (1H, d, J = 3.3 Hz, thiophene-H), 7.67 (1H, d, J = 3.3 Hz, thiophene-H), 8.11 (1H, d, J = 3.2 Hz, thiophene-H); MS (FAB) m/z 346.9239 (M + H) ($^{12}C_{12}H_{10}{}^{81}BrO_3S_2$ requires 346.9234); 344.9252 (M + H) ($^{12}C_{12}H_{10}{}^{79}BrO_3S_2$ requires 344.9255).

Methyl 4-bromothiophene-3-carboxylate (13b). BuLi (1.6 M in hexanes, 14.2 mL, 23 mmol) was added rapidly to 3,4-dibromothiophene (**10**) (5.00 g, 21 mmol) in dry Et_2O (100 mL) at −116 °C under nitrogen and the mixture was stirred for 5 min at −110 °C. MeOCOCl (3.91 g, 41 mmol) was added and the mixture was stirred for 20 min at 20 °C. The solution was washed (brine). Drying, evaporation and chromatography (hexane/EtOAc, 20/1) yielded **13b** (1.65 g, 36%) as pale-yellow crystals: mp 47–49 °C (lit.[47] mp 49–51 °C); IR 1720 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.88 (3H, s, Me), 7.31 (1H, d, J = 3.4 Hz, 5-H), 8.11 (1H, d, J = 3.4 Hz, 2-H); m/z (EI) 222 (M). Further elution gave **15b** (210 mg, 4%) as a white solid: mp 135–137 °C (lit.[50] mp 137–137.5 °C); ^1H NMR ($CDCl_3$) δ 3.92 (3H, s, Me), 7.56 (1H, s, 5-H).

4-(1-Acetyl-2-hydroxyprop-1-enyl)thiophene-3-carboxylic acid (18). Method A. Pentane-2,4-dione (2.4 g, 24 mmol) was added to $KOBu^t$ (1.08 g, 9.7 mmol) in Bu^t OH (25 mL), followed by **11** (1.0 g, 4.8 mmol) and Cu powder (31 mg). The mixture was boiled under reflux for 16 h. The evaporation residue was poured into water (40 mL) and the mixture was acidified with dilute HCl. The solid was collected, washed (water) and dried to furnish **18** (830 mg, 76%) as white crystals: mp 193–195 °C (lit.[32] mp 198–199 °C); IR 3200–2800, 1690 cm^{-1}; ^1H NMR ° 1.79 (6H, s, 2×Me), 7.51 (1H, d, J = 3.3 Hz, thiophene 5-H), 8.40 (1H, d, J = 3.3 Hz, thiophene 2-H), 12.66 (1H, br, CO_2H), 16.57 (1H, s, enol OH); MS (EI) m/z 226 (M), 43.

4-(1-Acetyl-2-hydroxyprop-1-enyl)thiophene-3-carboxylic acid (18). Method B. NaH (60% in oil, 650 mg, 6.8 mmol) was added during 5 min to **11** (1.4 g, 6.8 mmol) and CuBr (60 mg) in pentane-2,4-dione (8.1 mL). The mixture was stirred at 80 °C for 16 h, then added to water (50 mL). The mixture was washed (Et_2O, 4×), acidified with aq HCl (9 M) and extracted with EtOAc. Drying and evaporation afforded **18** (1.25 g, 64%) with properties as above.

4-(2-Oxopropyl)thiophene-3-carboxylic acid (19a). The enol **18** (810 mg, 3.6 mmol) was stirred with aq NH_3 (35%, 54 mL) for 24 h. The evaporation residue was acidified with dilute HCl and was extracted with EtOAc. Washing (water), drying, evaporation and chromatography (EtOAc/hexane, 2/1) gave **19a** (480 mg, 73%) as white crystals: mp 150–152 °C (lit.[32] mp 153–154 °C); IR 3200–2700, 1720, 1690 cm^{-1}; ^1H NMR δ 2.12 (3H, s, Me), 3.95 (2H, s, CH_2), 7.32 (1H, d, J = 3.5 Hz, 5-H), 8.24 (1H, d, J = 3.3 Hz, 2-H), 12.5 (1H, br, CO_2H); MS (EI) m/z 184 (M), 124.

