Exploring the functionalisation of the thieno[2,3-d]pyrimidinedione core: Late stage access to highly substituted 5-carboxamide-6-aryl scaffolds

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The thieno[2,3-d]pyrimidinedione core is found as a component in a range of pharmaceutically active compounds, however, synthetic approaches to these scaffolds rely on access to functionalised, highly substituted thiophenes. Here we describe a new approach for the preparation of 5-carboxamide-6-aryl analogues that involves a two-step synthesis of the thieno[2,3-d]pyrimidinedione core from a readily available mercaptouracil derivative. Thio-alkylation with ethyl 3-bromopyruvate, followed by cyclisation and dehydration mediated by polyphosphoric acid allowed the scalable synthesis of the thieno[2,3-d]pyrimidinedione unit. The late-stage functionalisation of this core motif via bromination of the thiophene ring and a subsequent Suzuki-Miyaura reaction as the key steps permitted access to a novel library of 5-carboxamide-6-aryl analogues. The physicochemical properties of these compounds were determined, generating an insight into the potential bioavailability of these scaffolds. Based on these results, a selection of the novel 5-carboxamide-6-aryl analogues were tested as lactate uptake inhibitors of monocarboxylate transporters 1, 2 and 4 in Xenopus oocytes.

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1. Introduction

Due to their similarity in structure to nucleic acid bases, pyrrolo[2,3-d]pyrimidine and thieno[2,3-d]pyrimidine scaffolds display a wide range of biological activities [1]. Within this structural class, the thieno[2,3-d]pyrimidinediones have been of particular interest, with pharmaceutical activity against a wide range of disease states. For example, thieno[2,3-d]pyrimidinediones bearing a N-piperazinylethyl moiety such as 1 (Fig. 1) are potent oral antihypertensive agents [2]. In a programme to discover new inhibitors of the monocarboxylate transporter 1, AstraZeneca showed that thieno[2,3-d]pyrimidinediones with 5-carboxamide-6-alkyl substituents had potent immunomodulatory activity [3]. More recently, compounds from this series (e.g. AZD3965 2) have been shown to kill tumour cells reliant on glycolysis [4]. Other examples include thieno[2,3-d]pyrimidinediones with a 6-(p-methoxyureidophenyl) substituent, such as relugolix 3 [5]. These compounds are selective antagonists of the gonadotropin-releasing hormone receptor and are in phase 3 clinical trials for the treatment of endometrosis and prostrate cancer [6,7].

Despite the importance of thieno[2,3-d]pyrimidinediones, there are relatively few synthetic approaches that allow both the efficient preparation of the bicyclic core and further functionalisation of either ring. Common approaches to access thieno[2,3-d]pyrimidinediones include the reaction of 2-aminothiophenes with isocyanates [2,5] or the alkylation of mercaptouracil derivatives with α-halocarbonyl compounds, followed by cyclisation in the presence of Lewis acids such as titanium tetrachloride [3c,8,9].

Building our research programme that seeks to discover novel biologically active polycyclic scaffolds [10], we were interested in developing a scalable synthesis of a thieno[2,3-d]pyrimidinedione core that could then be further functionalised allowing late stage access to a diverse series of highly substituted analogues. We now report a three-stage synthetic approach for the

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rapid preparation of a small library of novel thieno[2,3-d]pyrimidinedione-5-carboxamide-6-aryl analogues. We have determined various physicochemical properties of these compounds, highlighting the potential of this scaffold for medicinal chemistry applications. The ability of these novel thieno[2,3-d]pyrimidinedione-5-carboxamide-6-aryl analogues to inhibit lactate uptake of monocarboxylate transporters 1, 2 and 4 in Xenopus oocytes is also presented.

2. Results and discussion

As outlined in Scheme 1, a three-stage approach was proposed for the preparation of the target thieno[2,3-d]pyrimidinedione-5-carboxamide-6-aryl analogues. The first-stage involved the development of a reproducible, scalable synthesis of thieno[2,3-d]pyrimidinedione 5 from commercially available 6-chloro-3-methyluracil (4). The aim was then to investigate the reactivity of this scaffold for the introduction of aryl groups at the 6-position. Finally, as thieno[2,3-d]pyrimidinediones bearing a 5-carboxamide moiety are often biologically active [1b,3], strategies for rapid conversion of the thiophene-carboxylic ester to various carboxamide groups were then explored.

To investigate various thiophene-forming reactions, 6-mercaptopyrimidinedione 7 was chosen as the key substrate. This was prepared in two steps from 6-chloro-3-methyluracil (4), by N-alkylation with isobutyl bromide under standard base-mediated conditions, followed by an efficient substitution reaction with sodium hydrosulfide (Scheme 2).

Previous reactions using mercaptopyrimidinedione 7 for the preparation of analogues of thieno[2,3-d]pyrimidinedione 5 have utilised a two-stage process involving S-alkylation with 2-chlorocarbonyl compounds followed by cyclisation and dehydration with titanium tetrachloride [3a,c]. However, these approaches generally result in low overall yields (18–37%) [3a,c]. We attempted a similar transformation with 7 and ethyl 3-bromopyruvate. Although the S-alkylated intermediate 8 was formed cleanly (by $^1$H NMR spectroscopy), cyclisation and dehydration with titanium tetrachloride gave only a 24% overall yield of thieno[2,3-d]pyrimidinedione 5 (Table 1, entry 1). In addition, it was difficult to reproduce these results using this two-stage process. As a consequence of the poor yields and reproducibility of using titanium tetrachloride to form the thiophene ring, other methods were investigated. Thieno[2,3-d]pyrimidinediones have been prepared efficiently from mercaptopyrimidinedione 7 by deprotonation with sodium acetate, followed by reaction with S-halocarbonyl compounds [11]. However, application of this method with 7 and ethyl 3-bromopyruvate gave only a 10% yield of 5, as well as a number of side-products (entry 2) [12].

