High-throughput screening and hit validation of extracellular-related kinase 5 (ERK5) inhibitors.

Stephanie M. Myers[1], Ruth H. Bawn[1], Louise C. Bisset[3], Timothy J. Blackburn[1], Betty Cottyn[1], Lauren Molyneux[1], Ai-Ching Wong[4], Celine Cano[1], William Clegg[2], Ross. W. Harrington[2], Hing Leung[5], Laurent Rigoreau[4], Sandrine Vidot,[1] Bernard T. Golding[1], Roger J. Griffin[1], Tim Hammonds[4], David R. Newell[3], Ian R. Hardcastle[1*]

[1] Newcastle Cancer Centre, Northern Institute for Cancer Research and School of Chemistry, Bedson Building, Newcastle University, Newcastle upon Tyne, NE1 7RU, UK

[2] School of Chemistry, Bedson Building, Newcastle University, Newcastle upon Tyne, NE1 7RU, UK

[3] Newcastle Cancer Centre, Northern Institute for Cancer Research, Paul O'Gorman Building, Medical School, Framlington Place, Newcastle University, Newcastle upon Tyne, NE2 4HH, UK

[4] Cancer Research Technology Ltd, Discovery Laboratories, Wolfson Institute for Biomedical Research, The Cruciform Building , Gower Street, London, WC1E 6BT, UK

[5] The Beatson Institute for Cancer Research, Garscube Estate, Switchback Road, Bearsden, Glasgow G61 1BD, UK

Abstract

The extracellular-related kinase 5 (ERK5) is a promising target for cancer therapy. A high-throughput screen was developed for ERK5, based on the IMAP FP Progressive Binding System, and used to identify hits from a library of 57,617 compounds. Four distinct chemical series were evident within the screening hits. Resynthesis and re-assay of the hits demonstrated that one series did not return active compounds, whereas three series returned active hits. Structure-activity studies demonstrated that the 4-benzoylpyrrole-2-carboxamide pharmacophore had excellent potential for further development. The minimum kinase binding pharmacophore was identified, and key examples demonstrated good selectivity for ERK5 over p38α kinase.

INTRODUCTION

The extracellular-related kinase 5 (ERK5), also known as Big Map Kinase (BMK1), is a 816 amino acid protein kinase that forms part of a non-canonical MAP kinase pathway in cells.[1] Extracellular stimulation, in the form of growth factors, such as epidermal growth factor (EGF), nerve growth factor (NGF), vascular endothelium growth factor (VEGF), and fibroblast growth factor-2 (FGF-2), initiates a signalling cascade from the cell surface to nuclear transcription factors via ERK5.[2] Unlike the linear canonical Ras/Raf/MEK/ERK pathway, the ERK5 signalling cascade occurs independently of Raf.[3] ERK5 is activated specifically by MEK5, which is in turn activated by MEKK2/3.[1a, 4]

ERK5 is structurally different from the other members of the ERK sub-families. A unique loop-12 structure and extended C-terminal domain (~400 amino acids) gives ERK5 its characteristic structure.[2, 4] The C-terminal extension harbours nuclear localisation and export sequences, two proline-rich domains, a transcriptional activating domain, and MEF2 interacting region. The large C-terminal unique to ERK5 is auto-phosphorylated at multiple sites, resulting in an increase in transcriptional activity.[5]

Phosphorylation of ERK5 results in activation of a number of transcription factors including MEF2, c-Myc, c-Jun, c-Fos, Fra-1, and NFκB.[6] ERK5 also phosphorylates and activates p90 RSK, also involved in signal transduction.[7] An increasing body of mechanistic data indicates that ERK5 plays a key role in tumor biology, i.e. cell proliferation and survival, invasion and metastasis, and angiogenesis.[8]

Expression of ERK5 is significantly up-regulated in advanced prostate cancer and has been identified as an independent prognostic biomarker for aggressive disease.[9] ERK5 is over-expressed in 20% of breast cancer patients and its expression is an independent prognostic biomarker for reduced disease-free survival.[10] In hepatocellular carcinoma (HCC), ERK5 overexpression has been reported, associated with gene amplification at the 17p11 chromosome fragment, harboring the MAPK7 gene.[11] High levels of ERK5 were found to correlate with more aggressive and metastatic stages in fresh samples from human clear cell renal cell carcinoma.[12]