4-(2-Oxo-2-phenylethyl)thiophene-3-carboxylic acid (19b). Method A. 1-Phenylbutane-1,3-dione (1.18 g, 7.2 mmol) was heated under reflux with NaOEt (from Na; 260 mg, 11 mmol), 11 (1.00 g, 4.8 mmol) and Cu powder (100 mg,

1.6 mmol) in EtOH (15 mL) under N_2 for 24 h. The mixture was poured into water (50 mL) and the solution was filtered. The filtrate was acidified with dilute HCl and was extracted with Et_2O. Washing (brine), drying, evaporation, and chromatography ($CHCl_3$/MeOH, 9/1) yielded **19b** (260 mg, 22%) as a white solid: mp 200–202 °C (lit.[32] mp 212–214 °C); IR 3200–2600, 1690 cm^{-1}; ^1H NMR δ 4.62 (2H, s, CH_2), 7.38 (1H, d, $J = 3.3$ Hz, thiophene 5-H), 7.56 (3H, m, Ph 3,4,5-H_3), 8.02 (2H, dd, J 7.8, 1.5 Hz, Ph 2,5-H_2), 8.27 (1H, d, $J = 3.3$ Hz, thiophene 2-H), 12.58 (1H, br, CO_2H); m/z (CI) 247 (M + H). Further elution gave **21** (88 mg, 11%) as a white solid: mp 104–106 °C (lit.[51] mp 106–107.5 °C); ^1H NMR δ 1.33 (3H, t; $J = 7.0$ Hz, CH_3), 4.0 (2H, q, $J = 7.0$ Hz, CH_2), 6.66 (1H, d, $J = 3.3$ Hz, 5-H), 8.13 (1H, d, $J = 3.7$ Hz, 2-H), 12.65 (1H, br, CO_2H).

4-(2-Oxo-2-phenylethyl)thiophene-3-carboxylic acid (19b). Method B. NaH (60% in oil, 500 mg, 21 mmol) was added to **11** (1.8 g, 8.7 mmol), 1-phenylbutane-1,3-dione (7.05 g, 44 mmol) and CuBr (75 mg) in PhMe (20 mL) during 5 min. The mixture was heated under reflux for 2 days and poured into water (50 mL). The solution was washed (Et_2O, 4×), acidified with aq HCl (9 M) and extracted with EtOAc. Drying and evaporation gave **19b** (860 mg, 40%) with properties as above.

Hurtley reaction of 4-bromothiophene-3-carboxylic acid (11) and 1-(4-methoxyphenyl)butane-1,3-dione. Na (130 mg, 5.7 mmol) was dissolved in dry EtOH (7.5 mL). 1-(4-Methoxyphenyl)butane-1,3-dione[52] (700 g, 3.6 mmol) was added, followed by **11** (500 mg, 2.4 mmol) and Cu powder (50 mg, 840 μmol), and the mixture was boiled under reflux under N_2 for 24 h. The cooled mixture was added to water (25 mL) and was filtered. The filtrate was acidified with dilute HCl and was extracted with EtOAc. Washing (brine), drying, evaporation, and chromatography (hexane/EtOAc, 10/1) gave ethyl 4-methoxybenzoate (150 mg, 23%) as a yellow oil: (lit.[53] oil); δ_H ($CDCl_3$) 1.38 (3H, t, $J = 7.3$ Hz, CMe), 3.86 (3H, s, OMe), 4.35 (2H, q, $J = 7.3$ Hz, CH_2), 6.92 (2H, d, $J =$ 9.0 Hz, Ph 3,5-H2), 8.00 (2H, d, $J = 8.8$ Hz, Ph 2,6-H_2). Further elution yielded 1-acetyl-4-methoxybenzene (160 mg, 29%) as a white solid with properties identical to a commercial sample. Further elution gave **21** (68 mg, 11%) as an off-white solid with properties as above. Further elution furnished 4-methoxybenzoic acid (200 mg, 37%) as a white solid with properties identical to a commercial sample. Further elution gave **20** (94 mg, 9%) as a colourless oil: ^1H NMR ($CDCl_3$) δ 3.87 (3H, s, Me), 6.97 (2H, d, $J = 8.8$ Hz, Ph 3,5-H_2), 7.98 (2H, d, $J = 8.8$ Hz, Ph 2,6-H_2), 8.18 (1H, d, $J = 3.3$ Hz, thiophene 5-H), 8.27 (1H, d, $J = 3.3$ Hz, thiophene 2-H), 8.80 (1H, br, CO_2H); ^{13}C NMR ($CDCl_3$) δ 55.61, 114.25, 125.41, 132.56, 132.79, 136.42, 137.75, 139.67, 163.90, 164.95, 189.16, 190.49; MS (FAB) m/z 291.0362 (M + H) ($^{12}C_{14}H_{11}O_5S$ requires 291.0372).