Ogura and co-workers demonstrated that mercaptopyrimidinediones could undergo S-alkylation with simple α-halocarbonyl compounds such as bromoacetone under neutral conditions [9]. Following isolation and purification of the S-alkylated mercaptopyrimidinediones, subsequent cyclisation with polyphosphoric acid (PPA) gave the corresponding thieno[2,3-d]pyrimidinediones in good yields. Using this approach as a starting point, conditions for the reaction of mercaptopyrimidinedione 7 with the highly electrophilic ethyl 3-bromopyruvate to form ester-derived thieno[2,3-d]pyrimidinedione 5 were investigated. Reaction of 7 with ethyl 3-bromopyruvate under neutral conditions did form intermediate 8 cleanly (Table 1, entry 3). However, unlike the alkyl and aryl derived intermediates from the Ogura study [12], S-alkylated mercaptopyrimidinedione 8 could not be isolated and purified due to significant decomposition. Therefore, intermediate 8 was directly converted to ester-derived thieno[2,3-d]pyrimidinedione 5 using the PPA-mediated cyclisation reaction. Initially, the cyclisation step was found to proceed at 100 °C, generating thieno[2,3-d]pyrimidinedione 5 in modest yield (entry 3). Optimisation of this step included increasing the reaction temperature, resulting in shorter reaction times. At an optimal temperature of 145 °C, this gave 5 in 54% overall yield from the one-pot, two step procedure (entry 5) [13]. More importantly, this approach was found to be readily reproducible and could be used for the multigram synthesis of 5.

The next stage of this research programme involved aryl substitution of the 6-position of thieno[2,3-d]pyrimidinedione 5. A two-step strategy was proposed involving bromination of the thiophene ring, followed by a Suzuki-Miyaura reaction (Scheme 3) [14]. Bromination of 5 under standard conditions with N-
bromosuccinimide (NBS), in the presence of acetic acid gave the corresponding bromide 9 in 84% yield. Suzuki-Miyaura reaction of 9 was then performed with various boronic acids using Pd(PPh₃)₄ as the catalyst. To explore the electronic and steric limitations of this process, both electron-rich and electron-deficient phenylboronic acids bearing either ortho- or para-substituents were investigated. Despite the variations, consistently high yields were observed for all four analogues formed from this reaction.

Having demonstrated efficient functionalisation of the C-6 position of the thieno[2,3-d]pyrimidinedione core through incorporation of various aryl groups, the final stage required preparation of the C-5 carboxamide. Initial studies began with the synthesis of morpholine carboxamide 14 (Scheme 4). Rapid hydrolysis of ethyl ester 10 was achieved using sodium hydroxide in ethanol. Coupling of the resulting carboxylic acid with morpholine was then attempted using various standard coupling reagents (EDCI, HBTU and EDCI/HOBt), however, all of these reactions gave low yields (9–32%) of 14. It was proposed that the low yields were due to the steric hindrance associated with the highly substituted thiophene ring and the subsequent slow reaction with the bulky coupling agents. It was believed that this could be overcome by using a smaller and more reactive acid chloride intermediate. Therefore, the carboxylic acid was converted to the acid chloride under mild conditions using oxalyl chloride and DMF. Without purification, this was treated with morpholine, resulting in the isolation of carboxamide 14 in 48% yield over the three steps (Scheme 4). Following the development of a straightforward approach to access the thieno[2,3-d]pyrimidinedione-5-carboxamide-6-aryl scaffold, the scope of this three step transformation was explored with structurally different amines, for the formation of carboxamides bearing cyclic and acyclic groups. As Weinreb amides are commonly biologically active due to an ability to hydrogen bond to biological targets [3], a series of these were also formed. Overall, all

**Table 1**

| Entry | Step 1 | Step 2 | Yield (%) of 5’ |
|-------|--------|--------|----------------|
| 1     | K₂CO₃, DMF, rt, 16 h | TiCl₄, CH₂Cl₂, 0 °C to rt, 16 h | 24% |
| 2     | NaOAc, H₂O, rt, 5 h | TiCl₄, CH₂Cl₂, 0 °C to rt, 16 h | 10% |
| 3     | EtOH, rt, 1 h | PPA, 100 °C, 12 h | 35% |
| 4     | EtOH, rt, 1 h | PPA, 130 °C, 6 h | 41% |
| 5     | EtOH, rt, 1 h | PPA, 145 °C, 3 h | 54% |

* Isolated yield over the two steps.

**Scheme 3.** Bromination and Suzuki-Miyaura reaction of thieno[2,3-d]pyrimidinedione-5-carboxylate ester 5.

**Scheme 4.** Preparation of the thieno[2,3-d]pyrimidinedione-5-carboxamide-6-aryl library. Isolated yields of 14–25 over the three steps are shown. *Hünig’s base was also used for the amidation step.
four Suzuki–Miyaura products 10–13 were easily converted via the three step sequence to the corresponding carboxamides. Yields were generally good for preparation of the morpholine carboxamides 14–17 and the Weinreb amides 22–25. Lower yields were observed over the three steps for the diethyl carboxamide analogues 18–21. In comparison to the other amines, this is likely due to the less rigid and less reactive nature of diethylamine.

With the successful synthesis of a library of novel thieno[2,3-d]pyrimidinedione-5-carboxamides, we were interested in evaluating how the incorporation of aryl groups at the C-6 position might affect the physicochemical properties. For compounds that might find application in binding to neurological receptors by penetrating the blood brain barrier, permeability across the plasma membrane is important. Similarly, for compounds that transport across cell membranes through passive diffusion, evaluation of the membrane partition coefficient is crucial. Therefore, the partition coefficient (log \( P \)), permeability (\( P_m \)), the membrane partition coefficient (\( K_m \)) and the percentage of plasma protein binding (%PPB) of all twelve thieno[2,3-d]pyrimidinedione-5-carboxamides were evaluated using established HPLC methods (Table 2). Previous work by Tavares et al., of ten biologically active compounds, established the limits of each of these parameters (log \( P < 4 \), \( P_m < 0.5 \), \( K_m < 250 \), %PPB < 95%) [35]. Based on these criteria, the physicochemical properties of the majority of these compounds were found to be excellent and well within the acceptable limits. Despite the incorporation of an aryl moiety and the resulting increase in lipophilicity, these compounds possess properties that should allow effective transport through cell membranes. Due to the relatively lipophilic diethyl carboxamide group, compounds 18 and 19 were found to have the highest plasma protein binding (%PPB). While these values are above the acceptable limits, there are medicinally important compounds with similar plasma protein binding that still demonstrate good bioavailability [13]. Therefore, all of these structural classes are of interest for further development.

