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Research paper

Substituted arylsulphonamides as inhibitors of perforin-mediated lysis



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ABSTRACT

The structure-activity relationships for a series of arylsulphonamide-based inhibitors of the pore-forming protein perforin have been explored. Perforin is a key component of the human immune response, however inappropriate activity has also been implicated in certain auto-immune and therapy-induced conditions such as allograft rejection and graft versus host disease. Since perforin is expressed exclusively by cells of the immune system, inhibition of this protein would be a highly selective strategy for the immunosuppressive treatment of these disorders. Compounds from this series were demonstrated to be potent inhibitors of the lytic action of both isolated recombinant perforin and perforin secreted by natural killer cells *in vitro*. Several potent and soluble examples were assessed for *in vivo* pharmacokinetic properties and found to be suitable for progression to an *in vivo* model of transplant rejection.

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1. Introduction

Perforin is a 67 kDa, calcium-dependent glycoprotein expressed by only the natural killer (NK) cells and cytotoxic T lymphocytes (CTLs) of the mammalian immune system [1,2]. These “killer lymphocytes” utilise the pore-forming ability of perforin as a critical component of the granule exocytosis pathway; the principal mechanism used by NK and CTL cells for tumour immunosurveillance and as a defence against viral infection and intracellular pathogens [3]. Identification of a target cell by an effector cell results in the formation of an immune synapse whereupon CTL (or NK) secretory granules polarise to the site of contact. These granules contain both perforin and a group of pro-apoptotic serine proteases known as granzymes, and upon fusion with the CTL

plasma membrane, release their luminal contents into the synapse [2,4]. Perforin performs a key role in this process because entry of the granzymes required for cell death into the target cell cytosol is solely dependent on its presence [1,5].

Although perforin is synthesized and secreted into the immune synapse as a monomer, it rapidly binds to the target cell membrane through its calcium-dependent C2 domain [6,7] and oligomerises into large transmembrane pores composed of approximately 24 perforin monomers. This process was elucidated using a combination of the perforin X-ray crystal structure and cryoelectron microscopy to reconstruct an entire perforin pore [8]. Electron microscopy, X-ray crystallography and functional studies have also shown that the process involves electrostatic interactions which include a salt bridge formed between R213 on the ‘front’ surface of one monomer interacting with E343 on the ‘back’ surface of the adjacent monomer [9]. Similarly, mutational studies reveal that D191, which is immediately adjacent to R213, makes interactions that are key to oligomerisation and that substitution with a bulky hydrophobic residue (D191V) abrogates this process [9].

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Until recently, the precise mechanism of granzyme entry into the target cell was debated, but it is beyond any doubt that the pore-forming activity of perforin is indispensable. In essence, secreted perforin forms large (18 nm diameter) transmembrane pores on the surface of the target cell, through which the granzyme monomers (4 nm diameter) diffuse into the cytosol [10,11]. Once internalised the granzymes cleave key substrates to initiate rapid apoptotic death [5,10–13]. Unlike the granzymes, which are encoded by many genes and are, therefore, subject to considerable redundancy of function, the gene encoding perforin (PRF1) is present as a single copy in all mammals. Gene-targeting studies in mice [1] and naturally occurring disease-causing mutations in humans [14,15] confirm that perforin deficiency cannot be compensated by any other protein. This makes perforin an ideal target for therapeutic intervention.

While perforin is a key component of the immune response, inappropriate activity has also been implicated in a number of human immunopathologies and therapy-induced conditions. These include cerebral malaria, insulin-dependent diabetes, juvenile idiopathic arthritis and postviral myocarditis [16–18], as well as therapy-induced conditions such as allograft rejection and graft versus host disease [19–21]. Our current goal is to seek small molecule inhibitors of perforin as potential immunosuppressive agents for the treatment of autoimmune diseases and other conditions characterised by dysfunction of this pathway. This should be a highly selective strategy since perforin is expressed exclusively by CTL and NK cells, in contrast to approaches using conventional immunosuppression treatments which indiscriminately depress immune function [22–24].

Based on an initial hit from a mass screen [25], we have previously designed and optimised inhibitors of perforin that can; (i) block recombinant purified perforin, (ii) block perforin delivered by intact NK cells and, (iii) withstand incubation in serum (e.g. **1**; Fig. 1) [26–30].

While these compounds appeared highly promising, replacement of the 2-thioxoimidazolidinone moiety that contained a potential Michael acceptor and showed variable toxicity toward perforin-producing NK cells proved problematic. This issue was only overcome when we amalgamated our own finding that an aryl sulphonamide could act as a bioisosteric replacement [30] with a strategy implemented by GSK workers, where a thiazolidinedione was replaced with a pyridyl-linked benzenesulphonamide to give **2** [31]. This approach resulted in a new series of benzenesulphonamide-based perforin inhibitors, exemplified by **3**, which were potent, soluble and essentially non-toxic toward NK cells [32]. In the following report we extend our study to explore whether it is possible to further modulate activity and

physicochemical properties through variation of the sulphonamide linker, linker position, and substitution on the central pyridine ring and terminal benzene (Fig. 2).

2. Results and discussion

2.1. Chemistry

The majority of the target compounds were constructed from right to left starting with our previously published key iodide **75** [32] (Scheme 1). Suzuki reaction of **75** with a variety of commercially available aminopyridine boronates under standard conditions gave the required amine intermediates **76–79** which were subsequently reacted with a range of substituted aryl sulphonyl chlorides. The 5-amino-3-pyridine derivative **78** [32] in particular was employed in the preparation of all compounds in Table 3. One exception was where the central pyridine ring was replaced with a benzene; in this case the Suzuki step was carried out with 2-methyl-5-nitrobenzeneboronic acid, the nitro compound (**80**) hydrogenated to give the amine (**81**), which was then reacted with either 2,4-difluorobenzenesulphonyl chloride or 2-pyridinesulphonyl chloride to afford **13** and **15** respectively. Finally, amido-linked compound **8** was prepared by reaction of **78** with 2,4-difluorobenzoic acid chloride.

In a smaller number of cases, mostly those examples with substitution on the central pyridine ring, the target compounds were effectively synthesized from two halves; the fully elaborated left-hand side benzenesulphonamide subunit (e.g. **82–85**) and key iodide **75** as the right-hand side (Scheme 2). The intermediate bromides **82–85** and **91–93** were prepared from a variety of commercially available aryl sulphonyl chlorides and substituted 3-aminopyridines (or anilines) under standard conditions. In the case of **85**, the sulphonamide NH was methylated with NaH and MeI in DMF to give **86**, and for **91–93** where protection of this NH was required for the subsequent coupling to be successful, the alkylation was carried out with (chloromethoxy)ethane to give **94–96**. All bromides were converted to the corresponding boronates **87–90** and **97–99** under palladium-catalysed conditions using bis(pinacolato)diboron and KOAc in DMSO, then finally reacted in a Suzuki step with iodide **75** to introduce the thiophene-*N*-methylisoindolinone subunit (**6**, **10–12**, **17**, **18**, **20**). Where required (for **17**, **18**, **20**), deprotection was carried out under acidic conditions.

A limited number of “reverse” sulphonamides were also prepared (Scheme 3). In the case of target compound **7**, intermediate bromide **100** was prepared from 2,4-difluoroaniline and 5-bromopyridine-3-sulphonyl chloride. Protection of the sulphonamide was not required and conversion to the boronate **101** and

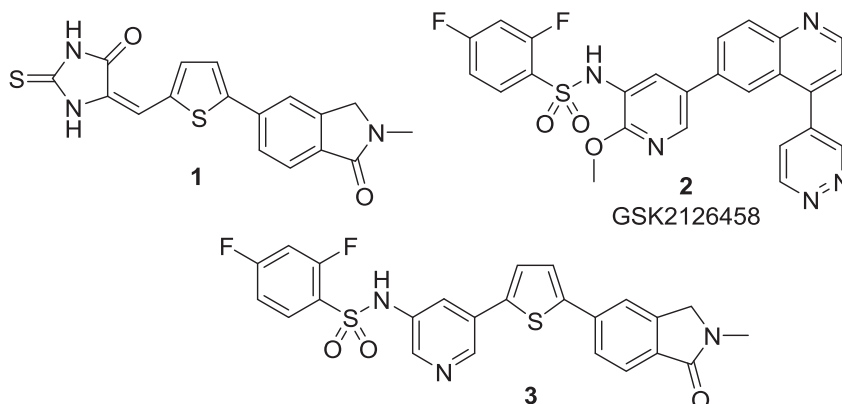


Fig. 1. Perforin inhibitors and PI3K α clinical candidate GSK2126458.

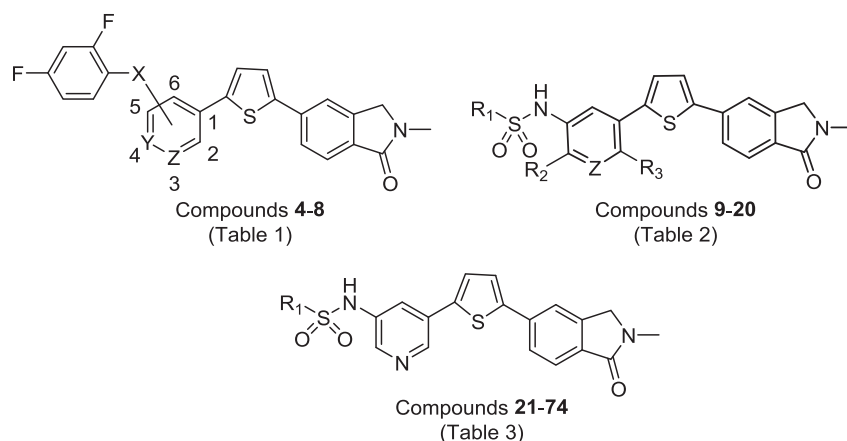
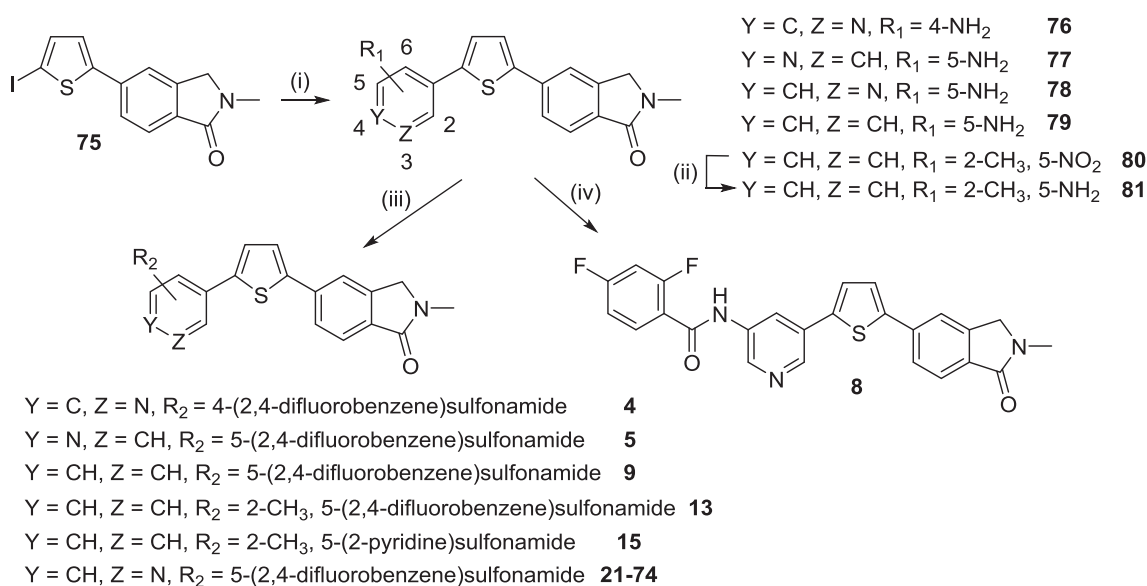


Fig. 2. Variations on **3** to give new benzenesulphonamide analogues.



Scheme 1. Reagents and conditions: (i) Boronate, Pd(dppf)Cl₂, EtOH/toluene, 2 M Na₂CO₃, reflux; (ii) H₂, 60 psi, 1:1:1 EtOH/EtOAc/THF, RT, 3 h; (iii) 2,4-Difluorobenzenesulfonyl chloride or pyridine-2-sulfonyl chloride, pyridine, 0–45 °C; (iv) 2,4-Difluorobenzoic acid chloride, pyridine, 0–45 °C, 16 h.

subsequent Suzuki coupling with **75** to give **7** proceeded smoothly. Likewise, bromide **102** was prepared from 2-aminopyridine 3-bromo-4-methylbenzenesulfonyl chloride, converted to the boronate **107** and therein coupled with **75** to afford the final product **16**. For bromides **103** and **104**, protection of the sulphonamide NH (**105**, **106**) was required for the sequential borylation (**108**, **109**) and Suzuki reactions to proceed in good yield, and afforded targets **14** and **19** respectively in good yield.

2.2. Inhibition of recombinant perforin-mediated lysis

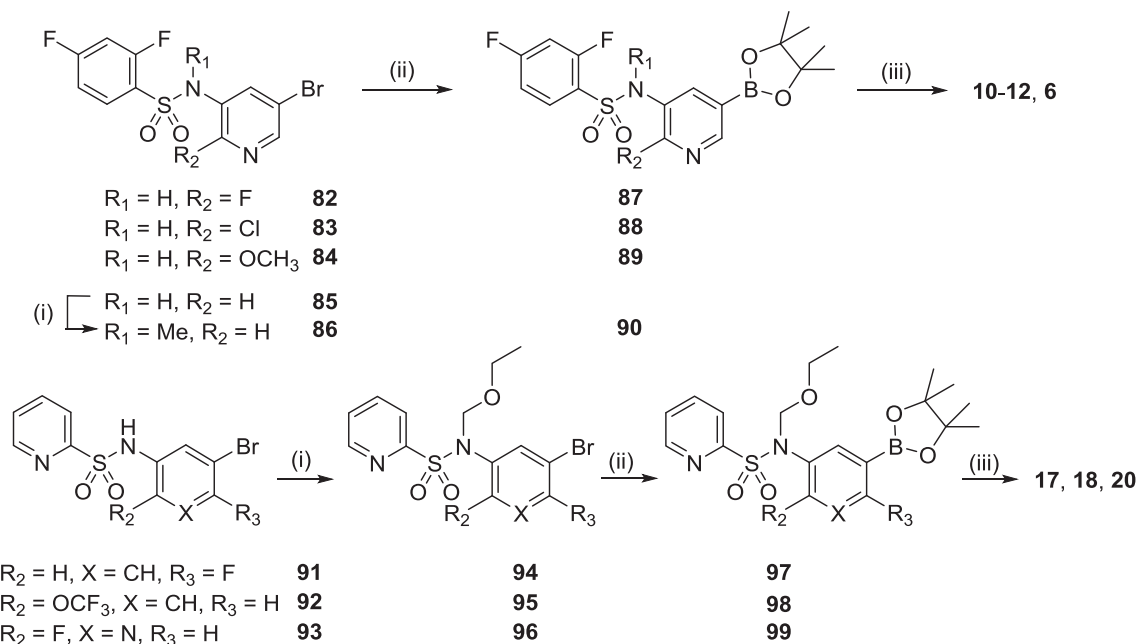
In our initial report describing the discovery of benzenesulphonamide-based inhibitors of perforin [32], analogue design focussed on exploration of the thiophene and *N*-methylisindolinone subunits which comprise the right-hand side of the molecule. For the current study we sought to further optimise potency and physicochemical characteristics through manipulation of the central pyridine and sulphonamide linker, as well as employing a wide range of substituents on the left-hand benzene (or aryl) ring.