6-Methylthieno[3,4-*c*]pyridin-4(5*H*)one (22a). Compound **19a** (410 mg, 2.2 mmol) was boiled under reflux with NH_4OAc (5.5 g, 71 mmol) in AcOH (6.2 mL) for 20 h. The mixture was added to water (30 mL) and was extracted with EtOAc. Washing (aq $NaHCO_3$, water), drying and evaporation gave **22a** (250 mg, 68%) as pale-orange crystals: mp 184–187 °C (lit.[32] mp 184–186 °C); IR 3180, 3120, 1680 cm^{-1}; ^1H NMR (Table 1); MS (EI) m/z 165 (M).

6-Phenylthieno[3,4-*c*]pyridin-4(5*H*)one (22b). The keto-acid **19b** was treated with NH_4OAc and AcOH, as for the synthesis of **22a**, except that the product was purified by chromatography (CH_2Cl_2/MeOH, 20/1), to give **22b** (72 mg, 43%) as a pale-yellow solid: mp 150–153 °C (lit.[32] mp 157–158 °C); ^1H NMR (Table 1); MS (EI) m/z 227 (M), 149.

6-Methyl-1-nitrothieno[3,4-*c*]pyridin-4(5*H*)one (23a) and 6-methyl-7-nitrothieno[3,4-*c*]pyridin-4(5*H*)one (24a). Fuming HNO_3 (90%, 0.32′mL, 7.6 mmol) was added slowly to Ac_2O (0.71 mL, 7.6 mmol) at −10 °C. Compound **22a** (250 mg, 1.5 mmol) in AcOH (2.0 mL) was added and the mixture was stirred at 20 °C for 5 min. Water (3 mL) was added and the mixture was extracted with EtOAc. Washing (aq $NaHCO_3$, water) drying, evaporation, and chromatography (hexane/EtOAc, 4/1) yielded **24a** (120 mg, 38%) as a yellow solid: mp 278–279 °C; ^1H NMR (Table 1); MS (FAB) m/z 211.0179 (M + H) ($^{12}C_8H_7N_2O_3S$ requires 211.0177). Further elution yielded **23a** (67 mg, 22%) as a yellow solid: mp 290–293 °C; ^1H NMR (Table 1); MS (FAB) m/z 211.0246 (M + H, 88) ($^{12}C_8H_7N_2O_3S$ requires 211.0177).

1-Nitro-6-phenylthieno[3,4-*c*]pyridin-4(5*H*)one (23b) and 7-nitro-6-phenylthieno[3,4-*c*]pyridin-4(5*H*)one (24b). Compound **22b** was treated with fuming HNO_3, Ac_2O and AcOH, as for the synthesis of **23a** and **24a**, except that the chromatographic eluant was hexane/EtOAc (10/1), to furnish **24b** (25%) as a yellow solid: mp 233–235 °C; ^1H NMR (Table 1); MS (FAB) m/z 273.0370 (M + H) ($^{12}C_{13}H_9N_2O_3S$ requires 273.0334). Further elution yielded **23b** (11%) as an orange solid: mp 270–272 °C; ^1H NMR (Table 1); MS (FAB) m/z 273.0354 (M + H) ($^{12}C_{13}H_9N_2O_3S$ requires 273.0334).