As highlighted above, thieno[2,3-d]pyrimidinediones have significant biological activity, particularly against monocarboxylate transporters (MCT), with certain compounds showing potent immunomodulatory activity or the ability to kill tumour cells that rely on glycolysis [3,4]. For these reasons, a selection of nine of the thieno[2,3-d]pyrimidinediones prepared in this current study were tested as inhibitors of lactate uptake of MCT1, MCT2 or MCT4 in Xenopus oocytes (Fig. 2) [16]. As well as control (blank) experiments, AR-C155858 (AR-C in Fig. 2), a commercially available inhibitor of MCTs was also tested as a standard [3c]. While none of the compounds showed any reduction in lactate uptake against MCT1, morpholine carboxamide 15 showed significant activity against MCT2 (\( p < 0.01 \)). For several of the compounds, a tendency to inhibit MCT2 was also observed, however the reduction in transport activity did not significantly differ from the control cells. The morpholine carboxamides also showed a slight inhibitory effect on MCT4, with compounds 14 and 16 showing ~10% reduction in uptake. Although these compounds are not significantly active in inhibiting lactate uptake (each compound was tested at 1 \( \mu \)M), this study has demonstrated the structure activity relationship of this series and identified thieno[2,3-d]pyrimidinedione morpholine carboxamide derivatives as potential scaffolds for further development as lactate uptake inhibitors of MCTs.

### Table 2

| Entry | Compound | Log \( P \) | \( P_m \) | \( K_m \) | %PPB |
|-------|----------|-------------|----------|----------|-------|
| 1     | 14       | 3.02        | 0.12     | 51.74    | 87.8  |
| 2     | 15       | 2.97        | 0.17     | 77.39    | 93.2  |
| 3     | 16       | 2.94        | 0.18     | 82.00    | 90.5  |
| 4     | 17       | 2.87        | 0.16     | 75.01    | 92.4  |
| 5     | 18       | 3.67        | 0.44     | 189.16   | 95.8  |
| 6     | 19       | 3.63        | 0.42     | 181.54   | 96.4  |
| 7     | 20       | 3.69        | 0.25     | 111.51   | 90.5  |
| 8     | 21       | 4.22        | 0.33     | 147.24   | 93.4  |
| 9     | 22       | 3.38        | 0.23     | 96.05    | 87.8  |
| 10    | 23       | 3.32        | 0.17     | 69.68    | 88.2  |
| 11    | 24       | 3.52        | 0.20     | 88.19    | 88.4  |
| 12    | 25       | 3.53        | 0.18     | 76.75    | 86.3  |

Determined using:

- \( ^a \) C18 column.
- \( ^b \) Immobilised artificial membrane (IAM) column.
- \( ^c \) Human serum albumin (HSA) coated column.

**Fig. 2.** Inhibition of lactate uptake in Xenopus oocytes, expressing (A) MCT1, (B) MCT2 + GP70 and (C) MCT4. Each compound was tested at a concentration of 1 \( \mu \)M. The lactate uptake for each compound shown is the mean ± SEM of eight independent experiments (n – 8). The asterisks refer to the values of control cells (white bars).

\( ^* p < 0.05 \), \( ^{**} p < 0.001 \).
3. Conclusions

In summary, a flexible and concise approach for the preparation of novel thieno[2,3-d]pyrimidinedione scaffolds has been developed. In particular, a reliable and scalable route for the synthesis of a thieno[2,3-d]pyrimidinedione core bearing a C-5 ester moiety has been achieved from a mercaptouracil derivative by an alklylation reaction with ethyl 3-bromopyruvate followed by an acid-mediated cyclisation. The product of this process has served as a key intermediate to explore the introduction of aryl groups at the C-6 position as well as explore further synthetic applications of this functionally rich bicyclic core.

4. Experimental section

4.1. General methods

All reactions were performed under an atmosphere of air unless otherwise stated. All reagents and starting materials were obtained from commercial sources unless otherwise stated. Brine refers to a saturated solution of sodium chloride. All dry solvents were purified using a PureSolv 500 MD solvent purification system. Flash column chromatography was carried out using Merck Feduran Si 60 (40–63 μm). Machedery-Nagel aluminium-backed plates pre-coated with silica gel 60 were used for thin layer chromatography and were visualised under a UV lamp. 1H NMR and 13C NMR spectra were recorded on a Bruker DPX 400 spectrometer or Bruker 500 spectrometer with chemical shift values in ppm relative to trimethylsilylane or residual chloroform as standard. J values are reported in Hz. The assignment of 1H NMR spectra is based on COSY and HSQC experiments and 13C NMR spectra is based on DEPT experiments. Infrared spectra were recorded using a Shimadzu FTIR-8400S spectrometer directly as a solid or liquid and mass spectra were obtained using a JEOL JMS-700 spectrometer or a Bruker MicroTOFq high-resolution mass spectrometer. Melting points were determined on a Gallenkamp melting point apparatus. All physicochemical analyses were performed using a Dionex Ultimate 3000 series, and data acquisition and processing performed using Chromelion 6.8 Chromatography software [4]. Standard and test compounds were dissolved in 1:1 organic/aqueous phases, and prepared to a concentration of 0.5 mg/mL. The HPLC system was set to 25 °C, and UV detection achieved using a diode array detector (190–800 nm). Analysis was performed using 5 μL sample injections.