To date, three small-molecule inhibitors of the MEK5/ERK5 pathway have been described. The indolinone based inhibitors, BIX02188 (**1a**) and BIX02189 (**1b**), are dual inhibitors of the MEK5/ERK5 cascade. Both compounds inhibit MEK5 with nanomolar potency, whereas modest activity was reported for ERK5.[13] Benzo[*e*]pyrimido-[5,4-

b]diazepine-6(11*H*)-one (XMD8-92, **2**) is a potent ERK5 inhibitor which exhibits anti-proliferative and anti-angiogenic effects on HeLa cells in mouse xenograft models.[14] An X-ray crystal structure of **2** bound to ERK5 shows the inhibitor bound to the Met140 in the kinase hinge *via* a pair of hydrogen bonds from the aniline and pyrimidine nitrogens, and an additional water-bridged hydrogen bond from the diazepinone carbonyl to Asp200 and Glu102 in the DFG loop.[15] However, some of the *in vivo* activity of **2** has been attributed to off-target activities e.g. inhibition of doublecortin-like kinase 1 (DCLK-1) in pancreatic cancer.[16] Inhibition of ERK5 or siRNA knockdown has been shown to inhibit the growth of HCC cell lines, *in vitro* and *in vivo*.[17] ERK5 signalling has been shown to be essential for chemically induced carcinogenesis in skin by *erk5* gene deletion or ERK5 inhibition (with **2**).[18] The combination of doxorubicin and either *erk5* gene deletion or ERK5 inhibition (with **2**) was found to be additive in inflammation-driven tumour models. Thus, pharmacological inhibition of ERK5 may provide an opportunity for the treatment of inflammation-driven, invasive or metastatic cancers where ERK5 is deregulated. Given the strong link between ERK5 mediated signalling and malignancy, there remains a strong need to develop selective tool molecules to fully elucidate the effect of ERK5 inhibition *in vivo*. For this reason, we sought to discover novel ERK5 inhibitory chemotypes through high-throughput screening as chemical tools and for development as therapeutic agents.

In this paper, we describe the development of a high-throughput screening assay for ERK5 inhibition based on the IMAP FP format that was used to identify four discrete series of hit molecules. Validation of each series was attempted by resynthesis and retesting of selected members from each series. Preliminary structure activity studies were obtained resulting in the identification of one series for further optimisation.

DEVELOPMENT AND EXECUTION OF ERK5 IMAP SCREEN

Assay development

The discovery of kinase inhibitors using high-thoughput screening is well established.[19] In our case, expression of active ERK5 protein required co-expression of MEK5. The screen was developed using the IMAP format (Molecular Devices) that relies on the high specificity interaction of phospho groups on a fluorescently tagged peptide with M^{3+} containing nanoparticles. The IMAP format was chosen as a robust and efficient method of determining kinase activity using a 'mix-and-measure' format with a non-radioactive, fluorescence output.

Preliminary experiments to determine a suitable peptide substrate for the IMAP FP assay used a commercial source of the enzyme and were based on sequence of the natural substrate of ERK5, and were augmented with a substrate finder kit. Five peptide sequences were designed based around the reported site of phosphorylation of MEF2C by ERK5, with the expected serine phosphorylation site highlighted in red (Table 1).[1] The peptides were tested using the IMAP FP Progressive Binding System (Molecular Devices) in the absence and presence of ERK5 (Carna Biosciences).

The IMAP FP substrate finder kit for serine/threonine kinases plate 2 (Molecular Devices) covering CMGC, CK1, STE, and TKL portions of the kinome was used to identify potential peptide substrates phosphorylated by ERK5. The FAM-EGFR-derived peptide (LVEPLTPSGEAPNQK-5FAM-COOH) proved optimal.

Kinetic studies determined the ERK5 K_M^{app} to be 300 μM. Ideally, kinase screens are run with the ATP concentration equal to the K_M, however, in this case the IMAP format did not return acceptable results at a high ATP concentration (300 μM), presumably due to interference of ATP with the interaction of the phospho peptide with the metal nanoparticles. For this reason, the HTS assay was run at the maximum acceptable ATP concentration (100 μM) to allow 'mix-and-measure' determinations.