Table 1 shows a group of compounds that explore the effect of

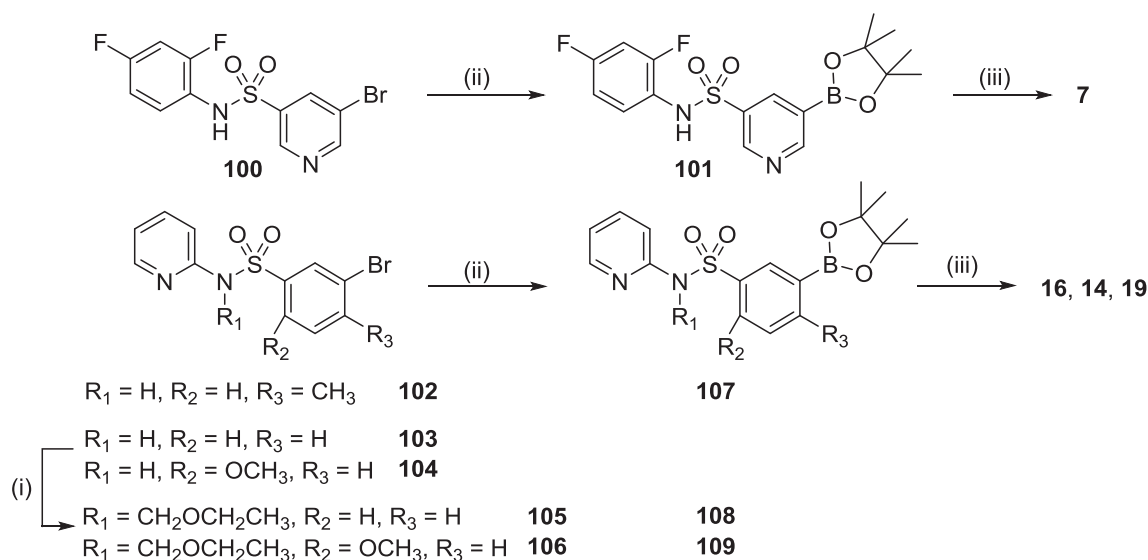
changing the position of the sulphonamide link to the pyridine, the location of the pyridine nitrogen, and modification/replacement of the sulphonamide itself.

By moving the sulphonamide link from the 5-position (**3**) to the 4-position (**4**), activity is abolished. If the sulphonamide is retained in the preferred 5-position and the pyridine nitrogen moved to the 4-position (**5**), activity is lost 4-fold compared to the lead, **3** (IC₅₀s = 4.70 and 1.17 μM respectively). The requirement for a free NH in the sulphonamide was probed through methylation of **3** to give *N*-methyl compound **6**. It appears likely that this acidic hydrogen is required for interaction with the target protein since a 14-fold drop in activity to IC₅₀ = 16.1 μM was observed. A “reverse” sulphonamide (**7**) is however still acceptable, with less than a 2-fold reduction in activity (IC₅₀ = 2.24 μM). Finally, replacement of the sulphonamide with a carboxamide (**8**) results in complete loss of potency, further supporting our hypothesis that the sulphonamide NH is required in the linker for optimal activity.

The effect of variation about the central pyridine ring was investigated next (Table 2). Direct replacement of the pyridine (**3**) with a benzene (**9**) resulted in a loss of activity from IC₅₀ = 1.17 μM to 5.74 μM. Introduction of a 2-fluoro- (**10**) or 2-chloro-substituent



Scheme 2. Reagents and conditions: (i) a. NaH, DMF, 0 °C, 0.5 h, b. MeI or (chloromethoxy)-ethane, DMF, 0 °C-RT, 1.5 h; (ii) Bis(pinacolato)diboron, KOAc, Pd(dppf)Cl₂, DMSO, 90 °C, 3 h.; (iii) a. **75**, Pd(dppf)Cl₂, EtOH/toluene, 2 M Na₂CO₃, reflux, b. Deprotection if required: 3 M HCl/1,4-dioxane (1:1), 1 h.



Scheme 3. Reagents and conditions: (i) a. NaH, DMF, 0 °C, 0.5 h, b. (Chloromethoxy)ethane, DMF, 0 °C-RT, 1.5 h; (ii) Bis(pinacolato)diboron, KOAc, Pd(dppf)Cl₂, DMSO, 90 °C, 3 h.; (iii) a. **75**, Pd(dppf)Cl₂, EtOH/toluene, 2 M Na₂CO₃, reflux, b. Deprotection if required: 3 M HCl/1,4-dioxane (1:1), 1 h.

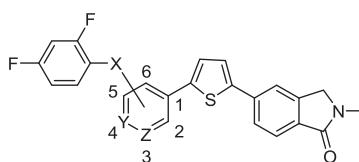
(**11**) to the pyridine gave similar activity to **3** (IC₅₀s = 1.03 and 1.99 μM respectively), while the electron-donating 2-methoxy-substituent (**12**) gave an analogue which was somewhat poorer (IC₅₀ = 3.56 μM). Benzene-linked compounds (**13**–**19**) were then explored to determine if substitution on the benzene ring could improve activity. Introduction of a single methyl group on **9** to give **13** resulted in complete loss of potency, however at this point we reasoned that the increased lipophilicity and reduced solubility associated with the presence of two benzene rings (**9** and **13**) could be improved if we effectively moved the central pyridine of **3** to the other side of the sulphonamide link (2-pyridylsulphonamide compounds **14**–**20**). This enabled us to explore a small number of compounds containing substitution on the central benzene ring,

without further detriment to the overall physicochemical properties. Although far from a comprehensive list, the introduction of methyl (**15**; IC₅₀ = 15.4 μM), fluoro (**17**; >20 μM), trifluoromethoxy (**18**; >20 μM) and methoxy (**19**; 9.68 μM) groups proved unsuccessful. The effect of reversing the sulphonamide orientation is consistent with Table 1, appearing to be slightly detrimental, although we only had a single matched pair for this comparison (**15** vs **16**; IC₅₀s = 15.4 vs > 20 μM). Lastly, compound **20** contains pyridine rings on either side of the sulphonamide link. While the solubility was significantly improved, the resulting activity (IC₅₀ = 4.10 μM) was still no improvement over the lead **3**.

Tables 1 and 2 are limited to either 2,4-difluorobenzene- or 2-pyridyl-sulphonamides. Thus it remained for us to investigate the

Table 1

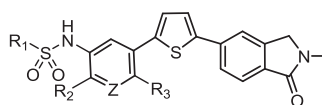
Variation of the sulphonamide linker and position.



Compound	X (linker)	Linker Position	Y	Z	Inhibition of Jurkat Cell Lysis IC ₅₀ (μM)
3^a	SO ₂ NH	5	CH	N	1.17
4	SO ₂ NH	4	C	N	>20
5	SO ₂ NH	5	N	CH	4.70
6	SO ₂ NCH ₃	5	CH	N	16.1
7^b	NHSO ₂	5	CH	N	2.24
8	CONH	5	CH	N	>20

^a Included as a comparator; see Ref. [32].^b Reverse sulphonamide.**Table 2**

Variation of the pyridine ring.



Compound	R ₁	R ₂	Z	R ₃	Inhibition of Jurkat Cell Lysis IC ₅₀ (μM)
3^a	2,4-diF-benzene	H	N	H	1.17
9	2,4-diF-benzene	H	CH	H	5.74
10	2,4-diF-benzene	F	N	H	1.99
11	2,4-diF-benzene	Cl	N	H	1.03
12	2,4-diF-benzene	OCH ₃	N	H	3.56
13	2,4-diF-benzene	H	CH	CH ₃	>20
14^b	2-pyridyl	H	CH	H	8.92
15	2-pyridyl	H	CH	CH ₃	15.4
16^b	2-pyridyl	H	CH	CH ₃	>20
17	2-pyridyl	H	CH	F	>20
18	2-pyridyl	OCF ₃	CH	H	>20
19^b	2-pyridyl	OCH ₃	CH	H	9.68
20	2-pyridyl	F	N	H	4.10

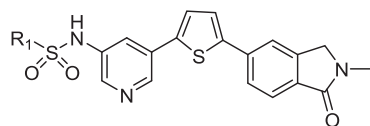
^a Included as a comparator; see Ref. [32].^b Reverse sulphonamide.

effect of alternative substituents on the left-hand side of the molecule to determine if we could optimise potency and physico-chemical properties further. A total of 52 new substituted benzene or aryl sulphonamides are shown in Table 3.

Clearly substitution on the benzene ring is required, with unsubstituted analogue **21** (IC₅₀ = 8.46 μM) suffering a 7-fold loss in activity. While 2,4-difluoro-compound (**3**) was the original lead substitution pattern, it can be separated into the constituent mono-fluoro- analogues **22–24**. This reveals that the contribution of the 2- position is most important (IC₅₀ = 2.03 μM), followed by 4- (9.65 μM), and lastly the 3-position which is detrimental to activity (>20 μM). Accordingly, the 3,4-difluoro-substituted compound **25** shows poor potency (18.6 μM) while that of the 2,4,6-trifluoro-analogue **26** is excellent (1.76 μM). This positional effect however, is not apparent in the corresponding chloro- compounds (**27–29**; IC₅₀s 2.11–8.77 μM) where the SAR between 2-, 3- and 4- is relatively flat. This may help explain why the most potent compound of the set is the 3,4-dichloro- **30** (1.32 μM) and not the 2,4-dichloro-target **31** (15.0 μM) as might be expected from the fluorinated series of analogues. The bromo- (**32–35**; IC₅₀s 2.11–8.77 μM) and iodo-substituted (**36**; 5.00 μM) compounds also displayed flat SAR,

perhaps because all these halogens are less electron-withdrawing than fluorine, a concept that we explore in more detail below. A different trend was observed when the electron-donating substituents methoxy (**37–40**) and trifluoromethoxy (**41–43**) were employed; here the *meta*- position was favoured over *ortho*- and *para*-. The two *meta*-substituted examples **38** (2.56 μM) and **42** (1.42 μM) showed significantly better activity in comparison to other positional isomers. We then surveyed a variety of electron-withdrawing substituents by preparing compounds **44–59**. As a group, these broadly paralleled the fluorine-substituted compounds discussed above, where the *ortho*- or *para*- positional isomers showed superior potency over the corresponding *meta*-examples. More specifically, for the trifluoromethyl- (**44–47**), ester- (**51–54**) and sulphonyl- (**56, 57**) substituted compounds, the *ortho* isomer was best (IC₅₀s = 1.45, 2.90, 6.80 μM respectively), while for the cyano- (**48–50**), carboxylic acid (**55**) and nitro- (**58, 59**) examples the *para* isomer showed high potency (5.17, 0.75 and 2.74 μM respectively). Compound **55** was particularly noteworthy, being one of the few sub-micromolar inhibitors of perforin identified to date. This subset of results is consistent with an inductive effect being exerted by electron-withdrawing substituents on the

Table 3
Modulation of activity through substitution on the sulphonamide.



Compound	R ₁	Inhibition of Jurkat Cell Lysis IC ₅₀ (μM)
21	benzene	8.46
22	2-F-benzene	2.03
23	3-F-benzene	>20
24	4-F-benzene	9.65
3 ^a	2,4-diF-benzene	1.17
25	3,4-diF-benzene	18.6
26	2,4,6-triF-benzene	1.76
27	2-Cl-benzene	4.01
28	3-Cl-benzene	2.42
29	4-Cl-benzene	5.39
30	3,4-diCl-benzene	1.32
31	2,4-diCl-benzene	15.0
32	2-Br-benzene	2.66
33	3-Br-benzene	2.11
34	4-Br-benzene	8.77
35	2,4-diBr-benzene	2.87
36	4-I-benzene	5.00
37	2-OCH ₃ -benzene	14.8
38	3-OCH ₃ -benzene	2.56
39	4-OCH ₃ -benzene	6.27
40	3,4-diOCH ₃ -benzene	13.8
41	2-OCF ₃ -benzene	17.7
42	3-OCF ₃ -benzene	1.42
43	4-OCF ₃ -benzene	>20
44	2-CF ₃ -benzene	1.45
45	3-CF ₃ -benzene	1.22
46	4-CF ₃ -benzene	>20
47	3,5-diCF ₃ -benzene	>20
48	2-CN-benzene	9.17
49	3-CN-benzene	>20
50	4-CN-benzene	5.17
51	2-COOCH ₃ -benzene	2.90
52	3-COOCH ₃ -benzene	7.77
53	4-COOCH ₃ -benzene	8.16
54	4-COOCH ₂ CH ₃ -benzene	19.4
55	4-COOH-benzene	0.75
56	2-SO ₂ CH ₃	6.80
57	4-SO ₂ CH ₃	>20
58	2-NO ₂ -benzene	6.65
59	4-NO ₂ -benzene	2.74
60	2-F,3-Cl-benzene	13.0
61	2-CH ₃ -4-F-benzene	5.53
62	3-CF ₃ -4-F-benzene	3.75
63	3-Cl, 4-CH ₃ -benzene	8.14
64	2-Cl, 4-CF ₃ -benzene	>20
65	3-CF ₃ -5-Br-benzene	>20
66	2-pyridyl	1.07
67	3-pyridyl	15.1
68	2-thiophenyl	1.07
69	3-thiophenyl	12.5
70		3.05
71		7.10
72		19.1
73		>20
74		11.6

^a Included as a comparator; see Ref. [32].

benzene ring and through to the sulphonamide, enhancing interactions with the protein and resulting in improved activity.

Hybrid compounds **60–65** were also prepared to investigate whether the effects of individual substituents could be combined. The resulting activities were neither synergistic nor additive, bringing no further gain to the overall potency. A set of four compounds (**66–69**) with a heterocycle (pyridine or thiophene) linked to the sulphonamide were also prepared. The preference for the heteroatom to be located directly next to the sulphonamide bond was clear with the 2-pyridyl and 2-thiophenyl compounds **66** and **68** (both IC₅₀s = 1.07 μM) far superior to the corresponding 3-linked isomers **67** and **69** (15.13 and 12.51 μM respectively). Finally, a set of compounds containing a variety of substituted heterocycles were prepared (**70–74**), but with the exception of the 4-oxazole **70** (IC₅₀ = 3.05 μM), none showed much promise.

2.3. Advanced assessment of selected compounds

Having shown that a range of benzenesulphonamides block lysis by recombinant perforin, a subset of promising examples was identified to test for inhibitory effect on the lytic action of whole NK cells. Compounds were selected based on potency, and included several for which the Jurkat IC₅₀s were >20 μM, to further validate our use of this higher throughput screen as our primary assay. The inhibitors were co-incubated with KHYG1 human NK cells in medium for 30 min at room temperature, ⁵¹Cr-labelled target cells were added, and the resulting level of chromium release used to determine residual lytic activity and thus degree of inhibition. The use of whole NK cells to deliver perforin provides a more realistic model of conditions *in vivo* compared to isolated recombinant protein which acts indiscriminately. Recognition of a+ target cell, formation of a synaptic cleft, and release of the granular contents into the cavity between effector and target are all required elements for lysis to occur. Confirmation that the observed level of inhibition is due to blocking the activity of perforin rather than non-specific killing of the effector cell was also sought by measuring the viability of the NK cells 24 h later. Our lead compound for the current work and most potent compound from our previous study [32], 2,4-difluorobenzene **3**, is included as a reference point (Table 4). One notable omission from this table is the potent 4-carboxylic acid-substituted compound **55** as this was toxic

Table 4
Capacity of selected compounds to inhibit perforin delivered by KHYG1 NK cells.

Compound	Jurkat IC ₅₀ (μM) ^a	KHYG1 Inhibition (% at 10 μM) ^b	KHYG1 Viability (% at 10 μM) ^c
3	1.17	67.5 ± 17.5	100
10	1.99	92.5 ± 1.8	92.0 ± 3.0
11	1.03	95.0 ± 2.5	94.0 ± 5.3
13	>20	50.3 ± 8.5	91.7 ± 10.0
16	>20	0	96.1 ± 6.0
17	>20	0	91.4 ± 6.5
26	1.76	81.3 ± 6.3	95.1 ± 2.1
44	1.45	85.0 ± 5.6	100
45	1.22	88.8 ± 1.9	100
47	>20	0	90.2 ± 3.4
49	>20	83.7 ± 3.2	94.4 ± 7.1
50	5.17	75.0 ± 7.8	99.3 ± 0.3
57	>20	0	90.0 ± 7.8
58	6.65	95.6 ± 1.9	98.7 ± 1.5
59	2.74	77.5 ± 5.6	85.0 ± 12.0

^a Data given for compounds as determined by the Jurkat assay.

^b Inhibition by compound (10 μM) of the perforin-induced lysis of ⁵¹Cr-labelled K562 leukemia target cells when co-incubated with KHYG1 human NK cells. Percent inhibition calculated compared to untreated control (n = 4).

^c Viability of KHYG1 NK cells after 24 h by Trypan blue exclusion assay (n = 3). See Experimental section for further details.

to the NK cells and therefore the degree of inhibition was unable to be determined.