4.2. Experimental procedures and compound characterisation

4.2.1. 6-Chloro-3-methyl-1-(2-methylpropyl)-2,4-(1H,3H)-pyrimidinedione [6] [17]

Isobutyl bromide (8.90 mL, 82.2 mmol) was added to a solution of 6-chloro-3-methyluracil (4) (12.0 g, 74.7 mmol) and potassium carbonate (12.4 g, 89.7 mmol) in DMSO (120 mL). The mixture was then heated to 60 °C and stirred for 48 h. The mixture was then cooled to room temperature and diluted with water (40 mL) and brine (40 mL). The product was then extracted using diethyl ether (3 × 40 mL). The organic layers were combined, dried (MgSO₄), filtered and concentrated in vacuo to provide title compound 6 (13.7 g, 84%) as an orange oil. This was used directly in the next step without further purification. Spectroscopic data were consistent with the literature. 1H NMR (400 MHz, CDCl₃) δ 0.96 (6H, d, J = 6.8 Hz, CH(CH₃)₂), 2.11–2.22 (1H, m, 2'-H), 3.34 (3H, s, 3-CH₃), 3.90 (2H, d, J = 7.6 Hz, 1'-H₂), 5.92 (1H, s, 5-H); 13C NMR (101 MHz, CDCl₃) δ 19.7 (2 × CH₃), 28.1 (CH₃), 28.3 (CH), 53.5 (CH₂), 101.9 (CH), 146.0 (C), 151.2 (C), 160.9 (C); MS (EI) m/z 217 (M+H⁺, 95%), 183 (17), 69 (10).

4.2.2. 6-Mercapto-3-methyl-1-(2-methylpropyl)-2,4-(1H,3H)-pyrimidinedione [7] [3a]

Sodium hydroxysulfide monohydrate (1.28 g, 22.7 mmol) was added to a stirred solution of 6-chloro-3-methyl-1-(2-methylpropyl)-2,4-(1H,3H)-pyrimidinedione (6) (4.10 g, 18.9 mmol) in ethanol (60 mL). The mixture was stirred at room temperature for 24 h before further sodium hydroxysulfide monohydrate (1.28 g, 22.7 mmol) was added and stirring continued at room temperature for a further 16 h under an atmosphere of argon. The solution was concentrated in vacuo and the resulting oil diluted with water (30 mL) and washed with ethyl acetate (30 mL). The aqueous layer was then acidified with an aqueous solution of 1 M hydrochloric acid and then extracted using ethyl acetate (3 × 30 mL). The combined organic layers were then dried (MgSO₄), filtered and concentrated in vacuo to give 6-mercaptop-3-methyl-1-(2-methylpropyl)-2,4-(1H,3H)-pyrimidinedione (7) as a pale yellow solid (3.69 g, 90%). Spectroscopic data were consistent with the literature [3a]. Mp 111–113 °C; 1H NMR (400 MHz, CDCl₃) δ 0.92 (6H, d, J = 6.8 Hz, CH(CH₃)₂), 2.24–2.31 (1H, m, 2'-H), 3.29 (3H, s, 3-CH₃), 4.14 (2H, s, 5-H₂), 4.27 (2H, d, J = 7.4 Hz, 1'-H₂); 13C NMR (101 MHz, CDCl₃) δ 20.1 (2 × CH₃), 26.6 (CH₃), 28.6 (CH), 49.2 (CH₂), 54.5 (CH₂), 150.3 (C), 164.8 (C), 197.2 (C); MS (ESI) m/z 237 (M+N⁺, 60%), 159 (20), 307 (5), 449 (2).

4.2.3. Ethyl 1-isobutyl-3-methyl-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-d]pyrimidin-5-carboxylate [5] [3a]

Ethyl bromopyruvate (1.70 mL, 13.3 mmol) was added to a solution of 6-mercaptop-1-isobutyl-3-methyl-2,4-(1H,3H)-pyrimidinedione (7) (1.90 g, 8.87 mmol) in ethanol (40 mL) and stirred at room temperature for 1 h. The reaction mixture was concentrated in vacuo to give a red oil. A mixture of the crude intermediate and polyphosphoric acid (10.0 g) were heated to 145 °C for 3 h, and then cooled to room temperature. A saturated aqueous solution of sodium carbonate (15 mL) was added and the mixture extracted with ethyl acetate (3 × 25 mL). The combined organic layers were dried (MgSO₄), filtered and concentrated in vacuo. Purification by flash column chromatography eluting with 20% ethyl acetate in petroleum ether gave ethyl 1-isobutyl-3-methyl-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-d]pyrimidin-5-carboxylate (5) as a dark pink solid (1.46 g, 54%). Mp 62–66 °C. Spectroscopic data were consistent with the literature [3a]. 1H NMR (400 MHz, CDCl₃) δ 1.00 (6H, d, J = 6.9 Hz, CH(CH₃)₂), 1.40 (3H, t, J = 7.4 Hz, OCH₂CH₃); 2.26–2.38 (1H, m, 2'-H), 3.42 (3H, s, 3-CH₃), 3.81 (2H, d, J = 7.6 Hz, OCH₂CH₃); 4.41 (2H, q, J = 7.4 Hz, OCH₂CH₃); 7.29 (1H, s, 6-H); 13C NMR (101 MHz, CDCl₃) δ 14.2 (CH₂), 20.0 (2 × CH₃), 27.0 (CH), 28.5 (CH₃), 56.2 (CH₂), 61.9 (CH₂), 112.6 (C), 119.4 (CH), 132.3 (C), 150.8 (C), 154.2 (C), 157.0 (C), 162.9 (C); MS (ESI) m/z 333 (M+Na⁺, 100%).
4.2.6. Ethyl 6-(2‘-fluorophenyl)-1-isobutyl-3-methyl-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-d]pyrimidine-5-carboxylate (II)

The reaction was carried out according to the previously described procedure for compound 10 using ethyl 6-bromo-1-isobutyl-3-methyl-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-d]pyrimidine-5-carboxylate (9) (0.20 g, 0.51 mmol) and 2-methoxyphenylboronic acid (0.10 g, 0.67 mmol). This gave title compound 11 as a light pink solid (0.18 g, 86%). Mp 94–98 °C; IR (neat) 3202, 2970, 1651, 1622, 1596, 1451, 1244, 1174, 1075 cm⁻¹; 1H NMR (400 MHz, CDCl₃) δ 1.01 (6H, d, JCF = 6.3 Hz, CH₂), 2.71 (2H, q, J = 7.6 Hz, 2CH₃), 4.28 (2H, s, 3-CH₂), 7.31 – 7.33 (1H, m, 5-H), 7.51 – 7.53 (1H, m, 6-H), 1.69 (s, 3-CH₃); 13C NMR (101 MHz, CDCl₃) δ 21.7 (CH₃), 28.3 (CH₂), 54.6 (3-CH₂), 121.8 (2CH₃), 129.1 (2CH₃), 131.1 (d, JCF = 8.0 Hz, CH₃), 131.1 (d, JCF = 9.8 Hz, CH); IR (KBr; νmax) 3351, 2960, 1747, 1662, 1509 cm⁻¹; MS (ESI) m/z 427 (M⁺ Na⁺), 439.1289, 439.1298.