High-throughput Screening for ERK5 inhibitors

To identify inhibitors of ERK5 the IMAP HTS assay was set up and a library of 57,617 small molecules was screened (final DMSO concentration of 4% and a total reaction volume of 40 μL). The library was composed of a 48,479 member diverse library and a 9136 member kinase focussed library, both libraries were sourced from commercial vendors. Z' factors for each plate were calculated using Equation 2, and were typically 0.6-0.8. Plates with Z' factors below 0.4 were re-screened (Supporting Information).

HTS Results

The HTS assay returned 245 active compounds (0.5 % hit rate), i.e. >50% inh at 30 μM, from the 57,617 member library. Active compounds (245) showing >50% inhibition in the screen were resupplied from stock or commercial vendors and retested at 30, 10 and 3.3 μM. 71 active compounds, giving >30% mean inhibition, were treated as confirmed hits (0.10% overall hit-rate) and assayed over a full IC_{50} range. IC_{50} determinations required a two stage process whereby the reaction occurred initially in the absence of the IMAP reagent, followed by subsequent addition of the IMAP reagent, to allow the K_M concentration of ATP to be used.

The hit compounds produced inhibition curves with IC_{50} values ranging from 0.6 to 76 μM (1 compound >120 μM). Confirmed hits were clustered according to common structures, revealing four promising chemical series. SAR around the hits was expanded by assaying related in-house compounds and close analogues from commercial suppliers. From these results, four compound series were selected for validation by resynthesis prior to progressing to hit-to-lead studies: 2-amino-*N*,*N*-alkylbenzo[*d*]thiazole-6-sulfonamides (Table 2, **3a-c**); 4-substituted-2-(substitutedthio)-6-phenylnicotinonitriles (Table 3, **4a,b**); 4-amino-2-(arylamino)pyrimidine-5-carbonitriles (Table 5, **5a-c**); and 4-aroyl-*N*-alkyl-1*H*-pyrrole-2-carboxamides (Table 6, **6a-e**).

SYNTHESIS

*Benzothiazole Series **3***

Numerous methods have been described for the synthesis of benzothiazoles.[20] For the synthesis of compounds **3a,b** and analogues, we used the reported reaction of anilines with potassium thiocyanate-copper(II) sulfate (Scheme 1).[21] The required 4-aminophenylsulfonamides (**7a-c**) were prepared by reduction of the corresponding nitro compounds (**8a-c**). The nitro precursors were obtained by coupling the relevant amine with 4-nitrobenzenesulfonyl chloride. The 5-sulfonamide isomer **9a**, was prepared *via* the same method (Scheme 2). Thus, 3-nitrobenzenesulfonyl chloride was reacted with pyrrolidine or *N*-methylethylamine, and the resulting sulfonamides **10a,b** were reduced to the

respective anilines **11a,b** (Scheme 2)**.** Reactions of **11a,b** with with potassium thiocyanate-copper(II) sulfate gave in each case, besides the desired 5-substituted benzothiazole **9a,b**, a significant quantity of a thiocyanatobenzene (**12a,b**).

*Nicotinonitrile Series **4***

The first synthetic approach considered for the synthesis of 3-cyanopyridines was based on the route reported by Shestopalov et al.[22] For example, 4-fluorochalcone **13a**, prepared *via* Claisen-Schmidt condensation of acetophenone with 4-fluorobenzaldehyde, was treated with elemental sulfur and morpholine in ethanol at reflux, followed by malononitrile, to give pyridinethione **14a** in moderate yield (Method A, Scheme 3). Isolation of the intermediate pyridinethiones **14** required extensive purification, attributed to the propensity of this intermediate to tautomerise, and its readiness to oxidise under atmospheric conditions.

Consideration of the likely mechanism of the one-pot sequence of the cyclisation reaction prompted the replacement of the sulfur and malononitrile with 2-cyanothioacetamide for the Michael addition in an alternative route (Method B, Scheme 3). Reactions were performed under nitrogen to avoid oxidative side-reactions. The crude intermediate **14** was used directly in the alkylation step, to avoid a lengthy purification, giving cyanopyridines **15a-k**. Method B allowed isolation of **15a** in an improved 54% yield over 2 steps. Deprotection with TFA gave acid**s 4a-k** in near quantitative yield (99%). The carboxamide **17** was prepared from **4a** by a HBTU-mediated coupling with *p*-methoxybenzylamine giving amide **16,** which was deprotected with TFA.