All of the compounds with Jurkat IC_{50} s < 10 μ M showed excellent suppression of NK-cell mediated killing of labelled target cells (68–96% at 10 μ M), however this activity did not correlate exactly with their potency against isolated recombinant protein. This finding may reflect the varying ability of the respective inhibitors to access perforin located in a synaptic cleft within a complex biological milieu. Two examples with halogen on the central pyridine ring (**10**, **11**) and the 2-nitrobenzene compound **58** were particularly effective in blocking NK cell action (93, 95 and 96% inhibition respectively). Results from the set of compounds with IC_{50} s > 20 μ M broadly validated our use of this assay as a primary screen, with four of six examples showing no potency in either assay (**16**, **17**, **47**, **57**). Compound **13** demonstrated 50% inhibition at 10 μ M, perhaps not surprisingly given that the only structural change from **3** was the replacement of a bridging pyridine with a benzene ring. The one unexpected outlier was the 3-cyano compound **49** which, although it had poor activity against isolated recombinant perforin, had excellent activity against perforin produced by whole NK cells. The NK cells also retained excellent viability across all examples, consistent with our previous findings for this series [32] and in contrast to earlier reported classes [26–28].

Preliminary physicochemical data was collected on the same (active) subset of compounds in order to assess their potential for progression to *in vivo* pharmacokinetic (PK) studies (Table 5). Following conversion to the corresponding sodium salts the solubility varied widely, with the 2,4,6-trifluorobenzene (**26**) and 4-cyanobenzene (**50**) analogues being highly soluble, while the presence of 2-fluoropyridine (**10**), 2-nitrobenzene or the more lipophilic trifluoromethylbenzene group (**44**, **45**) had a negative impact on solubility. All examples tested showed good stability in aqueous solution over 24 h, however results were more varied in the presence of human, rat and mouse microsomes. While **10**, **11**, and **58** showed acceptable stability (>70% parent after 30 min) across all three species, the remaining compounds (**3**, **26**, **45**, **50**, **59** and especially **44**) showed moderate to poor stability with human microsomes. This data in combination with poor solubility resulted in the elimination of **44** and **45** from consideration for the *in vivo* PK studies reported in section 2.4 below.

2.4. *In vivo* pharmacokinetics

The *in vivo* PK parameters were measured for seven compounds selected on the basis of the *in vitro* assessment described above.

Table 5
Physicochemical properties of selected compounds.

Compound	Solubility ^a (μ g/mL)	cLogP ^b	Stability in Solution ^c (%)	Microsome Stability (%) ^d		
				Mouse	Rat	Human
3 ^e	1080	2.70 $\pm$ 0.72	82	92	99	58
10	428	2.34 $\pm$ 0.79	100	96	100	94
11	4027	3.21 $\pm$ 0.73	100	97	99	95
26	10674	2.65 $\pm$ 0.77	88	100	83	63
44	88	2.97 $\pm$ 0.65	92	84	49	14
45	574	3.13 $\pm$ 0.65	—	97	80	57
50	12900	2.17 $\pm$ 0.64	85	87	64	52
58	671	1.83 $\pm$ 0.61	100	100	89	73
59	1680	2.36 $\pm$ 0.61	—	85	72	39

^a Solubility of the sodium salt in water at room temperature; conversion to salt as described in experimental section 4.1.

^b cLogP calculated using ACD/PhysChem software v12.5.

^c Percentage of parent compound (as sodium salt) remaining after 24 h at 20 °C in water.

^d Percentage of parent compound (as sodium salt) remaining after exposure to mouse, rat or human microsomes for 30 min.

^e Data for compound **3** from Ref. [32]. See Supplementary Material for further details of assay conditions.

Plasma pharmacokinetics were determined in male CD-1 mice for compounds **3**, **10**, **11**, **26**, **50**, **58** and **59** (Table 6). Blood samples were collected at 5–8 time-points after dosing the compounds at 10 mg/kg in a solution of 20% hydroxypropyl- β -cyclodextrin by intraperitoneal (IP) injection. For analysis of the samples, chromatographic conditions were optimised by HPLC for each compound of interest and an internal standard. A liquid chromatography with tandem mass spectrometry (LC-MS/MS) method was then developed and validated for quantitation of each analyte.

Compound **3** possessed the best half-life (12 h) but showed much lower C_{max} and AUC than other examples. While **10**, **11** and **26** had shorter half-lives (4.5–9.5 h) they also showed the highest C_{max} (236, 149 and 124 μ mol/L) and exposures (AUC = 2885, 2364 and 1019 μ mol/L*h respectively). 4-Cyanobenzene (**50**) and both nitrobenzene isomers (**58**, **59**), had even shorter half-lives, however the maximum concentration and overall exposure reached was still superior to the compound with the overall longest half-life (**3**).

3. Conclusions

Between our previous report [32] and the current study, we have explored in detail how changes made on a benzenesulphonamide-based template affects perforin inhibitory activity. Analysis of the resulting SAR shows that although a range of variations were explored throughout the molecule, the isoindolinone, thiophene, pyridine and sulphonamide link of **3** are probably close to optimal, while there is some tolerance for substitution on the central pyridine ring and the terminal benzene

Table 6
In vivo Pharmacokinetics of selected compounds.^a

Compound	$T_{1/2}$ (h)	C_{max} (μ mol/L)	AUC _{0-∞} (μ mol/L*h)
3	12.0	10	220
10	6.6	236	2885
11	9.5	149	2364
26	4.5	124	1019
50	3.3	87	642
58	2.5	105	415
59	2.2	64	383

^a Pharmacokinetic parameters derived from the plasma-concentration time profiles for each compound following a 10 mg/kg *i.p.* dose. The results were processed using a noncompartment model approach using Phoenix WinNonlin 6.2 (Pharsight Corporation, St. Louis, MO). The derived parameters are: maximum plasma concentration (C_{max}), the area under the curve (AUC) and plasma half-life ($T_{1/2}$). See Supplementary Material for further details of assay conditions.

ring. A smaller panel of compounds was selected based on the following criteria; *in vitro* potency against isolated recombinant perforin and whole human NK cells, lack of toxicity against NK cells, solubility and stability (aqueous and microsomal). For this group the *in vivo* PK parameters were assessed to select potential candidates for evaluation in a mouse model of transplant rejection. We sought acceptable half-life (potentially impacts dosing frequency), $C_{\max}$ and AUC to ensure sufficient exposure to maximise our chances of achieving efficacy. Other key parameters taken into consideration for selection of the final *in vivo* candidates were *in vitro* potency and solubility. While the *in vivo* mouse efficacy studies are carried out using IP dosing, ultimately this product would be administered to human patients intravenously, meaning that sufficient solubility in a formulation close to physiological pH is crucial.

Given the above criteria, one example from the panel that appears to possess a suitable balance of properties is the 2-chloro pyridine **11**. This compound shows excellent potency (without toxicity) *in vitro*, good solubility, stability across all three species of microsomes and acceptable PK properties. Compounds **58** and **3** are also worth consideration; **58** because it is the most potent compound of the set, and **3** based on a combination of potency, solubility and half-life. The next step will be to conduct studies to determine if these compounds are capable of preventing transplant rejection in a mouse model.

4. Experimental

4.1. Chemistry

Elemental analyses were performed by the Microchemical Laboratory, University of Otago, Dunedin, NZ; values are indicated by the symbols of the elements and were within $\pm 0.4\%$ of the theoretical values. Melting points were determined using an Electrothermal Model 9200 and are as read. Several compounds were examined as sharp-melting solvates, on which elemental analyses were determined. NMR spectra were measured on a Bruker Advance 400 MHz spectrometer and referenced to Me₄Si. Mass spectra were recorded either on a Varian VG 7070 spectrometer at nominal 5000 resolution or a Finnigan MAT 900Q spectrometer. All final compound purities were determined to be >95% by HPLC on an Alltech Alltima C18 column (3.2 $\times$ 150 mm, 5 μ m) eluting with 5–80% MeCN/45 mM NH₄HCO₃.

4.1.1. General procedure A: 5-(5-(6-Aminopyridin-3-yl)thiophen-2-yl)-2-methylisindolin-1-one (**76**)

Iodide **75** [32] (500 mg, 1.41 mmol) and 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-amine (465 mg, 2.11 mmol) were dissolved in a mixture of toluene (8 mL) and EtOH (4 mL). A solution of 2 M Na₂CO₃ (2 mL) and Pd(dppf)Cl₂·CH₂Cl₂ (57 mg, 0.07 mmol) were added and the entire mixture heated at reflux under N₂ for 2 h. Upon cooling, the desired product precipitated from the reaction mixture, was isolated by filtration, and washed with H₂O, CH₂Cl₂ and MeOH. No further purification was required, giving **76** as a green-yellow solid (326 mg, 72%); mp (MeOH/CH₂Cl₂) 252–254 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 8.28 (dd, J = 2.6, 0.6 Hz, 1 H), 7.86 (s, 1 H), 7.76 (dd, J = 8.0, 1.5 Hz, 1 H), 7.70 (dd, J = 8.6, 2.6 Hz, 1 H), 7.67 (d, J = 7.9 Hz, 1 H), 7.62 (d, J = 3.8 Hz, 1 H), 6.35 (d, J = 3.8 Hz, 1 H), 6.51 (dd, J = 8.6, 0.6 Hz, 1 H), 6.25 (br s, 2 H) 4.49 (s, 2 H), 3.08 (s, 3 H). HRMS (ESI⁺) calcd for C₁₈H₁₆N₃OS 322.1009 (MH⁺), found 322.1007.

4.1.2. General procedure B: 2,4-Difluoro-N-(5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-2-yl)benzenesulfonamide (**4**)

To a solution of **76** (120 mg, 0.37 mmol) in dry pyridine (12 mL) under N₂ at RT, was added 2,4-difluorobenzenesulphonyl chloride (159 mg, 0.74 mmol) in CH₂Cl₂ (1.5 mL) dropwise over 5 min. The mixture was stirred at 45 °C under N₂ for 16 h, and the solvent then removed under reduced pressure. The reaction was quenched with a little water and the resulting solid collected by filtration and washed with water and Et₂O. Purification was carried out by trituration with hot CH₂Cl₂/MeOH solution to give **4** as a pale brown solid (65%); mp (CH₂Cl₂/MeOH) 269–272 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 12.00 (bs, 1 H), 8.39 (bs, 1 H), 7.99–8.10 (m, 2 H), 7.89 (s, 1 H), 7.78 (dd, J = 8.0, 1.3 Hz, 1 H), 7.69 (d, J = 4.2 Hz, 1 H), 7.68 (d, J = 8.1 Hz, 1 H), 7.57 (d, J = 3.9 Hz, 1 H), 7.44–7.53 (m, 1 H), 7.26–7.33 (m, 1 H), 7.16–7.26 (m, 1 H) 4.50 (s, 2 H), 3.07 (s, 3 H). HRMS (ESI⁺) calcd for C₂₄H₁₈N₃O₃S₂F₂ 498.0752 (MH⁺), found 498.0746. Anal. C, H, N. In some cases a bis-sulphonamide was also formed; here a second step was introduced where the crude product was treated with a 1:1 mixture of 1,4-dioxane and 2 M NaOH. The mono-sulphonamide resulting from subsequent acidification of the reaction mixture was isolated by filtration, washed well with water, and dried. Purification was carried out by flash column chromatography (MeOH/CH₂Cl₂ gradient).

4.1.3. 5-(5-(2-Aminopyridin-4-yl)thiophen-2-yl)-2-methylisindolin-1-one (**77**)

Reaction of **75** with 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-amine according to general procedure A gave **77** as a green-yellow solid (72%); mp (CH₂Cl₂/MeOH) 302–306 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 7.90–7.97 (m, 2 H), 7.82 (d, J = 7.1 Hz, 1 H), 7.67–7.73 (m, 2 H), 7.65 (d, J = 3.4 Hz, 1 H), 6.83 (d, J = 4.0 Hz, 1 H), 6.69 (s, 1 H), 6.05 (br s, 2 H) 4.52 (s, 2 H), 3.09 (s, 3 H). HRMS (ESI⁺) calcd for C₁₈H₁₆N₃OS 322.1009 (MH⁺), found 322.1002.

4.1.4. 2,4-Difluoro-N-(4-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-2-yl)benzenesulfonamide (**5**)

Amine **77** was reacted with 2,4-difluorobenzenesulfonyl chloride according to general procedure B to give **5** as a pale brown solid (26%); mp (CH₂Cl₂/MeOH) 235–239 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 13.27 (br s, 1 H), 7.99–8.09 (m, 2 H), 7.85–7.98 (m, 3 H), 7.80 (d, J = 3.8 Hz, 1 H), 7.72 (d, J = 7.9 Hz, 1 H), 7.38–7.51 (m, 2 H), 7.19–7.32 (m, 2 H) 4.53 (s, 2 H), 3.09 (s, 3 H). HRMS (ESI⁺) calcd for C₂₄H₁₈N₃O₃S₂F₂ 498.0752 (MH⁺), found 498.0754. Anal. C, H, N.

4.1.5. N-(5-Bromopyridin-3-yl)-2,4-difluoro-N-methylbenzenesulfonamide (**86**)

2,4-Difluorobenzenesulfonyl chloride and 5-bromopyridin-3-amine were reacted according to general procedure B. Without further purification, the resulting crude sulphonamide **85** (540 mg, 15.5 mmol) was dissolved in dry DMF (20 mL) and cooled to 0 °C. NaH (41 mg, 17.0 mmol) was added and the mixture stirred for 0.5 h, gradually being allowed to return to R.T. Methyl iodide (241 mg, 17.0 mmol) was then added dropwise and stirring continued for 1.5 h. After quenching with water the mixture was extracted with CH₂Cl₂, dried with MgSO₄ and evaporated to give a solid which was purified by flash column chromatography (1–3% MeOH/CH₂Cl₂ as eluant), yielding **86** as a brown solid (320 mg, 57%). ¹H NMR [400 MHz, (CD₃)₂SO] δ 8.65 (d, J = 2.0 Hz, 1 H), 8.51 (d, J = 2.2 Hz, 1 H), 8.04 (t, J = 2.2 Hz, 1 H), 7.72–7.83 (m, 1 H), 7.57–7.68 (m, 1 H), 7.32 (dt, J = 8.2, 2.4 Hz, 1 H), 3.26 (s, 3 H). LRMS (APCI⁺) calcd for C₁₂H₉BrF₂N₂O₂S 364 (MH⁺), found 364.

4.1.6. General procedure C: 2,4-Difluoro-N-methyl-N-(5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)-benzenesulphonamide (**6**)

Bromide **86** (300 mg, 0.83 mmol), bis(pinacolato)diboron (232 mg, 0.91 mmol), KOAc (243 mg, 2.48 mmol) and Pd(dppf)Cl₂·CH₂Cl₂ (34 mg, 0.04 mmol) were weighed into a flask, DMSO (5 mL) added, and the entire mixture heated and stirred under N₂ for 4 h. Upon cooling, the reaction was diluted with CH₂Cl₂ (25 mL) and filtered through a pad of Celite®, washing well with additional CH₂Cl₂. The filtrate and combined washings (ca 80 mL) were washed with water (3 × 40 mL), brine (50 mL), dried (Na₂SO₄) and filtered. Removal of the solvent under reduced pressure gave the crude boronate **90** which was coupled directly to **75** according to general procedure A, giving **6** as a cream solid (220 mg, 52%); mp (CH₂Cl₂/MeOH) 200–202 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 8.87 (d, *J* = 2.0 Hz, 1 H), 8.43 (d, *J* = 2.3 Hz, 1 H), 7.96 (t, *J* = 2.2 Hz, 1 H), 7.94 (s, 1 H), 7.83 (dd, *J* = 7.8, 1.4 Hz, 1 H), 7.73–7.81 (m, 3 H), 7.71 (d, *J* = 7.9 Hz, 1 H), 7.63 (m, 1 H), 7.32 (dt, *J* = 8.1, 2.0 Hz, 1 H), 4.52 (s, 2 H), 3.34 (s, 3 H), 3.09 (s, 3 H). Anal. C, H, N.

4.1.7. 5-Bromo-N-(2,4-difluorophenyl)pyridine-3-sulphonamide (**100**)

2,4-Difluoroaniline and 5-bromopyridine-3-sulphonyl chloride were reacted according to general procedure B. The crude product was recrystallised from 5% MeOH/CH₂Cl₂ and hexanes, and triturated with EtOAc to give **100** as an ivory solid (370 mg, 56%). ¹H NMR [400 MHz, (CD₃)₂SO] δ 10.52 (br s, 1 H), 9.20 (d, *J* = 2.2 Hz, 1 H), 8.77 (d, *J* = 2.0 Hz, 1 H), 8.26 (t, *J* = 2.1 Hz, 1 H), 7.22–7.35 (m, 2 H), 7.02–7.15 (m, 1 H). LRMS (APCI⁺) calcd for C₁₁H₇BrF₂N₂O₂S 350 (MH⁺), found 350.