4.2.7. Ethyl 6-(4‘-methoxyphenyl)-1-isobutyl-3-methyl-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-d]pyrimidine-5-carboxylate (12)

The reaction was carried out according to the previously described procedure for compound 10 using ethyl 6-bromo-1-isobutyl-3-methyl-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-d]pyrimidine-5-carboxylate (9) (0.20 g, 0.51 mmol) and 4-methoxyphenylboronic acid (0.10 g, 0.67 mmol). This gave title compound 12 as a white solid (0.18 g, 85%). Mp 129–131 °C; IR (neat) 3286 (OH), 2926 (CH), 1720, 1649 (C=O), 1577, 1483, 1324, 1252, 1204, 1020, 831, 729 cm⁻¹; 1H NMR (400 MHz, CDCl₃) δ 1.01 (6H, d, J = 6.7 Hz, CH₂(OH)), 1.31 (3H, t, J = 7.3 Hz, OCH₂CH₃), 2.30 – 2.40 (1H, m, 2-H), 3.41 (3H, s, 3-CH₃), 3.80 (2H, d, J = 7.7 Hz, 2CH₂), 4.39 (2H, q, J = 7.3 Hz, OCH₂CH₃), 6.92 (2H, d, J = 8.8 Hz, 3‘-H and 5‘-H), 7.43 (2H, d, J = 8.8 Hz, 2‘-H and 6‘-H); 13C NMR (101 MHz, CDCl₃) δ 14.0 (CH₂), 20.0 (2 × CH₃), 27.1 (CH₂), 28.3 (CH), 55.4 (CH₂), 56.3 (CH₃), 62.2 (CH₂), 114.0 (C), 114.4 (2 × CH), 123.5 (C), 126.2 (2 × CH), 129.5 (2 × CH), 132.9 (C), 150.8 (C), 151.8 (C), 157.6 (C), 160.3 (C), 165.0 (C); MS (ESI) m/z 439 (M⁺ Na⁺), 439.1289, 439.1298.

4.2.8. Ethyl 1-isobutyl-6-(2‘-fluorophenyl)-3-methyl-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-d]pyrimidine-5-carboxylate (13)

The reaction was carried out according to the previously described procedure for compound 10 using ethyl 6-bromo-1-isobutyl-3-methyl-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-d]pyrimidine-5-carboxylate (9) (0.20 g, 0.51 mmol) and 2-methoxyphenylboronic acid (0.10 g, 0.67 mmol). This gave title compound 13 as a yellow solid (0.18 g, 86%). Mp 119–123 °C; IR (neat) 3293 (CH), 1730 (CO), 1705 (CO), 1659 (C=O), 1489, 1479, 1244, 1200, 1020, 729 cm⁻¹; 1H NMR (400 MHz, CDCl₃) δ 1.01 (6H, d, J = 6.3 Hz, CH₂(OH)), 1.23 (3H, t, J = 7.3 Hz, OCH₂CH₃), 2.31 – 2.41 (1H, m, 2-H), 3.42 (3H, s, 3-CH₃), 3.81 (2H, d, J = 7.9 Hz, 1‘-CH₂), 4.38 (2H, q, J = 7.3 Hz, OCH₂CH₃), 7.01 – 7.13 (2H, m, 2‘-H and 6‘-H), 7.46 – 7.51 (2H, m, 3‘-H and 5‘-H); 13C NMR (101 MHz, CDCl₃) δ 14.0 (CH₂), 20.0 (2 × CH₂), 27.1 (CH₂), 28.3 (CH), 56.4 (CH₂), 62.3 (CH₃), 114.0 (C), 116.2 (d, JCF = 22.3 Hz, 2 × CH), 127.2 (d, JCF = 38.8 Hz, C), 128.1 (C), 129.0 (C), 130.2 (d, JCF = 9.8 Hz, 2 × CH), 150.7 (C), 152.3 (C), 157.5 (C), 162.8 (d, JCF = 250.7 Hz, C), 167.7 (C); MS (ESI) m/z 427 (M⁺ Na⁺), 427.1098, found 427.1098.

4.2.9. 6-(4‘-Fluorophenyl)-1-isobutyl-3-methyl-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-d]pyrimidine-5-N-morpholinocarboxamide (14)