6-Methyl-7-nitrothieno[3,4-*c*]pyridin-4(5*H*)one (24a) and 1,7-dinitro-6-methylthieno[3,4-*c*]pyridin-4(5*H*)one (25a). Fuming HNO_3 (90%, 0.12 mL, 3.0 mmol) was added slowly to Ac_2O (0.31 g, 3.0 mmol) at −10 °C. Compound **22a** (100 mg, 610 μmol) and AcOH (1.0 mL) were added and the mixture was stirred at 20 °C for 10 min. Water (3 mL) was added and the mixture was extracted with EtOAc. The extract was washed (aq $NaHCO_3$ (3×), water). Drying, evaporation and chromatography (hexane/EtOAc 1/1) yielded **24a** (12 mg, 10%) with properties as above. Further elution afforded **25a** (39 mg, 25%) as a yellow solid: mp > 300 °C; ^1H NMR (Table 1); MS (EI) m/z 254.9951 (M) ($^{12}C_8H_5N_3O_5S$ requires 254.9950).

1,7-Dinitro-6-methylthieno[3,4-*c*]pyridin-4(5*H*)-one (25a). Compound **22a** was treated with KNO_3 and CF_3CO_2H, as for the synthesis of **25b**, to give **25a** (36%) with properties as above.

1,7-Dinitro-6-phenylthieno[3,4-*c*]pyridin-4(5*H*)one (25b). KNO_3 (200 mg, 1.9 mmol) was added to **22b** (220 mg,

970 μmol) in CF_3CO_2H (10 mL) at −15 °C and the mixture was stirred at −15 °C for 5 min. The evaporation residue, in EtOAc, was washed (water, brine). Drying, evaporation and chromatography (CH_2Cl_2/MeOH 100/1) gave **25b** (110 mg, 36%) as yellow crystals: mp 294–295 °C; ^{1}H NMR (Table 1); MS (FAB) *m/z* 318.0207 (M + H) ($^{12}C_{13}H_8N_3O_5S$ requires 318.0185).

4-Bromo-5-nitrothiophene-3-carboxylic acid (26). Fuming HNO_3 (90%, 1.0 mL, 24 mmol) was added slowly to Ac_2O (2.3 mL, 24 mmol) at −10 °C, followed by **11** (1.00 g, 4.8 mmol) in AcOH (5 mL). The mixture was stirred for 20 min at −10 °C. Water (30 mL) was added. The mixture was extracted with EtOAc. Washing (aq $NaHCO_3$ (3×), water), drying, evaporation and chromatography (hexane/EtOAc/AcOH 100/100/1) yielded **26** (66 mg, 54%) as a pale-yellow solid: mp 228–231 °C (lit.[35] mp 236–238 °C); ^{1}H NMR δ 8.69 (1H, s, 2-H), 13.62 (1H, br, CO_2H); MS (CI) *m/z* 253 (M + H).

Ethyl 4-oxotetrahydrothiophene-3-carboxylate (30). Na (140 mg, 6.2 mmol) was dissolved in dry EtOH (20 mL). Methyl 3-(methoxycarbonylmethylthio)propanoate (**28**)[45] (1.0 g, 5.2 mmol) was added and the mixture was boiled under reflux for 16 h. The evaporation residue was brought to pH 5 with dilute HCl and was extracted with EtOAc. Drying, evaporation and chromatography (hexane/EtOAc 40/1) yielded **30** (130 mg, 14%) as a pale-yellow oil: (lit.[44] oil); IR (film) 3500–3100, 1724, 1665, 1618 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.30 (1H, t, *J* = 6.8 Hz, Me, keto), 1.31 (2H, t, *J* = 6.8 Hz, Me, enol), 3.21 (0.3 H, dd, *J* = 1.7, 7.8 Hz, 2-H, keto), 3.32 (0.3 H, d, *J* = 17.6 Hz, 5-H, keto), 3.39 (0.3H, d, *J* = 18 Hz, 5-H, keto), 3.54 (0.3H, dd, *J* = 9.3, 7.8 Hz, 2-H, keto), 3.77 (1.4H, t, *J* = 2.9 Hz, 5-H, enol), 3.82 (1.4H, t, *J* = 2.9 Hz, 2-H2, enol), 4.26 (0.7 H, q, *J* = 6.8 Hz, OCH_2, keto), 4.26 (1.4H, q, *J* = 6.8 Hz, OCH_2, enol), 4.37 (0.3H, m, 3-H, keto), 11.03 (0.7 H, br, OH, enol); MS (EI) *m/z* 174.0351 (M) ($^{12}C_7H_{10}O_3S$ requires 174.0351). Further elution gave **29** (88 mg, 10%) as a pale-yellow oil: (lit.[44] oil); IR (film) 3500–3100, 1750 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.27 (2H, t, *J* = 7.0 Hz, Me, keto), 1.27 (1H, t, *J* = 7.0 Hz, Me, enol), 2.02–2.54 (8H, m, 4,5-H_2, (enol + keto), 3.69 (0.3 H, m, 2-H, keto), 4.13 (1.4 H, q, *J* = 7.0 Hz, OCH_2, keto), 4.26 (0.7H, q, *J* = 7.0 Hz, OCH_2, enol), 11.05 (0.7H, br, OH, enol).