Further variations to the thioether group were introduced *via* alkylation of **14a** (Schemes 4-7). The acetamide derivatives **31** and **32** were prepared by the alkylation of **14a** and **14g**, respectively, with bromoacetamide **30** which was obtained by reaction of aminoacetone hydrochloride **29** with bromoacetylchloride (Scheme 8).[23]

*Cyanopyrimidine Series **5***

A small series of 4-amino-2-anilinopyrimidine-5-carbonitriles (**5a-c**) were prepared by the reaction of the appropriate aniline with chloropyrimidine (**33**) at 100 ^{o}C in DMF (Scheme 9).[24]

*4-Benzoylpyrrole-2-carboxamide Series **6***

A selection of 4-benzoylpyrrole-2-carboxamides were prepared by Friedel-Crafts acylation of methyl 1*H*-pyrrole-2-carboxylate (**34**) with a substituted benzoyl chloride giving pyrrole (**35**). Hydrolysis of the methyl ester with lithium hydroxide gave carboxylic acid **36** that was coupled with the appropriate amine using CDI to give the desired carboxamides (**6a-r**)(Scheme 10). The *N*-methyl derivative **6m** was prepared by methylation of ester **35a**, followed by hydrolysis and coupling with 3-pyridylmethylamine (Scheme 11).

2-Substituted-4-benzoylpyrrole derivatives

The alkene derivative **40** was prepared by aldol condensation of ketone **39** and isonicotinaldehyde (Scheme 12). Selective reduction was achieved by refluxing alkene **40** in aqueous ethanol with indium metal and ammonium

chloride giving alkane **41** in moderate yield. [25] The cyclopropyl analogue **42** was prepared by a Corey Chaykovsky reaction.[26] Thus, alkene **40** was reacted with trimethylsulfoxonium iodide and potassium *tert*-butoxide giving **42** in 12% yield.[27] Diketone **44** was prepared *via* a Claisen condensation between 1-(1*H*-pyrrol-2-yl)ethanone and methyl isonicotinate diketone **43** (Scheme 13). Friedel-Crafts acylation with 2,3-dichlorobenzoyl chloride gave **44**.

DISCUSSION

Selected examples of the HTS hits in the benzothiazole series (**3a**, **3b**, **3i**) were synthesised and re-assayed. The ERK5 inhibitory activity for the resynthesized benzothiazoles were 1000-fold lower than for the library material (Table 2). Comparision of the ^{1}H-NMR and LCMS spectra of the resynthesized and screened samples of **3a** suggested that the library material was the 5-sulfonamide **9a**, so authentic samples of isomers **9a** and **9b** were prepared. In order to eliminate the possibility of mis-identification of the compounds by spectroscopic methods, the identity of isothiocyanate **12a** and benzothiazoles **3a** and **3i** were elucidated by small-molecule X-ray crystallography (Figures 1-3).

The assay results for these isomers also failed to replicate the initial IC_{50} values from the screening samples. Interestingly, the isothiocyanate side-product **12a** showed 10-fold greater potency than the benzothiazole, although this result was not replicated for the analogue **12b**. Time-dependent enzyme inactivation by isothiocyanates, *via* their reaction with lysine residues, has been reported.[28] Further investigations into the mechanism of action of **12a** were not conducted. Some aminothiazoles have recently been identified as frequent hitters from a fragment screening set and dubbed promiscuous 2-aminothiazoles (PrATs).[29] The reason for the discrepancy between the activity of the HTS sample and the resynthesized material is not clear. Numerous mechanisms for false positives in HTS are possible, including the presence of trace impurities or protein aggregation, and further effort was not expended eliminating these possibilities.[30]

Three HTS hits in the nicotinonitrile series (**4a**, **19**, and **31**) were synthesised and reassayed (Tables 3 and 4). The results for the glycine derivative **4a** and proline methyl ester derivative **19** were in good agreement with the HTS IC_{50} values. In contrast, the propan-2-one derivative **31** was 50-fold less active than the HTS result. On this basis, a limited series of compounds was prepared to establish preliminary SARs and to determine the minimum inhibitory pharmacophore. The SARs for the 4- and 6-substitutents were delineated keeping the 2-thio substituent as the glycine amide (Table 3). The 4,6-diphenyl, 4-phenyl-6-methyl and 4,6-dimethyl compounds (**4d**, **4j** and **4k**, respectively) were each devoid of activity. The 4-(2-fluorophenyl) derivative **4c** was 7-fold less active than the 4-(4-fluorophenyl) derivative **4a**, whereas the 4-(3-fluorophenyl) derivative **4b** lacked measurable potency. The combination of 2-fluoro and 4-fluoro substituents (**4f**) was not additive and resulted in a 15-fold loss of potency compared with **4a**. The 4-(4-pyridyl) derivative **4g** exhibited a 13-fold loss in potency compared to **4a**, despite the similar electronic properties of the rings. Substitution of the 4-phenyl group with 4-trifluoromethyl **4e**, or 4-methoxy **4h** resulted in loss of activity.