Ethyl 6-(4‘-fluorophenyl)-1-isobutyl-3-methyl-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-d]pyrimidine-5-carboxylate (10) (0.18 g, 0.44 mmol) was dissolved in ethanol (4 mL) and water (4 mL) prior to the addition of 4 M sodium hydroxide (1.5 mL, 20 mmol) and stirred at 80 °C for 1 h. The reaction mixture was diluted with water (10 mL) and washed with diethyl ether (2 × 10 mL). The aqueous layer was acidified with 1 M hydrochloric acid and extracted with ethyl acetate (3 × 10 mL). The combined organic layers were dried (MgSO₄), filtered and concentrated in
vacuo. The resulting yellow solid was dissolved in dichloromethane (5 mL) and N,N'-dimethylformamide (2 drops) and cooled to 0°C prior to the addition of oxalyl chloride (0.045 mL, 0.53 mmol). The reaction mixture was warmed to 40°C and stirred for 3 h. After being cooled to room temperature, the reaction mixture was concentrated in vacuo to yield the acid chloride, which was used without further purification. The crude acid chloride was dissolved in dichloromethane (5 mL) and cooled to 0°C. Morpholine (0.19 mL, 2.2 mmol) was added dropwise to the stirring acid chloride solution. The reaction mixture was stirred at 40°C for 18 h under argon. The reaction mixture was cooled to room temperature, diluted in water (10 mL) and extracted with dichloromethane (3 × 10 mL). The organic layers were combined, dried (MgSO4), filtered and concentrated in vacuo. Purification by flash column chromatography eluting with 60% ethyl acetate in petroleum ether gave 6'-fluoro-1-isobutyl-3-methyl-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-d]pyrimidine-5-N-morpholinocarboxamide (14) (0.094 g, 48%) as a colourless oil. IR (neat) 2970 (CH), 1740 (CO), 1366, 1229, 1217 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.03 (3H, d, J = 6.5 Hz, CH₃(CH2)₂), 2.31–2.40 (1H, m, 2-H), 3.05 (1H, d, J = 8.5, 5.8 Hz, 2-H, NCH), 3.10 (1H, d, J = 10.6, 4.6 Hz, 2-H, NCH), 3.26 (1H, d, J = 10.6, 5.8 Hz, 2-H, NCH), 3.41 (3H, s, 3-CH₃), 3.47–3.57 (2H, m, NCH and OCH₂), 3.68 (1H, d, J = 11.4, 6.0 Hz, 1'-H), 3.74–3.86 (3H, m, OCH and OCH₂), 3.97 (1H, d, J = 11.4, 6.4 Hz, 1'-H), 7.10–7.14 (2H, m, 2'-H and 6'-H), 7.51–7.54 (2H, m, 3'-H and 5'-H); ¹³C NMR (101 MHz, CDCl₃) δ 20.0 (CH₂), 20.1 (CH₃), 27.1 (CH₃), 28.3 (CH₃), 42.3 (CH₂), 47.0 (CH₂), 56.5 (CH₂), 66.2 (CH₃), 66.3 (CH₃), 113.9 (C), 116.4 (d, JCF = 22.3 Hz, CH), 127.4 (d, JCF = 3.8 Hz, CH), 128.3 (CHₙ), 129.1 (C), 129.8 (d, JCF = 8.2 Hz, 2 × CH), 150.7 (C), 152.9 (C), 157.7 (C), 163.1 (d, JCF = 250.7 Hz, C), 164.0 (C); MS (ESI) m/z 468 (M+Na⁺, 100%); HRMS (ESI) calcd for C₂₂H₂₄FN₃NaO₄S (M+Na⁺): 468.1346, found 468.1350.

14. 6'-fluoro-1-isobutyl-3-methyl-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-d]pyrimidine-5-N-morpholinocarboxamide (15)

The reaction was carried out according to the previously described procedure for compound 14 using ethyl 6'-fluoro-1-isobutyl-3-methyl-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-d]pyrimidine-5-carboxylate (11) (0.178 g, 0.44 mmol) and morpholine (0.193 mL, 2.2 mmol). This gave title compound 15 as a clear oil (0.105 g, 54%). IR (neat) 2961 (CH), 1704 (CO), 1849, 1113, 1001, 762 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 0.13 (6H, d, J = 5.6 Hz, CH(CH₃)₂), 1.02 (3H, d, J = 5.2 Hz, CH(CH₃)₂), 2.30–2.40 (1H, m, 2'-H), 3.04 (1H, ddd, J = 8.8, 6.0, 2.4 Hz, NCH), 3.16 (1H, ddd, J = 10.6, 4.4, 2.4 Hz, NCH), 3.29 (1H, ddd, J = 10.6, 6.0, 2.4 Hz, NCH), 3.42 (3H, s, 3-CH₃), 3.43–3.47 (1H, m, OCH), 3.56 (1H, ddd, J = 8.8, 4.4, 2.4 Hz, NCH), 3.65–3.74 (2H, m, OCH₁ and OCH₂), 3.77–3.84 (2H, m, OCH₂), 3.97 (1H, dd, J = 11.2, 6.4 Hz, 1'-H), 7.15–7.20 (1H, m, 6'-H), 7.22 (1H, d, J = 7.7, 13.4 Hz, 4'-H), 7.38–7.42 (1H, m, 3'-H), 7.85 (1H, td, J = 7.7, 13.5 Hz, 5'-H); ¹³C NMR (101 MHz, CDCl₃) δ 20.0 (CH₂), 20.1 (CH₃), 27.1 (CH₃), 28.3 (CH₂), 42.2 (CH₂), 47.0 (CH₂), 56.5 (CH₂), 66.2 (CH₃), 66.2 (CH₃), 113.9 (C), 116.0 (d, JCF = 22.7 Hz, CH), 118.7 (d, JCF = 14.5 Hz, C), 123.2 (C), 124.9 (d, JCF = 3.8 Hz, CH), 131.2 (d, JCF = 8.3 Hz, CH), 131.5 (C), 131.5 (d, JCF = 9.3 Hz, CH), 150.8 (C), 154.2 (C), 157.6 (C), 159.4 (d, JCF = 252.8 Hz, C), 163.6 (C); MS (ESI) m/z 445 (M⁺, 62%), 402 (39), 359 (100), 303 (52), 276 (22), 246 (21), 190 (16), 86 (3); HRMS (ESI) calcd for C₂₂H₂₁N₂O₃S (M⁺): 445.1472, found 445.1490.