Ethyl *EZ*-4-oximinotetrahydrothiophene-3-carboxylate (31). Compound **30** (1.32 g, 7.6 mmol) was boiled under reflux with $BaCO_3$ (3.43 g, 17.4 mmol) and $NH_2OH.HCl$ (1.21 g, 17.4 mmol) in MeOH (20 mL) for 16 h. The mixture was filtered and the solvent was evaporated from the filtrate. The residue, in EtOAc, was washed (water) and dried. Evaporation gave **31** (1.36 g, 95%) as a pale-yellow oil: IR (film) 3332, 1732 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.28 (0.6H, t, *J* = 7.1 Hz, Me, minor geometrical isomer), 1.29 (2.4H, t, *J* = 7.1 Hz, Me, major geometrical isomer), 3.10 (0.8H, dd, *J* = 11.5, 7.0 Hz, 2-H, major), 3.15 (0.2H, dd, *J* = 14, 7.1 Hz, 2-H, minor), 3.26 (0.2H, dd, *J* = 14, 7.9 Hz, 2-H, minor), 3.27 (0.8H, dd, *J* = 11.5, 6.6 Hz, 2-H, major), 3.55 (0.2H, d, *J* = 16 Hz, 5-H, minor), 3.65 (0.8H, dd, *J* = 16, 1.5 Hz, 5-H, major), 3.67 (0.2H, dd, *J* = 16, 1.5 Hz, 5-H, minor), 3.75 (0.8 H, d, *J* = 16 Hz, 5-H, major), 3.81 (0.8H, t, *J* = 7 Hz, 3-H, major), 4.06 (0.2H, ddd, *J* = 7.9, 7.1, 1.5 Hz, 3-H, minor), 4.21 (0.4 H, q, *J* = 7.1 Hz, OCH_2, minor), 4.23 (1.6 H, q, *J* = 7.2 Hz, OCH_2, major), 8.45 (0.2 H, br, OH, minor), 8.65 (0.8 H, br, OH, major); MS (EI) *m/z* 190.0506 (M) ($^{13}C_{12}C_6H_{11}NO_3S$ requires 190.0493), 189.0474 (M) ($^{12}C_7H_{11}NO_3S$ requires 189.0460), 116.

Ethyl 4-aminothiophene-3-carboxylate hydrochloride (32). Compound **31** (1.30 g, 6.9 mmol) was stirred with hydrogen chloride (7.9 mmol) in dry Et_2O (24 mL) for 24 h. Evaporation gave crude **32** (1.36 g, 95%) as a dark hygroscopic solid: ^{1}H NMR δ 1.32 (3 H, t, *J* = 7.3 Hz, Me), 4.30 (2H, q, *J* = 7.3 Hz, CH_2), 7.73 (1H, d, *J* = 3.7 Hz, 5-H), 8.45 (1H, d, *J* = 3.7 Hz, 2-H); MS (FAB) *m/z* 172.0437 (M + H) ($^{12}C_7H_{10}NO_2S$ requires 172.0432). This material was used directly without further purification.