The SARs for the thioether side-chain were investigated (Table 4). The pyridine thiol **14a** lacking the amide side-chain showed a 20-fold loss in potency compared to **4a**. The glycine ethyl ester **15l** showed similar activity to the proline methyl ester derivative **19**, and was 4-fold less potent than the corresponding glycine derivative **4a**. In contrast, the glycine amide **17** lacked measurable activity. The shorter, unsubstituted amide **20** showed weak activity, whereas the corresponding ester **21** was inactive. Two thioalkyl carboxylic acids **23a** and **23b** were inactive, as was the corresponding amine **28**, demonstrating the requirement for the amide group in the sidechain for potency. Comparison of the 4-fluorophenyl propan-2-one derivative **31** with the 4-pyridyl derivative **32** showed a 5-fold loss in potency consistent with the results in the glycine amide series (**4a** and **4g**).

Overall, each of the changes made to the hit compounds in the nicotinonitrile series (**4a**, **19**, and **31**) resulted in loss of potency. Modifications to the aromatic and side-chain substituents revealed a highly constrained pharmacophore and limited SAR. As a result, no further optimisation of this series was attempted. Interestingly, 3-cyano-4,6-diphenyl-pyridines have been identified recently as inhibitors of the PA-PB1 protein-protein interaction for influenza.[31]

The cyanopyrimidines **5a-c** showed reasonable activity against ERK5, with IC_{50} values in the 12-88 μM range (Table 5), and generally consistent with the HTS values. The activity against ERK5 in this series was promising, but the series had also been selected for development against another target internally. For this reason, no further analogues were prepared. Kinase inhibitors incorporating a 5-cyanopyrimidine core have been reported, e.g. Wee1 inhibitors[32], and CDK2 inhibitors[33].

Five 4-benzoylpyrrole-2-carboxamides (**6a-e**) gave good potency in the HTS. Upon resynthesis and retesting, the 2,3-dichlorobenzoyl-*N*-(4-fluorobenzyl) substitued analogue **6a** maintained significant activity (IC_{50} = 3.7 μM) despite a 5-fold loss in potency compared to the HTS result (Table 6). Similarly, the 2-trifluoromethylbenzoyl-*N*-methyl substituted analogue **6d** gave a two-fold drop in activity (IC_{50} = 9.6 μM) compared to the HTS result, and the benzoyl-*N*-methyl-3-pyridyl derivative **6e** gave a 3-fold drop in activity (IC_{50} = 26 μM). In contrast, the resynthesized 2,4-dichlorobenzoyl analogues **6b** and **6c** bearing either the *N,N*-dimethylamide or *N*-phenethylamide substituents, respectively, showed no activity.

Encouraged by these results, a small series of aroylpyrroles was prepared. Compounds were designed to establish the minimum kinase binding pharmacophore, and to explore possibilities to gain potency and selectivity by variation of the amide substituent. The benzoyl substituent was fixed as most potent 2,3-dichlorophenyl for all these examples.

Series (**6f-n**) was prepared to explore simple variations to the amide moiety (Table 7). Monomethyl amide **6f** was equipotent with the parent 4-fluorobenzyl amide **6a,** whereas the dimethyl amide **6g** was 7-fold less potent. Introduction of the 3-pyridylmethyl amide from **6e** or the 4-pyridylmethyl amide **6h**, retained potency, whereas the benzyl derivative **6k** and 2-pyridyl derivative **6j** were less potent, and phenylethyl amide **6l** was inactive. Similar to **6g**, *N*-methyl-(3-pyridylmethyl) amide derivative **6n** was 7-fold less potent than primary amide **6i**. Importantly,