15. 6'-isobutyl-3-methyl-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-d]pyrimidine-5-N-morpholinocarboxamide (16)

The reaction was carried out according to the previously described procedure for compound 14 using ethyl 6'-fluoro-1-isobutyl-3-methyl-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-d]pyrimidine-5-carboxylate (10) (0.12 g, 0.30 mmol) and diethylamine (0.16 mL, 1.5 mmol). This gave title compound 16 as a colourless oil. IR (neat) 2963 (CH), 1707 (CO), 1665 (CO), 1636 (CO), 1533 (C=C), 1487, 1294, 1238, 841 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 0.30 (3H, t, J = 7.2 Hz, NCH₂CH₃), 1.02 (3H, d, J = 6.4 Hz, CH(CH₃)₂), 1.03 (3H, d, J = 6.8 Hz, CH(CH₂)₃), 1.21 (3H, t, J = 7.2 Hz, NCH₂CH₃), 2.30–2.41 (1H, m, 2'-H), 3.03–3.14 (2H, m, NCH₂CH₃), 3.40 (3H, s, 3-CH₃), 3.43–3.52 (1H, m, NCH₂CH₂), 3.56–3.71 (2H, m, NCH₂CH₃ and 1'-H), 3.96 (1H, d, J = 14.0, 7.6 Hz, 1'-H), 7.06–7.10 (2H, m, 2'-H and 6'-H), 7.54–7.58 (2H, m, 3'-H and 5'-H); ¹³C NMR (101 MHz, CDCl₃) δ 12.2 (CH₂), 13.5 (CH₃), 20.0 (CH₃), 20.1 (CH₃), 27.1 (CH₃), 28.3 (CH₃), 39.2 (CH₂), 43.0 (CH₂), 56.4 (CH₂), 114.0 (C), 116.1 (d, JCF = 216.5 Hz, 2 × CH), 127.8 (d, JCF = 3.8 Hz, C), 128.4 (C), 129.8 (d, JCF = 9.8 Hz, 2 × CH), 130.3 (C), 150.8 (C), 152.7 (C), 157.6 (C), 162.8 (d, JCF = 250.7 Hz, C), 164.7 (C).
The reaction was carried out according to the previously described procedure for compound 14 using ethyl 6-[(2-fluoro-phenyl)-1-isobutyl-3-methyl-2,4-dioxo-1,2,3,4-tetrahydrothieno [2,3-d]pyrimidine-5-carboxylate (10) (0.080 g, 0.20 mmol) and a 1:3 mixture of rotamers. Data for the major rotamer: 1H NMR (400 MHz, CDCl3) δ 7.39 (1H, d, J = 7.8 Hz, 2-H), 3.95 (1H, dd, J = 14.0, 7.8 Hz, 1'-H), 7.10 (2H, t, J = 8.2 Hz, 2''-H and 6''-H), 7.57 (2H, dd, J = 8.8, 5.2 Hz, 3''-H and 5''-H); 13C NMR (101 MHz, CDCl3) δ 20.0 (CH3), 217.1 (CH), 128.7 (d, JCF = 3.1 Hz, C), 128.7 (C), 129.6 (C), 129.7 (d, JCF = 8.4 Hz, C), 150.8 (C), 152.1 (C), 157.8 (C), 162.9 (d, JCF = 248.9 Hz, C), 166.4 (C); MS (ESI) m/z 442 (M+Na+, 100%); HRMS (ESI) calcd for C28H26FN3NaO4S (M+Na+) 442.1207, found 442.1191.

**4.2.18. 6-(4'-Fluorophenyl)-1-isobutyl-3-methyl-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-d]pyrimidine-5-(N-methoxy-N-methyl)carboxamide (23)**

The reaction was carried out according to the previously described procedure for compound 14 using ethyl 6-[(2-fluoro-phenyl)-1-isobutyl-3-methyl-2,4-dioxo-1,2,3,4-tetrahydrothieno [2,3-d]pyrimidine-5-carboxylate (10) (0.080 g, 0.20 mmol) and a mixture of N,O-dimethylhydroxylamine hydrochloride (0.064 g, 0.66 mmol) and N,N-diisopropylethylamine (0.23 mL, 1.3 mmol). This gave title compound 23 as a viscous yellow oil (0.040 g, 38%). IR (neat) 2959 (CH), 1707 (CO), 1662 (CO), 1535 (C=C), 1486, 1390, 973 cm⁻¹; The compound exists as a 3:1 mixture of rotamers. Data for the major rotamer: 1H NMR (400 MHz, CDCl3) δ 1.02 (6H, d, J = 6.8 Hz, CH2(CF3)2), 2.31-2.42 (1H, m, 2''-H), 3.35 (3H, s, NCH3), 3.36 (3H, s, OCH3), 3.41 (3H, s, 3-CH3), 6.31 (2H, dd, J = 14.1, 7.7 Hz, 1''-H), 3.95 (1H, dd, J = 14.1, 7.7 Hz, 1'-H), 7.10 (2H, t, J = 8.2 Hz, 2''-H and 6''-H), 7.57 (2H, dd, J = 8.8, 5.2 Hz, 3''-H and 5''-H); 13C NMR (101 MHz, CDCl3) δ 20.0 (CH3), 217.1 (CH), 128.7 (d, JCF = 3.1 Hz, C), 128.7 (C), 129.6 (C), 129.7 (d, JCF = 8.4 Hz, C), 150.8 (C), 152.1 (C), 157.8 (C), 162.9 (d, JCF = 248.9 Hz, C), 166.4 (C); MS (ESI) m/z 442 (M+Na+, 100%); HRMS (ESI) calcd for C28H26FN3NaO4S (M+Na+) 442.1207, found 442.1191.

**4.2.19. 1-Isobutyl-6-(4'-methoxyphenyl)-3-methyl-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-d]pyrimidine-5-(N-methoxy-N-methyl)carboxamide (24)**