Ethyl 4-acetamidothiophene-3-carboxylate (33a). Compound **32** (110 mg, 530 μmol) was stirred with AcCl (50 mg, 640 μmol), Et_3N (133 mg, 1.3 mmol) and 4-(dimethylamino)pyridine (20 mg, 160 μmol) in $CHCl_3$ (1.5 mL) for 16 h. $CHCl_3$ (20 mL) was added and the solution was washed (dilute HCl, aq Na_2CO_3, water). Drying, evaporation and chromatography (hexane/EtOAc 10/1) gave **33a** (90 mg, 80%) as white crystals: mp 57–59 °C; Found: C, 50.70; H, 5.19; N, 6.36. $C_9H_{11}NO_3S$ requires C, 50.68; H, 5.21; N, 6.57%; ^{1}H NMR ($CDCl_3$) δ 1.40 (3H, t, *J* = 7.1 Hz, CH_2*Me*), 2.21 (3 H, s, COMe), 4.36 (2H, q, *J* = 7.1 Hz, CH_2), 8.01 (1H, d, *J* = 3.6 Hz) and 8.04 (1H, d, *J* = 3.5 Hz) (thiophene 2,5-H), 11.05 (1H, br, NH); MS (EI) *m/z* 213 (M), 125.

Ethyl 4-benzamidothiophene-3-carboxylate (33b). Compound **32** (150 mg, 720 μmol) was stirred with PhCOCl (120 mg, 870 μmol), Et_3N (180 mg, 1.8 mmol) and 4-(dimethylamino)pyridine (20 mg, 160 μmol) in $CHCl_3$ (2.0 mL) for 16 h. $CHCl_3$ (20 mL) was added and the solution was washed (dilute HCl, aq Na_2CO_3, water). Drying, evaporation and chromatography (hexane/EtOAc 20/1) gave **33b** (150 mg, 75%) as white crystals: mp 106–108 °C; Found: C, 61.30; H, 4.77; N, 5.01. $C_{14}H_{13}NO_3S$ requires C, 61.07; H, 4.77; N, 5.09%; ^{1}H NMR ($CDCl_3$) δ 1.43 (3H, t, *J* = 7.0 Hz, Me), 4.41 (2H, q, *J* = 7.0 Hz, CH_2), 7.52 (3H, m, Ph 3,4,5-H_3), 8.0 (2H, dd, *J* = 7.7, 1.5 Hz, Ph 2,6-H_2), 8.11 (1H, d, *J* = 3.7 Hz, thiophene 5-H), 8.21 (1H, d, *J* = 3.7 Hz, thiophene 2-H), 11.05 (1H, br, NH); MS (EI) *m/z* 275 (M), 105.

4-Acetamidothiophene-3-carboxylic acid (34a). The ester **33a** was treated with NaOH in aq EtOH, as for the synthesis of **34b**, to give **34a** (40.3 mg, 86%) as a white solid: mp 203–205 °C (lit.[54] mp 207–208 °C); ^{1}H NMR ($CDCl_3$) δ 2.24 (3H, s, Me), 8.07 (1H, d, *J* = 3.6 Hz, 5-H), 8.19 (1H, d, *J* = 3.6 Hz, thiophene 2-H), 9.85 (1 H, br, NH); MS (FAB) *m/z* 186.0257 (M) ($^{12}C_7H_8NO_3S$ requires 186.0202).

4-Benzamidothiophene-3-carboxylic acid (34b). The ester **33b** (63 mg, 229 μmol) was boiled under reflux with NaOH (20 mg, 500 μmol) in EtOH (0.5 mL) and water (0.5 mL) for 1 h. The evaporation residue, in water, was

washed with EtOAc, acidified with dilute HCl and extracted with EtOAc. Drying and evaporation afforded **34b** (52 mg, 92%) as a white solid: mp 199–202 °C (lit.[54] mp 215–216 °C); ^{1}H NMR ($CDCl_3$) δ 7.56 (3 H, m, Ph 3,4,5-H_3), 7.97 (2H, d, J = 8.2 Hz, Ph 2,5-H_2), 8.26 (1H, d, J = 3.6 Hz) and 8.27 (1H, d, J = 3.6 Hz) (thiophene 2,5-H_2); MS (EI) m/z 247.0307 (M) ($^{12}C_{12}H_9NO_3S$ requires 247.0303), 105.

2-Methylthieno[3,4-*d*][1,3]oxazin-4-one (35a). Compound **34a** (34 mg, 184 μmol) was boiled under reflux in Ac_2O (3.4 mL) for 24 h. Evaporation gave **35a** (29 mg, 94%) as white crystals: mp 121–123 °C (lit.[54] mp 121–122 °C); ^{1}H NMR ($CDCl_3$) δ 2.24 (3H, s, Me), 8.07 (1H, d, J = 3.6 Hz, 3-H), 8.66 (1H, d, J = 3.6 Hz, 1-H); MS (CI) m/z 168 (M + H).