4.1.8. N-(2,4-Difluorophenyl)-5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridine-3-sulphonamide (**7**)

Bromide **100** was reacted with bis(pinacolato)diboron according to general procedure C and the crude boronate **101** subsequently coupled to **75** according to general procedure A, to give **7** as a yellow solid (23%); mp (CH₂Cl₂/MeOH) 221–224 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 10.50 (br s, 1 H), 9.23 (d, *J* = 2.2 Hz, 1 H), 8.71 (d, *J* = 2.1 Hz, 1 H), 8.21 (t, *J* = 2.2 Hz, 1 H), 7.96 (s, 1 H), 7.81–7.88 (m, 2 H), 7.78 (d, *J* = 3.9 Hz, 1 H), 7.72 (d, *J* = 8.0 Hz, 1 H), 7.23–7.35 (m, 2 H), 7.09 (ddt, *J* = 9.2, 1.4 Hz, 1 H), 4.52 (s, 2 H), 3.09 (s, 3 H). Anal. C, H, N.

4.1.9. 2,4-Difluoro-N-(5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)benzamide (**8**)

To 2,4-difluorobenzoic acid (99 mg, 0.62 mmol) in dry CH₂Cl₂ (2 mL) was added oxalyl chloride (193 mg, 1.52 mmol) and 1 drop of dry DMF. The whole mixture was refluxed for 2, cooled to RT and concentrated *in vacuo* to give 2,4-difluorobenzoic acid chloride. To amine **78** [32] (100 mg, 0.31 mmol) in dry pyridine (10 mL) at 0 °C under N₂ was added the acid chloride (110 mg, 0.62 mmol) in dry CH₂Cl₂ (2 mL) dropwise over 4 min. The mixture was then left to stir at 45 °C for 16, quenched with H₂O and concentrated *in vacuo*. The residue was taken up in citric acid, sonicated for 5 min, and the precipitate formed was filtered and washed thoroughly with H₂O, MeOH, diethyl ether and dried on to silica gel. The crude material was chromatographed (1–3% MeOH/CH₂Cl₂) to give **8** as a yellow solid (52 mg, 36%); mp 267–269 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 10.76 (br s, 1 H), 8.77 (t, *J* = 2.9 Hz, 2 H), 8.52 (t, *J* = 2.0 Hz, 1 H), 7.96 (s, 1 H), 7.81–7.88 (m, 2 H), 7.75 (d, *J* = 3.9 Hz, 1 H), 7.71 (d, *J* = 3.8 Hz, 1 H), 7.70 (s, 1 H), 7.49 (dt, *J* = 9.4, 2.5 Hz, 1 H), 7.28 (dt, *J* = 8.6, 2.2 Hz, 1 H) 4.52 (s, 2 H), 3.09 (s, 3 H). LRMS (APCI⁺) calcd for C₂₅H₁₇N₃O₂F₂S 462.5 (MH⁺), found 462.8. Anal. C, H, N.

4.1.10. 5-(5-(3-Aminophenyl)thiophen-2-yl)-2-methylisindolin-1-one (**79**)

Iodide **75** was reacted with (3-aminophenyl)boronic acid according to general procedure A to give **79** as a green solid (69%); mp (CH₂Cl₂/MeOH) 244–247 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 7.89 (s, 1 H), 7.77 (dd, *J* = 7.9, 1.3 Hz, 1 H), 7.68 (d, *J* = 8.0 Hz, 1 H), 7.63 (d, *J* = 3.8 Hz, 1 H), 7.41 (d, *J* = 3.8 Hz, 1 H), 7.08 (t, *J* = 7.72 Hz, 1 H), 6.82–6.91 (m, 2 H), 6.55 (dd, *J* = 7.9, 1.3 Hz, 1 H), 5.25 (br s, 2 H), 4.50 (s, 2 H), 3.08 (s, 3 H).

4.1.11. 2,4-Difluoro-N-(3-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)phenyl)benzene sulphonamide (**9**)

Amine **79** was reacted with 2,4-difluorobenzenesulphonyl chloride according to general procedure B to give **9** as a yellow solid (62%); mp (CH₂Cl₂/MeOH) 260–262 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 10.84 (br s, 1 H), 7.93–8.02 (m, 1 H), 7.91 (s, 1 H), 7.81 (dd, *J* = 8.0, 1.3 Hz, 1 H), 7.70 (d, *J* = 8.0 Hz, 1 H), 7.67 (d, *J* = 3.8 Hz, 1 H), 7.55 (dt, *J* = 8.5, 2.5 Hz, 1 H), 7.47 (d, *J* = 3.8 Hz, 1 H), 7.38–7.45 (m, 2 H), 7.25–7.36 (m, 2 H), 7.05 (d, *J* = 7.4 Hz, 1 H), 4.51 (s, 2 H), 3.09 (s, 3 H). Anal. C, H, N.

4.1.12. N-(5-Bromo-2-fluoropyridin-3-yl)-2,4-difluorobenzenesulphonamide (**82**)

5-Bromo-2-fluoropyridin-3-amine was reacted with 2,4-difluorobenzenesulphonyl chloride according to general procedure B to give **82** as a brown solid (17%). ¹H NMR [400 MHz, (CD₃)₂SO] δ 11.13 (br s, 1 H), 8.18 (s, 1 H), 8.01 (dd, *J* = 8.6, 2.3 Hz, 1 H), 7.81–7.92 (m, 1 H), 7.53–7.64 (m, 1 H), 7.22–7.32 (m, 1 H). LRMS (APCI⁺) calcd for C₁₁H₆BrF₃N₂O₂S 368 (MH⁺), found 368.

4.1.13. 2,4-Difluoro-N-(2-fluoro-5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)benzenesulphonamide (**10**)

Bromide **82** was reacted with bis(pinacolato)diboron according to general procedure C and the crude boronate **87** subsequently coupled to **75** according to general procedure A to give **10** as a pale green solid (32%); mp (MeOH/CH₂Cl₂) 237–239 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 11.04 (br s, 1 H), 8.42 (s, 1 H), 8.06 (dd, *J* = 9.1, 2.3 Hz, 1 H), 7.94 (s, 1 H), 7.84–7.92 (m, 1 H), 7.82 (dd, *J* = 7.9, 1.5 Hz, 1 H), 7.74 (d, *J* = 3.9 Hz, 1 H), 7.72 (d, *J* = 8.0 Hz, 1 H), 7.68 (d, *J* = 3.9 Hz, 1 H), 7.57–7.65 (m, 1 H), 7.28 (dt, *J* = 8.9, 2.4 Hz, 1 H), 4.52 (s, 2 H), 3.09 (s, 3 H). In this case the product was dissolved in 1,4-dioxane and the sodium salt precipitated by slow addition of 2 M NaOH to give a light-green solid (89%). ¹H NMR [400 MHz, (CD₃)₂SO] δ 7.91 (s, 1 H), 7.83–7.90 (m, 1 H), 7.79 (dd, *J* = 7.9, 1.5 Hz, 1 H), 7.71 (d, *J* = 2.3 Hz, 1 H), 7.67 (d, *J* = 7.3 Hz, 1 H), 7.60–7.65 (m, 2 H), 7.33 (d, *J* = 3.8 Hz, 1 H), 7.22 (dt, *J* = 9.7, 2.5 Hz, 1 H), 7.11 (dt, *J* = 8.3, 2.5 Hz, 1 H), 4.51 (s, 2 H), 3.08 (s, 3 H). Anal. C, H, N.

4.1.14. N-(5-Bromo-2-chloropyridin-3-yl)-2,4-difluorobenzenesulphonamide (**83**)

5-Bromo-2-chloropyridin-3-amine was reacted with 2,4-difluorobenzenesulphonyl chloride according to general procedure B, giving **83** as an off-white solid (32%). ¹H NMR [400 MHz, (CD₃)₂SO] δ 11.03 (br s, 1 H), 8.47 (d, *J* = 2.3 Hz, 1 H), 8.04 (d, *J* = 2.3 Hz, 1 H), 7.75–7.85 (m, 1 H), 7.53–7.63 (m, 1 H), 7.20–7.29 (m, 1 H). LRMS (APCI⁺) calcd for C₁₁H₆BrF₂ClN₂O₂S 385 (MH⁺), found 385.

4.1.15. 2,4-Difluoro-N-(2-chloro-5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)benzenesulphonamide (**11**)

Bromide **83** was reacted with bis(pinacolato)diboron according to general procedure C and the crude boronate **88** subsequently coupled to **75** according to general procedure A to give **11** as a yellow solid (12%); mp (CH₂Cl₂/MeOH) 238–240 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 10.92 (br s, 1 H), 8.68 (d, *J* = 1.8 Hz, 1 H), 8.02

procedure E gave **17** (11%, 5 steps) ^1H NMR [400 MHz, $(\text{CD}_3)_2\text{SO}$] δ 8.13 (br s, 1 H), 8.01 (br s, 1 H), 7.88–7.97 (m, 3 H), 7.83 (dd, $J = 8.0$, 1.6 Hz, 1 H), 7.79–7.81 (m, 1 H), 7.75–7.77 (m, 1 H), 7.68–7.73 (m, 3 H), 7.61 (t, $J = 7.8$ Hz, 1 H), 7.24 (d, $J = 8.4$ Hz, 1 H), 6.87 (t, $J = 6.9$ Hz, 1 H), 4.51 (s, 2 H), 3.08 (s, 3 H). Anal. C, H, N.

4.1.25. *N*-(5-Bromo-2-(trifluoromethoxy)phenyl)-*N*-(ethoxymethyl)pyridine-2-sulfonamide (**95**)

Pyridine-2-sulfonyl chloride and 5-bromo-2-(trifluoromethoxy)aniline were reacted according to general procedure B to give **92**. Protection of the sulfonamide according to general procedure D, gave **95** which was purified by column chromatography eluting with hexanes/EtOAc 3:1 and isolated as a colourless oil (89%, 2 steps). ^1H NMR [400 MHz, CDCl_3] δ 8.75 (ddd, $J = 4.8$, 1.6, 1.0 Hz, 1 H), 7.82–7.90 (m, 2 H), 7.46–7.54 (m, 3 H), 7.10 (dddd, $J = 8.9$, 1.9, 1.8, 1.8 Hz, 1 H), 5.21 (br s, 2 H), 3.79 (q, $J = 7.0$ Hz, 2 H), 1.20 (t, $J = 7.0$ Hz, 3 H). LRMS (APCI $^+$) calcd for $\text{C}_{13}\text{H}_{10}\text{BrF}_3\text{N}_2\text{O}_3\text{S}$ 409 (M-EtO) $^+$, found 409.

4.1.26. *N*-(5-(5-(2-Methyl-1-oxoisindolin-5-yl)thiophen-2-yl)-2-(trifluoromethoxy)phenyl)pyridine-2-sulfonamide (**18**)

Bromide **95** was reacted with bis(pinacolato)diboron, to give the crude boronate **98** which was subsequently coupled to **75** according to general procedure A. The intermediate from this step was then deprotected according to general procedure E giving **18** as a yellow solid (51%, 3 steps); mp (MeOH/ CH_2Cl_2) 200–201 °C. ^1H NMR [400 MHz, $(\text{CD}_3)_2\text{SO}$] δ 10.61 (s, 1 H), 8.79 (ddd, $J = 4.6$, 1.6, 0.8 Hz, 1 H), 8.11 (ddd, $J = 7.7$, 7.7, 1.7 Hz, 1 H), 7.99 (ddd, $J = 7.9$, 0.9, 0.9 Hz, 1 H), 7.93–7.95 (m, 1 H), 7.82 (dd, $J = 8.0$, 1.5 Hz, 1 H), 7.79 (d, $J = 2.3$ Hz, 1 H), 7.68–7.74 (m, 3 H), 7.61 (dd, $J = 8.6$, 2.3 Hz, 1 H), 7.53 (d, $J = 3.4$ Hz, 1 H), 7.39 (dddd, $J = 8.5$, 1.5, 1.5, 1.5 Hz, 1 H), 4.52 (s, 2 H), 3.09 (s, 3 H). Anal. C, H, N.

4.1.27. 5-Bromo-*N*-(ethoxymethyl)-2-methoxy-*N*-(pyridin-2-yl)benzenesulfonamide (**106**)

Pyridine-2-amine and 5-bromo-2-methoxybenzenesulfonyl chloride were reacted according to general procedure B. Protection of the sulfonamide **104** was then carried out according to general procedure D, giving **106** as a colourless oil (99%, 2 steps). ^1H NMR [400 MHz, CDCl_3] δ 8.76 (ddd, $J = 4.7$, 1.6, 0.8 Hz, 1 H), 7.82 (dd, $J = 7.7$, 1.7 Hz, 1 H), 7.76 (ddd, $J = 7.9$, 1.1, 1.1 Hz, 1 H), 7.48 (ddd, $J = 7.6$, 4.8, 1.3 Hz, 1 H), 7.37–7.40 (m, 2 H), 6.64–6.68 (m, 1 H), 5.19 (br s, 2 H), 3.80 (q, $J = 7.0$ Hz, 2 H), 3.37 (s, 3 H), 1.21 (t, $J = 7.0$ Hz, 3 H). LRMS (APCI $^+$) calcd for $\text{C}_{13}\text{H}_{13}\text{BrN}_2\text{O}_3\text{S}$ 355 (M-EtO) $^+$, found 355.

4.1.28. Sodium ((2-methoxy-5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)phenyl)sulfonyl)(pyridin-2-yl)amide (**19**)

Bromide **106** was reacted with bis(pinacolato)diboron according to general procedure C, then the crude boronate **109** was subsequently coupled to **75** according to general procedure A. The intermediate from this step was deprotected and converted to the sodium salt according to general procedure E, furnishing **19** as a yellow solid (68%, 3 steps); mp (MeOH/ CH_2Cl_2) 322–325 °C. ^1H NMR [400 MHz, $(\text{CD}_3)_2\text{SO}$] δ 8.10 (br d, $J = 2.5$ Hz, 1 H), 7.89 (br s, 1 H), 7.86 (ddd, $J = 4.9$, 2.1, 0.8 Hz, 1 H), 7.79 (dd, $J = 8.0$, 1.5 Hz, 1 H), 7.63–7.68 (m, 3 H), 7.36 (d, $J = 3.8$ Hz, 1 H), 7.20 (ddd, $J = 8.4$, 7.0, 2.1 Hz, 1 H), 7.04 (d, $J = 8.7$ Hz, 1 H), 6.71 (br d, $J = 8.4$ Hz, 1 H), 6.36 (ddd, $J = 6.9$, 4.8, 1.0 Hz, 1 H), 4.50 (s, 2 H), 3.74 (s, 3 H), 3.08 (s, 3 H). Anal. C, H, N.

4.1.29. *N*-(5-Bromo-2-fluoropyridin-3-yl)-*N*-(ethoxymethyl)pyridine-2-sulfonamide (**96**)

Pyridine-2-sulfonyl chloride and 5-bromo-2-fluoropyridin-3-amine were reacted according to general procedure B.

Intermediate **93** was then protected according to general procedure D to give **96** as a colourless oil (32%, 2 steps). ^1H NMR [400 MHz, $(\text{CD}_3)_2\text{SO}$] δ 8.75 (ddd, $J = 4.7$, 1.6, 0.9 Hz, 1 H), 8.22 (dd, $J = 2.4$, 1.5 Hz, 1 H), 8.01 (dd, $J = 8.1$, 2.4 Hz, 1 H), 7.84–7.92 (m, 2 H), 7.54 (ddd, $J = 7.4$, 4.7, 1.4 Hz, 1 H), 5.21 (s, 2 H), 3.76 (q, $J = 7.1$ Hz, 2 H), 1.20 (t, $J = 7.0$ Hz, 3 H). LRMS (APCI $^+$) calcd for $\text{C}_{11}\text{H}_8\text{BrFN}_3\text{O}_2\text{S}$ 346 (M-EtO) $^+$, found 346.

4.1.30. *N*-(2-Fluoro-5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)pyridine-2-sulfonamide (**20**)

Bromide **96** was reacted with bis(pinacolato)diboron to afford crude boronate **99** which was subsequently coupled to **75** according to general procedure A. The intermediate from this step was then deprotected according to general procedure E to give **20** as a white solid (11%, 3 steps); mp ($\text{CH}_2\text{Cl}_2/\text{MeOH}$) 279–382 °C. ^1H NMR [400 MHz, $(\text{CD}_3)_2\text{SO}$] δ 10.9 (br s, 1 H), 8.76 (ddd, $J = 4.7$, 1.6, 0.9 Hz, 1 H), 8.39 (dd, $J = 2.0$, 1.3 Hz, 1 H), 8.21 (dd, $J = 9.2$, 2.4 Hz, 1 H), 8.13 (dd, $J = 9.2$, 2.4 Hz, 1 H), 8.02 (ddd, $J = 7.9$, 1.0, 1.0 Hz, 1 H), 7.95 (br s, 1 H), 7.84 (dd, $J = 7.9$, 1.6 Hz, 1 H), 7.70–7.74 (m, 3 H), 7.65 (J = 3.9 Hz, 1 H), 4.52 (s, 2 H), 3.09 (s, 3 H). Anal. C, H, N.