The reaction was carried out according to the previously described procedure for compound 14 using ethyl 1-isobutyl-6-(4'-methoxyphenyl)-1-isobutyl-3-methyl-2,4-dioxo-1,2,3,4-tetrahydrothieno [2,3-d]pyrimidine-5-carboxylate (10) (0.087 g, 0.20 mmol) and 1:3 mixture of rotamers. Data for the major rotamer: 1H NMR (400 MHz, CDCl3) δ 7.39 (1H, d, J = 7.8 Hz, 2-H), 3.95 (1H, dd, J = 14.0, 7.8 Hz, 1'-H), 7.10 (2H, t, J = 8.2 Hz, 2''-H and 6''-H), 7.57 (2H, dd, J = 8.8, 5.2 Hz, 3''-H and 5''-H); 13C NMR (101 MHz, CDCl3) δ 20.0 (CH3), 217.1 (CH), 128.7 (d, JCF = 3.1 Hz, C), 128.7 (C), 129.6 (C), 129.7 (d, JCF = 8.4 Hz, C), 150.8 (C), 152.1 (C), 157.8 (C), 162.9 (d, JCF = 248.9 Hz, C), 166.4 (C); MS (ESI) m/z 442 (M+Na+, 100%); HRMS (ESI) calcd for C28H26FN3NaO4S (M+Na+) 442.1207, found 442.1191.
described procedure for compound 14 using ethyl 1-isobutyl-6-(4′-methoxyphenyl)-3-methyl-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-d]pyrimidine-5-carboxylate (12) (0.296 g, 0.710 mmol) and a combination of N,N-dimethyldimethylhydroxylamine hydrochloride (0.227 g, 2.32 mmol) and N,N′-diisopropylethylamine (0.930 mL, 4.64 mmol). This gave title compound 25 as a viscous yellow oil (0.070 g, 0.710 mmol, 56%). IR (neat) 2961 (CH), 1703 (CO), 1657 (CO), 1533 (C=C), 1475, 1254, 1180, 733 cm⁻¹. The reaction was carried out according to the previously described procedure for compound 14 using ethyl 1-isobutyl-6-(2′-methoxyphenyl)-3-methyl-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-d]pyrimidine-5-carboxylate (13) (0.119 g, 0.29 mmol) and a combination of N,N-dimethyldimethylhydroxylamine hydrochloride (0.093 g, 0.960 mmol) and N,N′-diisopropylethylamine (0.334 mL, 2.19 mmol). This gave title compound 26 as a viscous oil (0.070 g, 0.070 g, 0.56%). IR (neat) 2961 (CH), 1703 (CO), 1533 (C=C), 1489, 1252, 1123, 991, 729 cm⁻¹. The authors are grateful to EPSRC (studentship to K.M.O., EP/K503058/1), the Deutsche Forschungsgemeinschaft (BE 4310/6-1), the University of Glasgow and Scottish Health Innovations Ltd for financial support.

Appendix A. Supplementary data
Supplementary data related to this article can be found at https://doi.org/10.1016/j.tet.2018.06.019.

References
[1] (a) P.K. Gupta, S. Daunert, M.R. Nassiri, L.L. Wotring, J.C. Drach, L.B. Townsend, J. Med. Chem. 32 (1989) 402; (b) O.T. Devinyak, M.V. Silvka, M.V. Silvka, V.M. Vais, V.G. Lendel, Med. Chem. Res. 21 (2012) 2263; (c) L.M. De Coen, T.S.A. Heugebert, D. García, C.V. Stevens, Chem. Rev. 116 (2016) 80.
[2] R.K. Russell, J.B. Press, R.A. Rampulla, et al., J. Med. Chem. 31 (1988) 1786.
[3] (a) C.M. Murray, R. Hutchinson, J.R. Bantick, et al., Nat. Chem. Biol. 1 (2005) 371; (b) S.D. Guile, J.R. Bantick, D.R. Cheshire, et al., Bioorg. Med. Chem. Lett. 16 (2006) 2260; (c) S.D. Guile, J.R. Bantick, M.E. Cooper, et al., J. Med. Chem. 50 (2007) 254.
[4] Polanski, C.L. Hodgkinson, A. Fusi, et al., Clin. Canc. Res. 20 (2014) 926.
[5] (a) S. Sasaki, N. Cho, Y. Nara, et al., J. Med. Chem. 46 (2003) 113; (b) K. Miwa, T. Hitaka, T. Imada, et al., J. Med. Chem. 54 (2011) 4998.
[6] Streuli, D. de Zegli, B. Borghezio, P. Santulli, F. Barretto, C. Chapron, Expert. Opin. Emerg. Drugs. 17 (2012) 83.
[7] R. Elancheran, V.L. Maruthanathan, M. Ramanathan, et al., Med. Chem. Commun. 6 (2015) 746.
[8] R. Roth, J. Med. Chem. 12 (1969) 227.
[9] H. Ogura, M. Sakaguchi, K. Takeda, Chem. Pharm. Bull. 20 (1972) 404.
[10] (a) E.D.D. Calder, F.I. McGonagle, A.H. Harkiss, G.A. McGonagle, A. Sutherland, J. Org. Chem. 79 (2014) 7633; (b) E.D.D. Calder, S.A.J. Sharif, F.I. McGonagle, A. Sutherland, J. Org. Chem. 80 (2015) 4683; (c) A. Blair, F. Zmuda, G. Malviya, et al., Chem. Sci. 6 (2015) 4772; (d) N.L. Sloan, S.K. Luthra, G. McRobbie, S.B. Pimlott, A. Sutherland, Chem. Commun. 53 (2017) 11008.
[11] D. Cheshire, A. Cooke, M. Cooper, et al., U.S. Patent 6,180,635, 2001.
[12] The side-products from this reaction were not fully characterised due to decomposition during column chromatography. However, from 1H NMR spectroscopy of the crude reaction mixture, these appear to be compounds derived from both S- and C-5 alkylation of 7 by ethyl 3-bromopyruvate.
[13] A quantity (~10–15%) of the carboxylic acid of 5 is also generated from the two-step transformation. This is separated from the product by extraction from sodium carbonate solution during the work-up.
[14] (a) N. Miyaura, K. Yamada, A. Suzuki, Tetrahedron Lett. 20 (1979) 3437; (b) N. Miyaura, T. Yanagi, A. Suzuki, Synth. Commun. 11 (1981) 513; (c) N. Miyaura, A. Suzuki, Chem. Rev. 95 (1995) 2457.
[15] A.A.S. Tavares, J. Lewsey, D. Dewar, S.L. Pimlott, Nucl. Med. Biol. 39 (2012) 127.
[16] H.M. Becker, S. Br, J.W. Deiterme, Biophys. J. 86 (2004) 235.
[17] H. Wang, C. Yang, J.R. Doherty, W.R. Roush, J.L. Cleveland, T.D. Bannister, J. Med. Chem. 57 (2014) 7317.