methylation of the pyrrole NH (**6m**) completely abolished ERK5 activity, indicating an essential interaction with the kinase at this position. In contrast, the relatively small drop in activity for the secondary amides **6g** and **6n** suggested the amide NH was not forming a critical interaction, and that the drop in potency could be related to the conformational preference of the amide group. With this in mind, a limited number of conformationally restricted, 5- and 6-membered cyclic secondary amides were investigated (Table 8). The 3,4-dihydro-2,6-naphthyridinyl and isoindolinyl derivatives (**6o** and **6q**) were inactive. In contrast, 3,4-dihydroisoquinolinyl **6p** was 5-fold less potent than **6h**, a comparable to the loss in potency seen for the *N*-methyl analogues, whereas the pyrrolidinopyridinyl **6r** was equipotent with **6h**. Selected examples in this series were assayed in an orthogonal LANCE™ assay format (see supporting information), based on time-resolved fluorescence resonance energy transfer (FRET), to eliminate the possibility of false positives. In all cases, the LANCE results were comparable with those obtained using the IMAP assay.

In order to establish the minimum kinase binding pharmacophore, systematic isosteric replacements to the amide group were made. The acetyl derivative **39** and the 1,3-diketone **44** were inactive (Table 9). In contrast, the unsaturated ketone **40** and the cyclopropyl ketone **42** retained similar activity to the parent **6a**, whereas the saturated ketone **41** was 10-fold less active. These results confirm that the amide NH is not required for activity, and that conformational rigidity at this position is favourable. The loss of activity for diketone **44** was explained by the preferred enol tautomer lacking an essential H-bond to the kinase via the ketone adjacent to the pyrrole.

The most potent pyrrole inhibitor **6h** was submitted for a kinase selectivity screen and gave a promising selectivity profile. Of the 20 kinases, screened only one kinase (SAPK2a or p38α MAP kinase) was inhibited at >50% inhibition (10 μM). Subsequent to our identification of pyrrole-2-carboxamides as ERK5 inhibitors, similar compounds, e.g. **45**, have been independently identified as p38α MAP kinase inhibitors with micromolar activity.[34] The X-ray structure of **45** shows it bound to the hinge of the kinase via hydrogen bonds from the pyrrole NH and the carboxamide carbonyl, with the aryl portion occupying the lipophilic region close to the gatekeeper, and the furan binding in the outer lipophilic region. ERK5 shares 48% sequence homology with p38α MAP kinase, and 58% homology in the kinase domain. In addition, the gatekeeper residues of the kinases are similar, with leucine in ERK5 and threonine in p38α MAP kinase. The ERK5 SAR for our series is consistent with a similar binding mode to ERK5 as seen in the p38 X-ray structure, in particular the donor/acceptor doublet of H-bonds from the pyrrole NH and amide carbonyl to the kinase.

At this point, given the similarity between the published p38α MAP kinase inhibitors and our hit series, we needed to establish selectivity vs p38α MAP kinase to provide useful ERK5 tool compounds or therapeutic agents.[34-35] Thus, selected compounds were counterscreened against p38α MAP kinase using a LANCE assay. As anticipated, the 2-pyridyl derivatives **6j** and 3,4-dihydroisoquinolinyl derivative **6p** were equipotent for both p38α MAP kinase and ERK5. Importantly, the pyrrolidinopyridinyl derivative **6r** was inactive in the p38α assay. The ability to eliminate p38α MAP kinase activity whilst maintaining ERK5 activity by variation of the amide side chain was not readily predicted

from the published p38α X-ray structure and points to differing structural requirements around the amide side-chain that may be exploited in further development of the series.

CONCLUSIONS

The IMAP FP high-throughput screen for ERK5 returned four distinct chemical series as hits. Synthesis of the hits and selected close analogues demonstrated that the HTS activity of the benzothiazoles **3** was not reproducable, activity for the cyanopyridine hits (**4a**, **19**) was reproducable, but the limited scope to develop the SAR ruled this series out, and two series with confirmed active hits. The lack of activity of these hits was disappointing but not atypical in screening campaigns. The cyanopyrimidine hits **5a-c** were not pursued for reasons of competition. The remaining series, the pyrrole carboxamides **6a-e**, demonstrated consistent ERK5 activity, with SARs consistent with a kinase hinge binder. Selectivity against the close homologue p38α MAP kinase was achieved without loss of ERK5 activity through minor structural modification, and a representative example **6h** showed an acceptable kinase selectivity profile in a panel. At this stage the pyrrole carboxamides demonstrated tractable synthesis, intelligible preliminary SARs, and promising selectivity. The relatively modest kinase inhibitory activity achieved at this stage did not give any concern as the pharmacophore established presented opportunities to optimise potency at both the benzoyl and amide portions, indepenently. Having demonstrated the necessary requirements to progress to the hit-to-lead optimisation stage, further SAR studies were undertaken, with an initial focus on improving potency, which will be reported seperately.[36]