4.1.31. *N*-(5-(5-(2-Methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)benzenesulphonamide (**21**)

Amine **78** was reacted with benzenesulphonyl chloride according to general procedure B to give **21** as a yellow solid (64%); mp 300–303 °C. ^1H NMR [400 MHz, $(\text{CD}_3)_2\text{SO}$] δ 10.77 (br s, 1 H), 8.67 (d, $J = 2.0$ Hz, 1 H), 8.21 (d, $J = 2.4$ Hz, 1 H), 7.94 (s, 1 H), 7.75–7.80 (m, 3 H), 7.68–7.76 (m, 3 H), 7.55–7.67 (m, 4 H), 4.52 (s, 2 H), 3.09 (s, 3 H). In this case the product was converted to its sodium salt to give the desired product **21** as a yellow solid (94%). ^1H NMR [400 MHz, $(\text{CD}_3)_2\text{SO}$] δ 8.00 (d, $J = 2.1$ Hz, 1 H), 7.90 (s, 1 H), 7.88 (d, $J = 2.4$, 1 H), 7.80 (dd, $J = 7.9$, 1.5 Hz, 1 H), 7.71–7.77 (m, $J = 8.0$, 2 H), 7.68 (d, $J = 8.0$, 1 H), 7.63 (d, $J = 3.8$, 1 H), 7.10–7.30 (m, 5 H), 4.51 (s, 2 H), 3.08 (s, 3 H). Anal. C, H, N.

4.1.32. 2-Fluoro-*N*-(5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)benzenesulfonamide (**22**)

Amine **78** was reacted with 2-fluorobenzenesulphonyl chloride according to general procedure B to give **22** as a beige solid (82%); mp ($\text{CH}_2\text{Cl}_2/\text{MeOH}$) 289–292 °C. ^1H NMR [400 MHz, $(\text{CD}_3)_2\text{SO}$] δ 11.11 (br s, 1 H), 8.67 (d, $J = 2.0$ Hz, 1 H), 8.25 (d, $J = 2.4$ Hz, 1 H), 7.95 (dt, $J = 7.4$, 1.7 Hz, 2 H), 7.83 (dd, $J = 8.0$, 1.5 Hz, 1 H), 7.68–7.76 (m, 4 H), 7.63 (d, $J = 3.8$ Hz, 1 H), 7.38–7.49 (m, 2 H), 4.52 (s, 2 H), 3.09 (s, 3 H). LRMS (APCI $^-$) calcd for $\text{C}_{24}\text{H}_{18}\text{N}_3\text{O}_3\text{FS}_2$ 479 (M – H), found 479. Anal. C, H, N.

4.1.33. 3-Fluoro-*N*-(5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)benzenesulfonamide (**23**)

Amine **78** was reacted with 3-fluorobenzenesulphonyl chloride according to general procedure B to give **23** as a beige solid (56%); mp ($\text{CH}_2\text{Cl}_2/\text{MeOH}$) 292–294 °C. ^1H NMR [400 MHz, $(\text{CD}_3)_2\text{SO}$] δ 10.88 (br s, 1 H), 8.70 (d, $J = 2.0$ Hz, 1 H), 8.22 (d, $J = 2.3$ Hz, 1 H), 7.94 (s, 1 H), 7.83 (dd, $J = 7.9$, 1.5 Hz, 1 H), 7.69–7.75 (m, 3 H), 7.61–7.68 (m, 4 H), 7.50–7.58 (m, 1 H), 4.52 (s, 2 H), 3.09 (s, 3 H). Anal. C, H, N.

4.1.34. 4-Fluoro-*N*-(5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)benzenesulfonamide (**24**)

Amine **78** was reacted with 4-fluorobenzenesulphonyl chloride according to general procedure B to give **24** as a yellow solid (86%); mp ($\text{CH}_2\text{Cl}_2/\text{MeOH}$) 272–274 °C. ^1H NMR [400 MHz, $(\text{CD}_3)_2\text{SO}$] δ 10.79 (br s, 1 H), 8.69 (d, $J = 2.0$ Hz, 1 H), 8.21 (d, $J = 2.3$ Hz, 1 H), 7.94 (s, 1 H), 7.85–7.92 (m, 2 H), 7.83 (dd, $J = 7.9$, 1.5 Hz, 1 H), 7.68–7.75 (m, 3 H), 7.66 (d, $J = 3.9$ Hz, 1 H), 7.40–7.48 (m, 2 H), 4.52 (s, 2 H), 3.09 (s, 3 H). In this case the product was converted to its

sodium salt to give the desired product as a yellow solid (90%). ¹H NMR [400 MHz, (CD₃)₂SO] δ 8.03 (d, *J* = 2.1 Hz, 1 H), 7.90 (s, 1 H), 7.88 (d, *J* = 2.5 Hz, 1 H), 7.73–7.84 (m, 3 H), 7.68 (d, *J* = 7.9 Hz, 1 H), 7.64 (d, *J* = 3.8 Hz, 1 H), 7.37–7.42 (m, 2 H), 7.15–7.23 (m, 2 H), 4.51 (s, 2 H) 3.08 (s, 3 H). Anal. C, H, N.

4.1.35. 3,4-Difluoro-*N*-(5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)benzenesulfonamide (25)

Amine **78** was reacted with 3,4-difluorobenzenesulphonyl chloride according to general procedure B to give **25** as a yellow solid (18%); mp (CH₂Cl₂/MeOH) 282–285 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 10.88 (br s, 1 H), 8.72 (d, *J* = 1.4 Hz, 1 H), 8.23 (d, *J* = 2.3 Hz, 1 H), 7.95 (s, 1 H), 7.90 (d, *J* = 8.3 Hz, 1 H), 7.83 (d, *J* = 8.0 Hz, 1 H), 7.66–7.76 (m, 6 H), 4.52 (s, 2 H), 3.09 (s, 3 H). Anal. C, H, N.

4.1.36. 2,4,6-Trifluoro-*N*-(5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)benzenesulfonamide (26)

Amine **78** was reacted with 2,4,6-trifluorobenzenesulphonyl chloride according to general procedure B to give **26** as a beige solid (16%); mp (CH₂Cl₂/MeOH) 272–275 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 11.43 (br s, 1 H), 8.28 (d, *J* = 2.3 Hz, 1 H), 7.95 (s, 1 H), 7.83 (dd, *J* = 7.9, 1.5 Hz, 1 H), 7.79 (t, *J* = 2.2 Hz, 1 H), 7.73 (d, *J* = 3.9 Hz, 1 H), 7.71 (d, *J* = 7.9 Hz, 1 H), 7.65 (d, *J* = 3.9 Hz, 1 H), 7.47 (br t, *J* = 9.4 Hz, 2 H), 4.52 (s, 2 H), 3.09 (s, 3 H). Anal. C, H, N.

4.1.37. 2-Chloro-*N*-(5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)benzenesulfonamide (27)

Amine **78** was reacted with 2-chlorobenzenesulphonyl chloride according to general procedure B to give **27** as a yellow solid (29%); mp (MeOH/CH₂Cl₂) 299–303 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 11.14 (br s, 1 H), 8.64 (d, *J* = 1.9 Hz, 1 H), 8.26 (d, *J* = 2.3 Hz, 1 H), 8.17 (dd, *J* = 7.3, 1.2 Hz, 1 H), 7.94 (s, 1 H), 7.83 (dd, *J* = 7.9, 1.5 Hz, 1 H), 7.55–7.73 (m, 7 H), 4.52 (s, 2 H), 3.09 (s, 3 H). LRMS (APCI[−]) calcd for C₂₄H₁₇ClN₃O₃S₂ 495 (M − H), found 495. Anal. C, H, N.

4.1.38. 3-Chloro-*N*-(5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)benzenesulfonamide (28)

Amine **78** was reacted with 3-chlorobenzenesulphonyl chloride according to general procedure B to give **28** as an orange solid (49%); mp (MeOH/CH₂Cl₂) 291–295 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 10.88 (br s, 1 H), 8.71 (d, *J* = 2.0 Hz, 1 H), 8.22 (d, *J* = 2.4 Hz, 1 H), 7.95 (s, 1 H), 7.81–7.86 (m, 2 H), 7.69–7.79 (m, 5 H), 7.67 (d, *J* = 3.9 Hz, 1 H), 7.63 (t, *J* = 7.9 Hz, 1 H), 4.52 (s, 2 H), 3.09 (s, 3 H). LRMS (APCI[−]) calcd for C₂₄H₁₇ClN₃O₃S₂ 495 (M − H), found 495. Anal. C, H, N.

4.1.39. 4-Chloro-*N*-(5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)benzenesulfonamide (29)

Amine **78** was reacted with 4-chlorobenzenesulphonyl chloride according to general procedure B to give **29** as a yellow solid (59%); mp (MeOH/CH₂Cl₂) 281–284 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 10.84 (br s, 1 H), 8.70 (d, *J* = 2.0 Hz, 1 H), 8.21 (d, *J* = 2.3 Hz, 1 H), 7.94 (s, 1 H), 7.79–7.85 (m, 3 H), 7.65–7.74 (m, 6 H), 4.52 (s, 2 H), 3.09 (s, 3 H). LRMS (APCI[−]) calcd for C₂₄H₁₇ClN₃O₃S₂ 495 (M − H), found 495. Anal. C, H, N. In this case the product was converted to its sodium salt to give the desired product as a yellow solid (77%), mp (EtOH) 240–244 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 8.04 (d, *J* = 2.1 Hz, 1 H), 7.91 (d, *J* = 0.8 Hz, 1 H), 7.89 (d, *J* = 2.4 Hz, 1 H), 7.80 (dd, *J* = 7.9, 1.5 Hz, 1 H), 7.73 (d, *J* = 8.6 Hz, 2 H), 7.68 (d, *J* = 7.8 Hz, 1 H), 7.64 (d, *J* = 3.8 Hz, 1 H), 7.43 (d, *J* = 8.6 Hz, 2 H), 7.41 (d, *J* = 3.8 Hz, 1 H), 7.39 (t, *J* = 2.3 Hz, 1 H), 4.51 (s, 2 H), 3.08 (s, 3 H). Anal. C, H, N.

4.1.40. 3,4-Dichloro-*N*-(5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)benzenesulfonamide (30)

Amine **78** was reacted with 3,4-dichlorobenzenesulphonyl chloride according to general procedure B to give **30** as an orange-brown solid (15%); mp (MeOH/CH₂Cl₂) 256–259 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 10.92 (br s, 1 H), 8.72 (d, *J* = 2.0 Hz, 1 H), 8.23 (d, *J* = 2.4 Hz, 1 H), 8.03 (d, *J* = 2.2 Hz, 1 H), 7.94 (s, 1 H), 7.89 (d, *J* = 8.5 Hz, 1 H), 7.83 (dd, *J* = 8.0, 1.6 Hz, 1 H), 7.70–7.77 (m, 4 H), 7.69 (d, *J* = 3.9 Hz, 1 H), 4.52 (s, 2 H), 3.09 (s, 3 H). HRMS (ESI[−]) calcd for C₂₄H₁₆Cl₂N₃O₃S₂ 528.0016 (M − H), found 528.0048.

4.1.41. 2,4-Dichloro-*N*-(5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)benzenesulfonamide (31)

Amine **78** was reacted with 2,4-dichlorobenzenesulphonyl chloride according to general procedure B to give **31** as a yellow solid (55%); mp (CH₂Cl₂/MeOH) 282–285 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 11.20 (br s, 1 H), 8.67 (d, *J* = 2.0 Hz, 1 H), 8.26 (d, *J* = 2.4 Hz, 1 H), 8.14 (d, *J* = 8.6 Hz, 1 H), 7.96 (s, 1 H), 7.91 (d, *J* = 2.0 Hz, 1 H), 7.83 (dd, *J* = 8.0, 1.5 Hz, 1 H), 7.65–7.74 (m, 4 H), 7.64 (d, *J* = 3.9 Hz, 1 H), 4.52 (s, 2 H), 3.09 (s, 3 H). In this case the product was converted to its sodium salt to give the desired product as a beige solid (89%). ¹H NMR [400 MHz, (CD₃)₂SO] δ 8.08 (d, *J* = 2.1 Hz, 1 H), 8.01 (d, *J* = 8.4 Hz, 1 H), 7.88–7.92 (m, 2 H), 7.80 (dd, *J* = 7.9, 1.5 Hz, 1 H), 7.68 (d, *J* = 8.0, 1 H), 7.64 (d, *J* = 3.8, 1 H), 7.54 (d, *J* = 2.1, 1 H), 7.46 (dd, *J* = 8.4, 2.2 Hz, 1 H), 7.41 (d, *J* = 3.8 Hz, 1 H), 7.37 (t, *J* = 2.3 Hz, 1 H), 4.51 (s, 2 H), 3.08 (s, 3 H). Anal. C, H, N.

4.1.42. 2-Bromo-*N*-(5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)benzenesulfonamide (32)

Amine **78** was reacted with 2-bromobenzenesulphonyl chloride according to general procedure B to give **32** as an orange solid (30%); mp (MeOH/CH₂Cl₂) 289–293 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 11.15 (br s, 1 H), 8.63 (s, 1 H), 8.26 (d, *J* = 2.3 Hz, 1 H), 8.20 (dd, *J* = 7.9, 1.7 Hz, 1 H), 7.94 (s, 1 H), 7.80–7.88 (m, 2 H), 7.68–7.74 (m, 2 H), 7.67 (t, *J* = 2.2 Hz, 1 H), 7.59–7.65 (m, 2 H), 7.54 (dt, *J* = 7.6, 1.7 Hz, 1 H), 4.52 (s, 2 H), 3.09 (s, 3 H). LRMS (APCI[−]) calcd for C₂₄H₁₇BrN₃O₃S₂ 539 (M − H), found 539. Anal. C, H, N.

4.1.43. 3-Bromo-*N*-(5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)benzenesulfonamide (33)

Amine **78** was reacted with 3-bromobenzenesulphonyl chloride according to general procedure B to give **33** as a yellow-orange solid (30%); mp (MeOH/CH₂Cl₂) 303–307 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 10.87 (br s, 1 H), 8.71 (d, *J* = 1.9 Hz, 1 H), 8.21 (d, *J* = 2.3 Hz, 1 H), 7.98 (t, *J* = 1.8 Hz, 1 H), 7.95 (d, *J* = 0.7 Hz, 1 H), 7.78–7.90 (m, 3 H), 7.69–7.75 (m, 3 H), 7.67 (d, *J* = 3.9 Hz, 1 H), 7.56 (t, *J* = 8.0 Hz, 1 H), 4.52 (s, 2 H), 3.09 (s, 3 H). HRMS (ESI⁺) calcd for C₂₄H₁₈BrN₃O₃S₂Na 561.9865 (M + Na⁺), found 561.9862. Anal. C, H, N.

4.1.44. 4-Bromo-*N*-(5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)benzenesulfonamide (34)

Amine **78** was reacted with 4-bromobenzenesulphonyl chloride according to general procedure B to give **34** as a yellow solid (60%); mp (MeOH/CH₂Cl₂) 276–279 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 10.84 (br s, 1 H), 8.69 (d, *J* = 2.0 Hz, 1 H), 8.21 (d, *J* = 2.4 Hz, 1 H), 7.94 (d, *J* = 0.8 Hz, 1 H), 7.80–7.86 (m, 3 H), 7.69–7.76 (m, 5 H), 7.66 (d, *J* = 3.9 Hz, 1 H), 4.52 (s, 2 H), 3.09 (s, 3 H). LRMS (APCI[−]) calcd for C₂₄H₁₇BrN₃O₃S₂ 539 (M − H), found 539. Anal. C, H, N.

4.1.45. 2,4-Dibromo-*N*-(5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)benzenesulfonamides (35)

Amine **78** was reacted with 2,4-dibromobenzenesulphonyl chloride according to general procedure B to give **35** as a cream solid (81%); mp (MeOH/CH₂Cl₂) 276–279 °C. ¹H NMR [400 MHz,

(CD₃)₂SO] δ 11.23 (br s, 1 H), 8.66 (d, J = 1.9 Hz, 1 H), 8.25 (d, J = 2.4 Hz, 1 H), 8.16 (d, J = 1.9 Hz, 1 H), 8.08 (d, J = 8.5 Hz, 1 H), 7.94 (d, J = 0.7 Hz, 1 H), 7.81–7.87 (m, 2 H), 7.67–7.74 (m, 2 H), 7.65 (t, J = 2.2 Hz, 1 H), 7.63 (d, J = 3.8 Hz, 1 H), 4.52 (s, 2 H), 3.09 (s, 3 H). LRMS (APCI[−]) calcd for C₂₄H₁₇Br₂N₃O₃S₂ 617, 619, 621 (M), found 617, 619, 621. Anal. C, H, N.