2-Phenylthieno[3,4-*d*][1,3]oxazin-4-one (35b). Compound **34b** (48 mg, 195 μmol) was boiled under reflux in Ac_2O (5.0 mL) for 16 h. Evaporation gave **35b** (42 mg, 95%) as a white solid: mp 172–174 °C (lit.[54] mp 172–174 °C); ^{1}H NMR ($CDCl_3$) δ 7.52 (3 H, m, Ph 3,4,5-H_3), 7.59 (1 H, d, J = 3.3 Hz, 3-H), 8.27 (2 H, dd, J = 8.4, 1.5 Hz, Ph 2,6-H_2), 8.43 (1 H, d, J = 3.3 Hz, 1-H); MS (EI) m/z 229.0200 (M) ($^{12}C_{12}H_7NO_2S$ requires 299.0198).

4-Acetamidothiophene-3-carboxamide (36a). Compound **35a** was treated with NH_3, as for the synthesis of **36b**, to give **36a** (24 mg, 71%) as a white solid: mp 185–187 °C; ^{1}H NMR ($CDCl_3$) δ 2.20 (3 H, s, Me), 5.7 (2 H, br, NH_2), 7.64 (1 H, d, J = 3.3 Hz, thiophene 5-H), 8.07 (1H, d, J = 3.3 Hz, thiophene 2-H), 10.5 (1H, br, NH); MS (FAB) m/z 185.0375 (M + H) ($^{12}C_7H_9N_2O_2S$ requires 185.0385).

4-Benzamidothiophene-3-carboxamide (36b). A rapid stream of NH_3 was passed through **35b** (41 mg, 177 μmol) in dry 1,4-dioxan (5.0 mL) for 10 min and the mixture was stirred for 16 h. NH_3 was passed through the solution for 20 min. Evaporation furnished **36b** (42 mg, 97%) as a white solid: mp 180–182 °C; ^{1}H NMR ($CDCl_3$) δ 5.8 (2H, br, NH_2), 7.52 (3H, m, Ph 3,4,5-H_3), 7.73 (1H, d, J = 3.3 Hz, thiophene 5-H), 8.00 (2H, dd, J = 7.7, 1.5 Hz, Ph 2,6-H_2), 8.25 (1H, d, J = 3.3 Hz, thiophene 2-H), 11.64 (1H, br, NH); MS (EI) m/z 246.0462 (M) ($^{12}C_{12}H_{10}N_2O_3S$ requires 246.0303).

2-Methylthieno[3,4-*d*]pyrimidin-4(3*H*)one (37a). Compound **36a** was treated with NaOH, as for the synthesis of **37b**, except that the chromatographic eluant was $CHCl_3$/MeOH 49/1, to give **37a** (57%) as a white solid: mp 232–233 °C; ^{1}H NMR ($CDCl_3$) δ 2.45 (3 H, s, Me), 7.48 (1H, d, J = 3.3 Hz, 7-H), 8.28 (1H, d, J = 3.3 Hz, 5-H), 9.5 (1H, br, NH); MS (FAB) m/z 167.0283 (M + H) ($^{12}C_7H_7N_2OS$ requires 167.0279).

2-Phenylthieno[3,4-*d*]pyrimidin-4(3*H*)one (37b). Compound **36b** (20 mg, 81 μmol) was boiled under reflux with NaOH (20 mg, 500 μmol) in water (0.4 mL) for 30 min. The mixture was acidified with AcOH and was extracted with EtOAc. The extract was washed with water. Drying, evaporation and chromatography ($CHCl_3$/MeOH 19/1) gave **37b** (8.0 mg, 43%) as a white solid: mp 242–243 °C; ^{1}H NMR ($CDCl_3$) δ 7.56 (3H, m, Ph 3,4,5-H_3), 7.69 (1H, d, J = 3.3 Hz, 7-H), 8.05 (2H, dd, J = 7.7, 1.8 Hz, Ph 2,6-H_2), 8.34 (1H, d, J = 3.3 Hz, 5-H), 10.00 (1H, br, NH); MS (FAB) m/z 229.0435 (M + H) ($^{12}C_{12}H_9N_2OS$ requires 229.0436).

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