4.1.46. 4-Iodo-N-(5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)benzenesulfonamides (36)

Amine **78** was reacted with 4-iodobenzenesulfonyl chloride according to general procedure B to give **36** as a pale yellow solid (59%); mp (CH₂Cl₂/Et₂O) 295–298 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 10.80 (br s, 1 H), 8.70 (d, J = 2.0 Hz, 1 H), 8.20 (d, J = 2.4 Hz, 1 H), 7.99 (d, J = 8.6 Hz, 2 H), 7.95 (d, J = 0.70 Hz, 1 H), 7.84 (dd, J = 8.0, 1.5 Hz, 1 H), 7.70–7.74 (m, 2 H), 7.68 (t, J = 2.2 Hz, 1 H), 7.66 (d, J = 3.9 Hz, 1 H), 7.56 (d, J = 8.6 Hz, 2 H), 4.52 (s, 2 H), 3.09 (s, 3 H). LRMS (APCI[−]) calcd for C₂₄H₁₈IN₃O₃S₂ 587 (M), found 587. Anal. C, H, N.

4.1.47. 2-Methoxy-N-(5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)benzenesulfonamide (37)

Amine **78** reacted with 2-methoxybenzenesulfonyl chloride according to general procedure B to give **37** as a yellow solid (26%); mp (MeOH/CH₂Cl₂) 273–276 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 10.49 (br s, 1 H), 8.61 (d, J = 2.0 Hz, 1 H), 8.24 (d, J = 2.4 Hz, 1 H), 7.94 (s, 1 H), 7.86 (dd, J = 7.9, 1.7 Hz, 1 H), 7.82 (dd, J = 8.0, 1.6 Hz, 1 H), 7.67–7.73 (m, 3 H), 7.56–7.62 (m, 2 H), 7.19 (d, J = 7.9 Hz, 1 H), 7.08 (dd, J = 7.6, 0.7 Hz, 1 H), 4.52 (s, 2 H), 3.88 (s, 3 H), 3.09 (s, 3 H). LRMS (APCI[−]) calcd for C₂₅H₂₀N₃O₄S₂ 491 (M – H), found 491. Anal. C, H, N.

4.1.48. 3-Methoxy-N-(5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)benzenesulfonamide (38)

Amine **78** was reacted with 3-methoxybenzenesulfonyl chloride according to general procedure B to give **38** as a pale yellow solid (33%); mp (MeOH/CH₂Cl₂) 278–280 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 10.76 (br s, 1 H), 8.67 (d, J = 2.0 Hz, 1 H), 8.21 (d, J = 2.3 Hz, 1 H), 7.94 (d, J = 0.7 Hz, 1 H), 7.83 (dd, J = 8.0, 1.5 Hz, 1 H), 7.68–7.75 (m, 3 H), 7.65 (d, J = 3.9 Hz, 1 H), 7.51 (t, J = 8.0 Hz, 1 H), 7.36–7.41 (m, 1 H), 7.32 (t, J = 2.1 Hz, 1 H), 7.21 (ddd, J = 8.3, 2.6, 0.8 Hz, 1 H), 4.52 (s, 2 H), 3.79 (s, 3 H), 3.09 (s, 3 H). HRMS (ESI⁺) calcd for C₂₅H₂₂N₃O₄S₂ 492.1046 (MH⁺), found 492.1033. Anal. C, H, N.

4.1.49. 4-Methoxy-N-(5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)benzenesulfonamide (39)

Amine **78** was reacted with 4-methoxybenzenesulfonyl chloride according to general procedure B to give **39** as a dark yellow solid (37%); mp (MeOH/CH₂Cl₂) 254–258 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 10.62 (br s, 1 H), 8.66 (d, J = 1.8 Hz, 1 H), 8.20 (d, J = 2.2 Hz, 1 H), 7.94 (s, 1 H), 7.83 (d, J = 8.3 Hz, 1 H), 7.76 (d, J = 8.9 Hz, 2 H), 7.67–7.74 (m, 3 H), 7.64 (d, J = 3.8 Hz, 1 H), 7.10 (d, J = 8.9 Hz, 2 H), 4.52 (s, 2 H), 3.79 (s, 3 H), 3.09 (s, 3 H). HRMS (ESI⁺) calcd for C₂₅H₂₂N₃O₄S₂ 492.1046 (MH⁺), found 492.1033.

4.1.50. 3,4-Dimethoxy-N-(5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)benzenesulfonamide, sodium salt (40)

Amine **78** was reacted with 3,4-dimethoxybenzenesulfonyl chloride according to general procedure B to give **40** as a pale orange solid (31%). ¹H NMR [400 MHz, (CD₃)₂SO] δ 10.57 (br s, 1 H), 8.66 (d, J = 2.0 Hz, 1 H), 8.21 (d, J = 2.4 Hz, 1 H), 7.94 (d, J = 0.7 Hz, 1 H), 7.83 (dd, J = 7.9, 1.5 Hz, 1 H), 7.69–7.75 (m, 3 H), 7.65 (d, J = 3.9 Hz, 1 H), 7.39 (dd, J = 8.5, 2.2 Hz, 1 H), 7.32 (d, J = 2.2 Hz, 1 H), 7.10 (d, J = 8.6 Hz, 1 H), 4.52 (s, 2 H), 3.79 (s, 3 H), 3.78 (s, 3 H), 3.09 (s, 3 H). LRMS (APCI[−]) calcd for C₂₆H₂₂N₃O₅S₂ 521 (M – H), found

521. In this case, the product was also converted to the sodium salt according to general procedure E to give the title compound as a yellow solid (96%); mp (EtOH) 240–244 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 8.00 (d, J = 2.1 Hz, 1 H), 7.89 (d, J = 0.8 Hz, 1 H), 7.86 (d, J = 2.5 Hz, 1 H), 7.79 (dd, J = 8.0, 1.6 Hz, 1 H), 7.68 (d, J = 7.9 Hz, 1 H), 7.64 (d, J = 3.8 Hz, 1 H), 7.37–7.41 (m, 2 H), 7.28–7.32 (m, 2 H), 6.92 (d, J = 8.1 Hz, 1 H), 4.51 (s, 2 H), 3.76 (s, 3 H), 3.73 (s, 3 H), 3.08 (s, 3 H). Anal. C, H, N.

4.1.51. N-(5-(5-(2-Methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)-2-(trifluoromethoxy)benzenesulfonamide (41)

Amine **78** was reacted with 2-trifluoromethoxybenzenesulphonyl chloride according to general procedure B to give **41** as a pale yellow solid (42%); mp (CH₂Cl₂/MeOH) 251–254 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 11.03 (br s, 1 H), 8.67 (d, J = 1.7 Hz, 1 H), 8.23 (d, J = 2.3 Hz, 1 H), 8.09 (dd, J = 7.8, 1.6 Hz, 1 H), 7.94 (s, 1 H), 7.75–7.85 (m, 2 H), 7.68–7.74 (m, 3 H), 7.63 (d, J = 3.8 Hz, 1 H), 7.55–7.62 (m, 2 H), 4.52 (s, 2 H), 3.09 (s, 3 H). Anal. C, H, N.

4.1.52. N-(5-(5-(2-Methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)-3-(trifluoromethoxy)benzenesulfonamide (42)

Amine **78** was reacted with 3-trifluoromethoxybenzenesulphonyl chloride according to general procedure B to give **42** as a pale brown solid (22%); mp (MeOH/CH₂Cl₂) 230–232 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 10.90 (br s, 1 H), 8.70 (d, J = 2.0 Hz, 1 H), 8.20 (d, J = 2.3 Hz, 1 H), 7.94 (s, 1 H), 7.80–7.87 (m, 2 H), 7.67–7.79 (m, 6 H), 7.65 (d, J = 3.8 Hz, 1 H), 4.52 (s, 2 H), 3.09 (s, 3 H). HRMS (APCI⁺) calcd for C₂₅H₁₈F₃N₃O₄S₂ 546.0764 (MH⁺), found 546.0747.

4.1.53. N-(5-(5-(2-Methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)-4-(trifluoromethoxy)benzenesulfonamide (43)

Amine **78** was reacted with 4-trifluoromethoxybenzenesulphonyl chloride according to general procedure B to give **43** as a pale yellow solid (41%); mp (CH₂Cl₂/MeOH) 293–296 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 10.88 (br s, 1 H), 8.70 (d, J = 2.0 Hz, 1 H), 8.22 (d, J = 2.3 Hz, 1 H), 7.92–7.98 (m, 3 H), 7.82 (dd, J = 8.0, 1.5 Hz, 1 H), 7.72 (m, 3 H), 7.66 (d, J = 3.9 Hz, 1 H), 7.57–7.63 (m, 2 H), 4.52 (s, 2 H), 3.09 (s, 3 H). Anal. C, H, N.

4.1.54. N-(5-(5-(2-Methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)-2-(trifluoromethyl)benzenesulfonamide (44)

Amine **78** was reacted with 2-(trifluoromethyl)benzenesulfonyl chloride according to general procedure B to give **44** as a pale yellow solid (67%); mp (CH₂Cl₂/Et₂O) 271–275 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 11.15 (br s, 1 H), 8.68 (d, J = 1.8 Hz, 1 H), 8.25 (d, J = 2.3 Hz, 1 H), 8.21 (d, J = 7.7 Hz, 1 H), 8.03 (d, J = 7.4 Hz, 1 H), 7.84–7.96 (m, 3 H), 7.82 (dd, J = 8.0, 1.4 Hz, 1 H), 7.68–7.74 (m, 3 H), 7.63 (d, J = 3.8 Hz, 1 H), 4.52 (s, 2 H), 3.09 (s, 3 H). LRMS (APCI[−]) calcd for C₂₅H₁₈F₃N₃O₃S₂ 529 (M), found 529. Anal. C, H, N.

4.1.55. N-(5-(5-(2-Methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)-3-(trifluoromethyl)benzenesulfonamides (45)

Amine **78** was reacted with 3-(trifluoromethyl)benzenesulfonyl chloride according to general procedure B to give **45** as a pale yellow solid (65%); mp (1,4-dioxane) 262–265 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 10.92 (br s, 1 H), 8.71 (d, J = 2.0 Hz, 1 H), 8.20 (d, J = 2.3 Hz, 1 H), 8.05–8.12 (m, 3 H), 7.93 (d, J = 0.7 Hz, 1 H), 7.86 (d, J = 7.9 Hz, 1 H), 7.82 (dd, J = 7.9, 1.6 Hz, 1 H), 7.68–7.74 (m, 3 H), 7.66 (d, J = 3.8 Hz, 1 H), 4.52 (s, 2 H), 3.09 (s, 3 H). LRMS (APCI[−]) calcd for C₂₅H₁₈F₃N₃O₃S₂ 529 (M), found 529. Anal. C, H, N.

4.1.56. *N*-(5-(5-(2-Methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)-4-(trifluoromethyl)benzenesulfonamide (**46**)

Amine **78** was reacted with 4-trifluoromethylbenzenesulfonyl chloride according to general procedure B to give **46** as a pink solid (55%); mp (CH₂Cl₂/MeOH) 282–284 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 10.99 (br s, 1 H), 8.72 (d, *J* = 2.0 Hz, 1 H), 8.22 (d, *J* = 2.3 Hz, 1 H), 7.97–8.07 (m, 4 H), 7.94 (s, 1 H), 7.83 (dd, *J* = 8.0, 1.6 Hz, 1 H), 7.68–7.76 (m, 3 H), 7.67 (d, *J* = 3.9 Hz, 1 H), 4.52 (s, 2 H), 3.09 (s, 3 H). Anal. C, H, N.

4.1.57. *N*-(5-(5-(2-Methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)-3,5-bis(trifluoromethyl)benzenesulfonamide (**47**)

Amine **78** was reacted with 3,5-bis(trifluoromethyl)benzene-1-sulfonyl chloride according to general procedure B to give **47** as a beige solid (63%); mp (MeOH/CH₂Cl₂) 299–302 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 11.04 (br s, 1 H), 8.75 (d, *J* = 2.0 Hz, 1 H), 8.53 (s, 1 H), 8.37 (s, 2 H), 8.22 (d, *J* = 2.4 Hz, 1 H), 7.93 (s, 1 H), 7.82 (dd, *J* = 7.9, 1.4 Hz, 1 H), 7.72–7.76 (m, 2 H), 7.71 (d, *J* = 7.9 Hz, 1 H), 7.68 (d, *J* = 3.9 Hz, 1 H), 4.52 (s, 2 H), 3.09 (s, 3 H). Anal. C, H, N.

4.1.58. 2-Cyano-*N*-(5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)benzenesulfonamide (**48**)

Amine **78** was reacted with 2-cyanobenzenesulfonyl chloride according to general procedure B to give **48** as a pale mustard-yellow solid (43%); mp (MeOH/CH₂Cl₂) >310 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 11.16 (br s, 1 H), 8.95 (d, *J* = 1.8 Hz, 1 H), 8.89 (d, *J* = 1.5 Hz, 1 H), 8.57 (t, *J* = 2.1 Hz, 1 H), 8.47 (d, *J* = 7.4 Hz, 1 H), 8.13 (d, *J* = 7.0 Hz, 1 H), 7.90–8.00 (m, 3 H), 7.85 (dd, *J* = 7.9, 1.3 Hz, 1 H), 7.79 (d, *J* = 3.9 Hz, 1 H), 7.76 (d, *J* = 3.9 Hz, 1 H), 7.72 (d, *J* = 8.0 Hz, 1 H), 4.53 (s, 2 H), 3.09 (s, 3 H). LRMS (APCI[−]) calcd for C₂₅H₁₈N₄O₃S₂ 486 (M), found 486. Anal. C, H, N. In this case the product was also converted to its sodium salt according to general procedure E to give the desired product as a pale yellow solid (100%). ¹H NMR [400 MHz, (CD₃)₂SO] δ 8.47 (t, *J* = 2.5 Hz, 2 H), 8.32 (t, *J* = 2.2 Hz, 1 H), 7.91–7.96 (m, 2 H), 7.85 (dd, *J* = 8.0, 1.4 Hz, 1 H), 7.67–7.73 (m, 3 H), 7.68 (d, *J* = 3.9 Hz, 1 H), 7.55–7.61 (m, 2 H), 4.52 (s, 2 H), 3.09 (s, 3 H).

4.1.59. 3-Cyano-*N*-(5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)benzenesulfonamide (**49**)

Amine **78** was reacted with 3-cyanobenzenesulfonyl chloride according to general procedure B to give **49** as a cream solid (39%); mp (CH₂Cl₂/Et₂O) 282–285 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 10.96 (br s, 1 H), 8.71 (d, *J* = 2.0 Hz, 1 H), 8.30 (t, *J* = 1.5 Hz, 1 H), 8.22 (d, *J* = 2.3 Hz, 1 H), 8.15 (dt, *J* = 7.9, 1.2 Hz, 1 H), 8.10 (ddd, *J* = 8.1, 1.9, 1.1 Hz, 1 H), 7.95 (d, *J* = 0.8 Hz, 1 H), 7.79–7.86 (m, 2 H), 7.67–7.74 (m, 4 H), 4.51 (s, 2 H), 3.09 (s, 3 H). LRMS (APCI⁺) calcd for C₂₅H₁₉N₄O₃S₂ 487 (MH⁺), found 487. Anal. C, H, N.

4.1.60. 4-Cyano-*N*-(5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)benzenesulfonamide (**50**)

Amine **78** was reacted with 4-cyanobenzenesulfonyl chloride according to general procedure B to give **50** as a yellow solid (10%); mp (CH₂Cl₂/MeOH) 282–285 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 11.02 (br s, 1 H), 8.71 (d, *J* = 2.0 Hz, 1 H), 8.21 (d, *J* = 2.4 Hz, 1 H), 8.08 (d, *J* = 8.6 Hz, 2 H), 7.98 (d, *J* = 8.6 Hz, 2 H), 7.95 (s, 1 H), 7.83 (dd, *J* = 8.0, 1.6 Hz, 1 H), 7.74 (d, *J* = 4.0 Hz, 1 H), 7.72 (s, 1 H), 7.71 (d, *J* = 8.4 Hz, 1 H), 7.68 (d, *J* = 3.9 Hz, 1 H), 4.52 (s, 2 H), 3.09 (s, 3 H). Anal. C, H, N.

4.1.61. Methyl 2-(*N*-(5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)sulfamoyl)benzoate (**51**)

Amine **78** was reacted with methyl 2-(chlorosulfonyl)benzoate according to general procedure B to give **51** as a pale orange solid (23%); mp (CH₂Cl₂/Et₂O) 209–212 °C. ¹H NMR [400 MHz, (CD₃)₂SO]

δ 10.74 (br s, 1 H), 8.66 (d, *J* = 2.0 Hz, 1 H), 8.22 (d, *J* = 2.4 Hz, 1 H), 7.95–7.99 (m, 1 H), 7.93 (br d, *J* = 0.7 Hz, 1 H), 7.82 (dd, *J* = 8.0, 1.6 Hz, 1 H), 7.68–7.76 (m, 5 H), 7.63–7.67 (m, 2 H), 4.52 (s, 2 H), 3.85 (s, 3 H), 3.09 (s, 3 H). LRMS (APCI⁺) calcd for C₂₆H₂₂N₃O₅S₂ 520 (MH⁺), found 520. Anal. C, H, N.

4.1.62. Methyl 3-(*N*-(5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)sulfamoyl)benzoate (**52**)

Amine **78** was reacted with methyl 3-(chlorosulfonyl)benzoate according to general procedure B giving **52** as a beige solid (24%); mp (CH₂Cl₂/MeOH) 250–254 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 10.92 (s, 1 H), 8.70 (d, *J* = 8.7 Hz, 1 H), 8.38 (dd, *J* = 1.6, 1.6 Hz, 1 H), 8.19–8.21 (m, 2 H), 8.07 (ddd, *J* = 7.9, 1.9, 1.1 Hz, 1 H), 7.94 (br s, 1 H), 7.77 (d, *J* = 7.9 Hz, 1 H), 7.70–7.75 (m, 3 H), 7.66 (d, *J* = 3.4 Hz, 1 H), 4.52 (s, 2 H), 3.87 (s, 3 H), 3.09 (s, 3 H). HRMS (ESI⁺) calcd for C₂₆H₂₂N₃O₅S₂ 520.0995 (MH⁺), found 520.1004.

4.1.63. Methyl 4-(*N*-(5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)sulfamoyl)benzoate (**53**)

Amine **78** was reacted with methyl 4-(chlorosulfonyl)benzoate according to general procedure B to give **53** as a pale yellow solid (60%); mp (CH₂Cl₂/Et₂O) 269–272 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 10.95 (br s, 1 H), 8.67 (d, *J* = 1.9 Hz, 1 H), 8.19 (d, *J* = 2.3 Hz, 1 H), 8.13 (d, *J* = 8.6 Hz, 2 H), 7.92–7.97 (m, 3 H), 7.82 (dd, *J* = 8.0, 1.6 Hz, 1 H), 7.68–7.73 (m, 3 H), 7.65 (d, *J* = 3.9 Hz, 1 H), 4.52 (s, 2 H), 3.85 (s, 3 H), 3.09 (s, 3 H). LRMS (APCI⁺) calcd for C₂₆H₂₂N₃O₅S₂ 520 (MH⁺), found 520. Anal. C, H, N.

4.1.64. Ethyl 4-(*N*-(5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)sulfamoyl)benzoate (**54**)

Amine **78** was reacted with ethyl 4-(chlorosulfonyl)benzoate according to general procedure B to give **54** as a pale yellow solid (52%); mp (CH₂Cl₂/Et₂O) 272–275 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 10.93 (br s, 1 H), 8.69 (d, *J* = 2.0 Hz, 1 H), 8.20 (d, *J* = 2.4 Hz, 1 H), 8.13 (d, *J* = 8.6 Hz, 2 H), 7.93–7.98 (m, 3 H), 7.83 (dd, *J* = 8.0, 1.6 Hz, 1 H), 7.69–7.74 (m, 3 H), 7.66 (d, *J* = 3.9 Hz, 1 H), 4.52 (s, 2 H), 4.31 (q, *J* = 7.1 Hz, 2 H), 3.09 (s, 3 H), 1.29 (t, *J* = 7.1 Hz, 3 H). LRMS (APCI⁺) calcd for C₂₇H₂₄N₃O₅S₂ 534 (MH⁺), found 534. Anal. C, H, N.

4.1.65. 4-(*N*-(5-(5-(2-Methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)sulfamoyl)benzoic acid (**55**)

Amine **78** was reacted with 4-chlorosulfonyl benzoic acid according to general procedure B to give **55** as a pale pink solid (6%); mp (MeOH/CH₂Cl₂) >310 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 13.46 (v br s, 1 H), 10.91 (v br s, 1 H), 8.68 (d, *J* = 1.9 Hz, 1 H), 8.21 (d, *J* = 2.3 Hz, 1 H), 8.11 (d, *J* = 8.6 Hz, 2 H), 7.91–7.95 (m, 3 H), 7.83 (dd, *J* = 8.0, 1.5 Hz, 1 H), 7.69–7.73 (m, 3 H), 7.65 (d, *J* = 3.9 Hz, 1 H), 4.52 (s, 2 H), 3.09 (s, 3 H). LRMS (APCI[−]) calcd for C₂₅H₁₉N₃O₅S₂ 505 (M), found 505. Anal. C, H, N.

4.1.66. *N*-(5-(5-(2-Methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)-2-(methylsulfonyl)benzenesulfonamide (**56**)

Amine **78** was reacted with 2-(methanesulfonyl)benzenesulfonyl chloride according to general procedure B to give **56** as a pale orange solid (77%); mp (Et₂O/CH₂Cl₂) 269–272 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 10.22 (br s, 1 H), 8.68 (d, *J* = 1.5 Hz, 1 H), 8.22–8.27 (m, 2 H), 8.14–8.18 (m, 1 H), 7.89–7.97 (m, 3 H), 7.82 (dd, *J* = 8.0, 1.5 Hz, 1 H), 7.76 (t, *J* = 2.2 Hz, 1 H), 7.68–7.73 (m, 2 H), 7.65 (d, *J* = 3.9 Hz, 1 H), 4.52 (s, 2 H), 3.53 (s, 3 H), 3.09 (s, 3 H). LRMS (APCI[−]) calcd for C₂₅H₂₁N₃O₅S₃ 539 (M), found 539. Anal. C, H, N.

4.1.67. Sodium (5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)((4-(methylsulfonyl)phenyl)sulfonyl)amide (**57**)

Amine **78** was reacted with 4-(methanesulfonyl)benzenesulfonyl chloride according to general procedure B and the

resulting crude product converted directly to the sodium salt according to general procedure E. The salt was recrystallised from EtOH to give **57** as a cream solid (27%); mp (EtOH) > 300 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 8.07 (d, *J* = 2.1 Hz, 1 H), 7.91–7.98 (m, 5 H), 7.90 (d, *J* = 0.7 Hz, 1 H), 7.81 (dd, *J* = 8.0, 1.5 Hz, 1 H), 7.68 (d, *J* = 8.2 Hz, 1 H), 7.65 (d, *J* = 3.8 Hz, 1 H), 7.42–7.45 (m, 2 H), 4.52 (s, 2 H), 3.19 (s, 3 H), 3.09 (s, 3 H). LRMS (APCI[−]) calcd for C₂₅H₂₁N₃O₅S₃ 539 (M – Na), found 539. HRMS (ESI⁺) calcd for C₂₅H₂₁N₃NaO₅S₃ 562.0536 (MH⁺), found 562.0522.

4.1.68. *N*-(5-(5-(2-Methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)-2-nitrobenzenesulfonamide (**58**)

Amine **78** was reacted with 2-nitrobenzenesulfonyl chloride according to general procedure B to give **58** as a yellow solid (32%); mp (MeOH/CH₂Cl₂) 270–273 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 11.21 (bs, 1 H), 8.71 (d, *J* = 1.9 Hz, 1 H), 8.26 (d, *J* = 2.4 Hz, 1 H), 8.06–8.10 (m, 1 H), 7.98–8.01 (m, 1 H), 7.94 (d, *J* = 0.8 Hz, 1 H), 7.86–7.90 (m, 2 H), 7.83 (dd, *J* = 8.0, 1.6 Hz, 1 H), 7.72–7.75 (m, 2 H), 7.71 (d, *J* = 8.0 Hz, 1 H), 7.66 (d, *J* = 3.9 Hz, 1 H), 4.52 (s, 2 H), 3.09 (s, 3 H). LRMS (APCI[−]) calcd for C₂₄H₁₇N₄O₅S₂ 506 (M – H), found 506. Anal. C, H, N.

4.1.69. *N*-(5-(5-(2-Methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)-4-nitrobenzenesulfonamide (**59**)

Amine **78** was reacted with 4-nitrobenzenesulfonyl chloride according to general procedure B to give **59** as a pale yellow solid (56%); mp (MeOH/CH₂Cl₂) 272–275 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 11.09 (br s, 1 H), 8.71 (d, *J* = 1.9 Hz, 1 H), 8.40 (dq, *J* = 9.0, 5.0 Hz, 2 H), 8.22 (d, *J* = 2.3 Hz, 1 H), 8.07 (dq, *J* = 8.9, 5.0 Hz, 2 H), 7.95 (s, 1 H), 7.83 (dd, *J* = 7.9, 1.4 Hz, 1 H), 7.76 (t, *J* = 2.2 Hz, 1 H), 7.73 (d, *J* = 4.0 Hz, 1 H), 7.71 (d, *J* = 8.0 Hz, 1 H), 7.68 (d, *J* = 3.9 Hz, 1 H), 4.52 (s, 2 H), 3.09 (s, 3 H). LRMS (APCI[−]) calcd for C₂₄H₁₇N₄O₅S₂ 506 (M – H), found 506. HRMS (APCI⁺) calcd for C₂₄H₁₈N₄O₅S₂ 507.0791 (MH⁺), found 507.0792. In this case the product was also converted to its sodium salt according to general procedure E, giving an orange solid (89%). ¹H NMR [400 MHz, (CD₃)₂SO] δ 8.24 (d, *J* = 8.8 Hz, 2 H), 8.11 (d, *J* = 1.6 Hz, 1 H), 7.96 (d, *J* = 8.8 Hz, 2 H), 7.94 (d, *J* = 2.4 Hz, 1 H), 7.91 (s, 1 H), 7.81 (dd, *J* = 8.0, 1.4 Hz, 1 H), 7.68 (d, *J* = 8.0 Hz, 1 H), 7.65 (d, *J* = 3.9 Hz, 1 H), 7.47 (t, *J* = 2.2 Hz, 1 H), 7.45 (d, *J* = 3.8 Hz, 1 H), 4.51 (s, 2 H), 3.08 (s, 3 H).

4.1.70. 3-Chloro-2-fluoro-*N*-(5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)benzenesulfonamide (**60**)

Amine **78** was reacted with 3-chloro-2-fluorobenzenesulfonyl chloride according to general procedure B to give **60** as a pale yellow solid (63%); mp (CH₂Cl₂/MeOH) 269–272 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 11.30 (br s, 1 H), 8.71 (d, *J* = 1.9 Hz, 1 H), 8.27 (d, *J* = 2.3 Hz, 1 H), 7.87–7.98 (m, 3 H), 7.83 (dd, *J* = 7.9, 1.2 Hz, 1 H), 7.68–7.76 (m, 3 H), 7.66 (d, *J* = 3.9 Hz, 1 H), 7.45 (t, *J* = 8.0 Hz, 1 H), 4.52 (s, 2 H), 3.09 (s, 3 H). Anal. C, H, N.

4.1.71. 4-Fluoro-2-methyl-*N*-(5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)benzenesulphonamide (**61**)

Amine **78** was reacted with 4-fluoro-2-methylbenzene-1-sulphonyl chloride according to general procedure B to give **61** as a light-brown solid (50%); mp 280–283 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 10.95 (br s, 1 H), 8.65 (d, *J* = 2.0 Hz, 1 H), 8.23 (d, *J* = 2.4 Hz, 1 H), 8.05 (dd, *J* = 5.8, 3.1 Hz, 1 H), 7.95 (s, 1 H), 7.83 (dd, *J* = 7.9, 1.4 Hz, 1 H), 7.68–7.75 (m, 2 H), 7.66 (t, *J* = 2.2 Hz, 1 H), 7.63 (d, *J* = 3.9 Hz, 1 H), 7.33 (dd, *J* = 9.9, 2.5 Hz, 1 H), 7.27 (dt, *J* = 8.4, 2.6 Hz, 1 H), 4.52 (s, 2 H), 3.09 (s, 3 H). Anal. C, H, N.

4.1.72. 4-Fluoro-*N*-(5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)-3-(trifluoromethyl)benzenesulphonamide (**62**)

Amine **78** was reacted with 4-fluoro-3-(trifluoromethyl)

benzene-1-sulphonyl chloride according to general procedure B to give **62** as a yellow solid (40%); mp 250–252 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 10.93 (br s, 1 H), 8.20 (d, *J* = 2.4 Hz, 1 H), 8.11–8.19 (m, 2 H), 7.93 (d, *J* = 0.6 Hz, 1 H), 7.82 (dd, *J* = 8.4, 1.6 Hz, 1 H), 7.77 (d, *J* = 9.9 Hz, 1 H), 7.68–7.75 (m, 2 H), 7.70 (d, *J* = 7.9 Hz, 1 H), 7.66 (d, *J* = 3.9 Hz, 1 H), 4.52 (s, 2 H), 3.09 (s, 3 H). HRMS (APCI⁺) calcd for C₂₅H₁₈F₄N₃O₃S₂ 548.0720 (MH⁺), found 548.0743.

4.1.73. 3-Chloro-4-methyl-*N*-(5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)benzenesulfonamide (**63**)

Amine **78** was reacted with 3-chloro-4-methylbenzenesulphonyl chloride according to general procedure B to give **63** as an off-white solid (50%); mp (CH₂Cl₂/MeOH) 277–279 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 10.81 (br s, 1 H), 8.70 (d, *J* = 2.0 Hz, 1 H), 8.22 (d, *J* = 2.3 Hz, 1 H), 7.94 (s, 1 H), 7.80–7.86 (m, 2 H), 7.64–7.75 (m, 5 H), 7.58 (d, *J* = 8.2 Hz, 1 H), 4.52 (s, 2 H), 3.09 (s, 3 H), 2.36 (s, 3 H). Anal. C, H, N.

4.1.74. 2-Chloro-*N*-(5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)-4-(trifluoromethyl)benzenesulfonamide (**64**)

Amine **78** was reacted with 2-chloro-4-trifluoromethylbenzenesulphonyl chloride according to general procedure B to give **64** as a beige solid (61%); mp (CH₂Cl₂/MeOH) 292–295 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 11.39 (br s, 1 H), 8.68 (d, *J* = 2.0 Hz, 1 H), 8.35 (d, *J* = 5.0 Hz, 1 H), 8.28 (d, *J* = 2.4 Hz, 1 H), 8.17 (s, 1 H), 7.97 (dd, *J* = 8.4, 1.2 Hz, 1 H), 7.93 (s, 1 H), 7.82 (dd, *J* = 7.9, 1.4 Hz, 1 H), 7.67–7.75 (m, 1 H), 7.64 (d, *J* = 3.9 Hz, 1 H), 4.52 (s, 2 H), 3.09 (s, 3 H). Anal. C, H, N.

4.1.75. 3-Bromo-*N*-(5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)-5-(trifluoromethyl)benzenesulphonamide (**65**)

Amine **78** was reacted with 3-bromo-5-(trifluoromethyl)benzene-1-sulphonyl chloride according to general procedure B to give **65** as a yellow solid (26%); mp 280–283 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 11.01 (br s, 1 H), 8.69 (s, 1 H), 8.34 (s, 1 H), 8.24 (s, 1 H), 8.20 (d, *J* = 2.2 Hz, 1 H), 8.05 (s, 1 H), 7.93 (s, 1 H), 7.82 (d, *J* = 8.4 Hz, 1 H), 7.63–7.77 (m, 4 H), 4.52 (s, 2 H), 3.09 (s, 3 H). Anal. C, H, N.

4.1.76. *N*-(5-(5-(2-Methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)pyridine-2-sulfonamide (**66**)

Amine **78** with pyridine-2-sulphonyl chloride according to general procedure B to give **66** as a cream solid (20%); mp 272–275 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 11.01 (br s, 1 H), 8.73–8.78 (m, 1 H), 8.66 (d, *J* = 2.0 Hz, 1 H), 8.30 (d, *J* = 2.4 Hz, 1 H), 8.11 (td, *J* = 7.8, 1.7 Hz, 1 H), 8.60 (dt, *J* = 7.6, 1.0 Hz, 1 H), 7.94 (s, 1 H), 7.81–7.87 (m, 2 H), 7.73 (d, *J* = 3.9 Hz, 1 H), 7.71 (d, *J* = 8.1 Hz, 1 H), 7.66–7.70 (m, 1 H), 7.64 (d, *J* = 3.8 Hz, 1 H), 4.52 (s, 2 H), 3.09 (s, 3 H). In this case the product was also converted to its sodium salt according to general procedure E giving a light-brown solid (89%). ¹H NMR [400 MHz, (CD₃)₂SO] δ 8.53 (td, *J* = 4.7, 1.4 Hz, 1 H), 8.04 (d, *J* = 2.1 Hz, 1 H), 7.93 (d, *J* = 2.4 Hz, 1 H), 7.91 (s, 1 H), 7.83–7.87 (m, 2 H), 7.80 (dd, *J* = 8.0, 1.5 Hz, 1 H), 7.68 (d, *J* = 7.9 Hz, 1 H), 7.65 (d, *J* = 3.8 Hz, 1 H), 7.60 (t, *J* = 2.2 Hz, 1 H), 7.41 (d, *J* = 3.8 Hz, 1 H), 7.32–7.38 (m, 1 H), 4.51 (s, 2 H), 3.08 (s, 3 H). HRMS (APCI⁺) calcd for C₂₃H₁₇N₄NaO₃S₂ 485.0713 (MH⁺), found 485.0710.

4.1.77. *N*-(5-(5-(2-Methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)pyridine-3-sulfonamide (**67**)

Amine **78** was reacted with pyridine-3-sulphonyl chloride according to general procedure B to give **67** as a light brown solid (45%); mp (CH₂Cl₂/MeOH) 283–286 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 10.98 (br s, 1 H), 8.97 (d, *J* = 2.0 Hz, 1 H), 8.83 (dd, *J* = 4.8, 1.4 Hz, 1 H), 8.70 (d, *J* = 1.9 Hz, 1 H), 8.22 (d, *J* = 2.2 Hz, 1 H), 8.20 (dt,

$J = 8.1, 1.8 \text{ Hz, 1 H}$, 7.94 (s, 1 H), 7.84 (d, $J = 7.9 \text{ Hz, 1 H}$), 7.75 (t, $J = 2.2 \text{ Hz, 1 H}$), 7.73 (d, $J = 3.9 \text{ Hz, 1 H}$), 7.71 (d, $J = 8.0 \text{ Hz, 1 H}$), 7.69 (d, $J = 3.9 \text{ Hz, 1 H}$), 7.65 (dd, $J = 5.2, 2.8 \text{ Hz, 1 H}$), 4.52 (s, 2 H), 3.09 (s, 3 H). HRMS (APCI⁺) calcd for C₂₃H₁₈N₄O₃S₂ 463.0893 (MH⁺), found 463.0891.

4.1.78. *N*-(5-(5-(2-Methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)thiophene-2-sulfonamide (68)

Amine **78** was reacted with thiophene-2-sulphonyl chloride according to general procedure B to give **68** as a cream solid (71%); mp (CH₂Cl₂/MeOH) 300–304 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 10.91 (br s, 1 H), 8.72 (d, $J = 2.0 \text{ Hz, 1 H}$), 8.24 (d, $J = 2.3 \text{ Hz, 1 H}$), 7.93–7.98 (m, 2 H), 7.83 (dd, $J = 8.0, 1.5 \text{ Hz, 1 H}$), 7.78 (t, $J = 2.2 \text{ Hz, 1 H}$), 7.74 (d, $J = 3.9 \text{ Hz, 1 H}$), 7.68 (d, $J = 3.9 \text{ Hz, 1 H}$), 7.65 (dd, $J = 3.8, 1.3 \text{ Hz, 2 H}$), 7.15 (dd, $J = 4.9, 3.8 \text{ Hz, 1 H}$), 4.52 (s, 2 H), 3.09 (s, 3 H). In this case the product was also converted to its sodium salt according to general procedure E giving a pale yellow solid (90%). ¹H NMR [400 MHz, (CD₃)₂SO] δ 8.08 (d, $J = 2.1 \text{ Hz, 1 H}$), 7.92 (d, $J = 2.4 \text{ Hz, 1 H}$), 7.90 (s, 1 H), 7.80 (dd, $J = 7.9, 1.5 \text{ Hz, 1 H}$), 7.68 (d, $J = 8.0 \text{ Hz, 1 H}$), 7.65 (d, $J = 3.8 \text{ Hz, 1 H}$), 7.46–7.53 (m, 2 H), 7.42 (d, $J = 3.9 \text{ Hz, 1 H}$), 7.27 (dd, $J = 3.6, 1.3 \text{ Hz, 1 H}$), 6.93 (dd, $J = 5.0, 3.6 \text{ Hz, 1 H}$), 4.51 (s, 2 H), 3.08 (s, 3 H). Anal. C, H, N.

4.1.79. *N*-(5-(5-(2-Methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)thiophene-3-sulfonamide (69)

Amine **78** was reacted with thiophene-3-sulphonyl chloride according to general procedure B to give **69** as a yellow solid (47%); mp (CH₂Cl₂/MeOH) > 300 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 10.72 (br s, 1 H), 8.68 (d, $J = 1.9 \text{ Hz, 1 H}$), 8.31 (q, $J = 1.3 \text{ Hz, 1 H}$), 8.24 (d, $J = 2.3 \text{ Hz, 1 H}$), 7.95 (s, 1 H), 7.83 (dd, $J = 8.0, 1.4 \text{ Hz, 1 H}$), 7.73–7.78 (m, 3 H), 7.70 (d, $J = 8.0 \text{ Hz, 1 H}$), 7.66 (d, $J = 3.9 \text{ Hz, 1 H}$), 7.32 (dd, $J = 5.2, 1.4 \text{ Hz, 1 H}$), 4.52 (s, 2 H), 3.09 (s, 3 H). Anal. C, H, N.

4.1.80. *N*-(5-(5-(2-Methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)-4-(oxazol-5-yl)benzenesulfonamides (70)

Amine **78** was reacted with 4-(1,3-oxazol-5-yl)benzenesulfonyl chloride according to general procedure B to give **70** as a pale yellow solid (81%); mp (MeOH/CH₂Cl₂) 278–281 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 10.80 (br s, 1 H), 8.69 (d, $J = 2.0 \text{ Hz, 1 H}$), 8.53 (s, 1 H), 8.23 (d, $J = 2.3 \text{ Hz, 1 H}$), 7.86–7.96 (m, 6 H), 7.80 (dd, $J = 7.9, 1.4 \text{ Hz, 1 H}$), 7.68–7.73 (m, 3 H), 7.66 (d, $J = 3.9 \text{ Hz, 1 H}$), 4.52 (s, 2 H), 3.09 (s, 3 H). LRMS (APCI⁺) calcd for C₂₇H₂₀N₄O₄S₂ 528 (M), found 528. Anal. C, H, N.

4.1.81. 5-(Isoxazol-5-yl)-*N*-(5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)thiophene-2-sulfonamide (71)

Amine **78** was reacted with 5-(5-isoxazolyl)thiophene-2-sulfonyl chloride according to general procedure B to give **71** as a pale pink solid (20%); mp (MeOH/1,4-dioxane) 245–249 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 11.15 (br s, 1 H), 8.76 (br s, 1 H), 8.72 (d, $J = 2.0 \text{ Hz, 1 H}$), 8.29 (d, $J = 2.3 \text{ Hz, 1 H}$), 7.93 (d, $J = 0.7 \text{ Hz, 1 H}$), 7.82 (dd, $J = 7.9, 1.5 \text{ Hz, 1 H}$), 7.79 (t, $J = 2.2 \text{ Hz, 1 H}$), 7.71–7.75 (m, 4 H), 7.70 (d, $J = 3.6 \text{ Hz, 1 H}$), 7.11 (d, $J = 1.9 \text{ Hz, 1 H}$), 4.52 (s, 2 H), 3.09 (s, 3 H). LRMS (APCI⁺) calcd for C₂₅H₁₈N₄O₄S₃ 534 (M), found 534. Anal. C, H, N.

4.1.82. 4-(3,5-Dimethyl-1H-pyrazol-1-yl)-*N*-(5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)benzenesulfonamide (72)

Amine **78** was reacted with 4-(3,5-dimethyl-1H-pyrazol-1-yl)benzenesulfonyl chloride according to general procedure B to give **72** as a beige solid (14%); mp (Et₂O/CH₂Cl₂) 265–268 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 10.81 (br s, 1 H), 8.70 (s, 1 H), 8.25 (d, $J = 2.2 \text{ Hz, 1 H}$), 7.94 (br s, 1 H), 7.91 (d, $J = 8.7 \text{ Hz, 2 H}$), 7.82 (dd, $J = 8.0, 1.3 \text{ Hz, 1 H}$), 7.77 (d, $J = 8.7 \text{ Hz, 2 H}$), 7.68–7.74 (m, 3 H), 7.61

(d, $J = 3.9 \text{ Hz, 1 H}$), 6.12 (s, 1 H), 4.51 (s, 2 H), 3.09 (s, 3 H), 2.34 (s, 3 H), 2.15 (s, 3 H). LRMS (APCI⁺) calcd for C₂₉H₂₅N₅O₃S₂ 555 (M), found 555. Anal. C, H, N.

4.1.83. *N*-(5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)-2,3-dihydrobenzo[*b*][1,4]dioxine-6-sulfonamide (73)

Amine **78** was reacted with 2,3-dihydrobenzo[*b*][1,4]dioxine-6-sulfonyl chloride according to general procedure B to give **73** as a yellow solid (78%). ¹H NMR [400 MHz, (CD₃)₂SO] δ 10.64 (s, 1 H), 8.68 (d, $J = 2.0 \text{ Hz, 1 H}$), 8.22 (d, $J = 2.3 \text{ Hz, 1 H}$), 7.95 (br s, 1 H), 7.84 (dd, $J = 8.0, 1.4 \text{ Hz, 1 H}$), 7.74–7.70 (m, 3 H), 7.66 (d, $J = 3.9 \text{ Hz, 1 H}$), 7.28–7.31 (m, 2 H), 7.04 (d, $J = 8.5 \text{ Hz, 1 H}$), 4.52 (s, 2 H), 4.28 (m, 4 H), 3.09 (s, 3 H). In this case the product was also converted to its sodium salt according to general procedure E giving a yellow solid (58%). ¹H NMR [400 MHz, (CD₃)₂SO] δ 8.00 (d, $J = 2.1 \text{ Hz, 1 H}$), 7.90 (br s, 1 H), 7.85 (d, $J = 2.4 \text{ Hz, 1 H}$), 7.80 (dd, $J = 8.0, 1.5 \text{ Hz, 1 H}$), 7.65–7.70 (m, 2 H), 7.37–7.41 (m, 2 H), 7.17–7.21 (m, 2 H), 6.82 (d, $J = 8.3 \text{ Hz, 1 H}$), 4.51 (s, 2 H), 4.21 (br s, 4 H), 3.08 (s, 3 H). HRMS calcd for C₂₆H₂₀N₃NaO₅S₂, 541.0742 (M⁺ + Na⁺); found, 541.0744.

4.1.84. 4-Methyl-*N*-(5-(5-(2-methyl-1-oxoisindolin-5-yl)thiophen-2-yl)pyridin-3-yl)thiazole-5-sulphonamide (74)

Amine **78** was reacted with 4-methylthiazole-5-sulphonyl chloride according to general procedure B, giving **74** as a brown solid (12%); mp 277–280 °C. ¹H NMR [400 MHz, (CD₃)₂SO] δ 11.15 (br s, 1 H), 9.20 (s, 1 H), 8.78 (d, $J = 2.0 \text{ Hz, 1 H}$), 8.25 (d, $J = 2.4 \text{ Hz, 1 H}$), 7.95 (d, $J = 0.6 \text{ Hz, 1 H}$), 7.83 (dd, $J = 7.9, 1.5 \text{ Hz, 1 H}$), 7.77 (t, $J = 2.2 \text{ Hz, 1 H}$), 7.74 (d, $J = 3.9 \text{ Hz, 1 H}$), 7.71 (d, $J = 8.0 \text{ Hz, 1 H}$), 6.69 (d, $J = 3.9 \text{ Hz, 1 H}$), 4.52 (s, 2 H), 3.09 (s, 3 H), 2.52 (s, 3 H). HRMS calcd for C₂₂H₁₈N₄O₃S₃, 483.06138 (M⁺); found, 483.06251.

4.2. Inhibition of perforin-mediated lysis of Jurkat cells

The ability of the compounds to inhibit the lysis of labelled nucleated (Jurkat T lymphoma) cells in the presence of 0.1% BSA was measured by the release of ⁵¹Cr. Jurkat target cells were labelled by incubation in medium with 100 μ Ci ⁵¹Cr for 1 h. The cells were then washed three times to remove unincorporated isotope and re-suspended at 1×10^5 cells per mL in RPMI buffer supplemented with 0.1% BSA. Each test compound was pre-incubated to concentrations of 20, 10, 5, 2.5 and 1.25 μ M with recombinant perforin for 30 min with DMSO as a negative control. ⁵¹Cr labelled Jurkat cells were then added and cells were incubated at 37 °C for 4 h. The supernatant was collected and assessed for its radioactive content on a gamma counter (Wallac Wizard 1470 automatic gamma counter). Each data point was performed in triplicate and an IC₅₀ was calculated from the range of concentrations described above.

4.3. KHYG1 inhibitory assay

KHYG1 cells were washed and re-suspended in RPMI/0.1% BSA at 16×10^5 cells/mL and 100 μ L of the cell suspension is dispensed to each well of a 96-well V-bottom plate. Test compounds are then added (50 μ L) at a final concentration of 20 μ M and incubated at RT for 20 min. ⁵¹Cr-labelled K562 leukemia target cells (50 μ L, 2×10^5 cells/mL) are then added to each well and incubated at 37 °C for 4 h. ⁵¹Cr release is assayed using a Skatron Harvesting Press and radioactivity estimated on a Wallac Wizard 1470 Automatic Gamma counter (Turku, Finland). The inhibitory function is then determined by identifying the number of untreated or inhibitor treated effector cells required to kill the same number of targets. The percent inhibition is calculated by the formula:

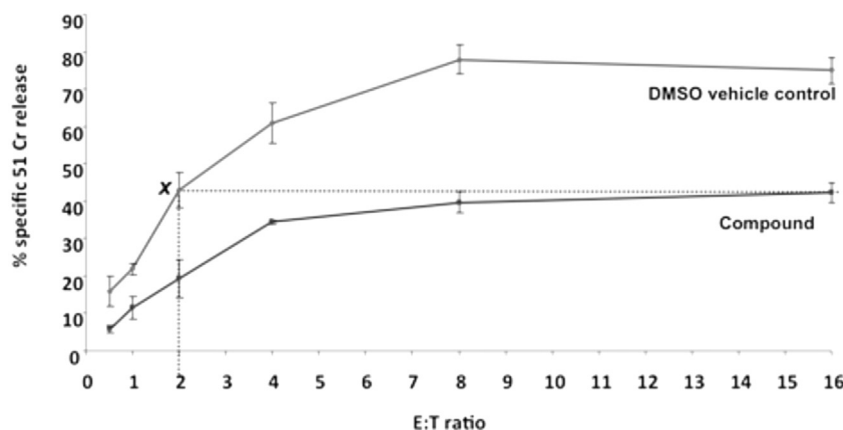


Fig. 3. Calculation of inhibitory function in the KHYG1 assay.

$$100 - \left(\frac{(X)}{(16)} \times 100 \right)$$

where x is the point the inhibitor intersects with the DMSO on a curve. In the example shown (Fig. 3) “x” is the x-intercept corresponding to the point on the DMSO control curve that yields the same level of ⁵¹Cr release as the test compound at an E/T ratio of 16:1.

4.4. Toxicity to KHYG1 NK cells

The toxicity assay was carried out in exactly the same manner as the killing assay above, but instead of adding the labelled K562 target cells, 100 μ L of RPMI 0.1% BSA was added. Cells were incubated for 4 h at 37 °C and then washed $\times 3$ in RPMI + 0.1% BSA. Cells were then re-suspended in 200 μ L of complete medium and incubated for 18–24 h at 37 °C. Trypan blue was added to each well. Viable (clear) cells and total (clear + blue) cells were counted, and the percentage of viable cells was calculated compared to DMSO treated cell control (% viability).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.ejmech.2017.05.048>.

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