EXPERIMENTAL SECTION

IMAP Substrate mapping

Non-phosphorylated and phosphorylated versions of each ERK5 sequence (Table 1) were obtained from the CRUK Peptide Synthesis Research Services group. The substrate finder kit was used according to the manufacturer's instructions. Reaction buffer (10 μL) containing ATP (100 μM) was added to wells of the plate to reconstitute 5-FAM labeled substrates. Reaction buffer (10 μL) with or without ERK5 (6.4 ng/μL) was added to appropriate wells of the plate, to generate background controls and positive controls. The reaction was incubated for 1 hour at ambient temperature after which IMAP Binding Solution (60 μL) was added. After a further 1 hour of incubation the fluorescence polarisation was measured. The results were analysed using the IMAP Substrate Mapper provided with the kit.

Kinetic characterisation of ERK5

Reactions were carried out with varying concentrations of ATP at constant substrate and enzyme concentrations. Due to limitations of the IMAP FP assay with respect to ATP concentrations, we utilised a transfer method to increase the maximum concentration of ATP that can be used. Reactions were conducted as normal in 10 μL reaction volume. After either 1, 2, 3 or 4 hour incubation period at 37°C, 4 μL of the reaction was transferred to 196 μL of reaction buffer followed by a subsequent transfer of 10 μL of this solution to 30 μL of IMAP Binding Solution. Rates

of reaction at 1 hour reaction time at the range of substrate concentrations were determined, and kinetic parameters were determined by non-linear regression fitting of the data to the Michaelis-Menten equation (Equation 1) ; curve fitting was performed using GraphPad Prism software.[37]

ERK5 High-throughput Screen

Compounds were assayed in a 10 µL reaction mixture per well containing: 1 in 700 dilution of ERK5 stock from CRT, 100 nM peptide R7129 and 100 µM of ATP. The reactions were performed with 10 mM Tris-HCl (pH 7.2), 10 mM $MgCl_2$, 0.05% NaN_3 and 0.01% Tween-20. Reactions were incubated for 3 hours at 37°C, followed by addition of 30 µL of IMAP binding solution (1 in 600 dilution of IMAP binding reagent in 60% Binding Buffer A and 40% Binding Buffer B) and a further incubation for 2 hours at ambient temperature. Plates were read on an Analyst HT microplate reader and the data analysed using ActivityBase.

ERK5 IC_{50} Determination (IMAP)

The enzyme reaction was run as described for the HTS but using 300 µM ATP, 250 nM peptide and a reduced incubation time of 2 hours at 37°C. 1 µL of this reaction was then transferred to a new assay plate and 9 µL of reaction buffer was added followed by 30 µL of IMAP binding solution.

X-ray crystallography

Data were collected on an Oxford Diffraction Gemini A Ultra diffractometer for **3i**, using MoKα radiation (λ = 0.71073 Å) at 150K, and on a Bruker Apex2 diffractometer for **3a** and **12a**, using synchrotron radiation (λ = 0.6946 Å; SRS station 9.8, Daresbury Laboratory) at 120 K because of the very small size of crystals available. Corrections were made for synchrotron beam decay and for absorption and other systematic effects on the basis of repeated and equivalent data. The structures were solved by direct methods and refined on all unique F^2 values with anisotropic non-hydrogen atoms, with freely refined isotropic H atoms bonded to N, and with a riding model for H atoms bonded to C. All four structures are fully ordered; **3i** have two independent molecules in the asymmetric unit, and the non-centrosymmetric but achiral crystal structure of **3i** displays inversion twinning with essentially equal components. Full crystallographic details are given in the Supporting information. Programs were standard Oxford Diffraction CrysAlisPro[38] and Bruker Apex2[39] for data collection and processing, and SHELXTL[40] and SHELXL-2014_ENREF_52_ENREF_53[41] for structure solution and refinement. CCDC references: 1410001, 141003, and 1410004.

ASSOCIATED CONTENT

Supporting Information

Additional screening and synthesis information, X-ray crystal structure data for compounds **12a**, **3a**, and **3i**, synthetic procedures, ERK5 and p38α LANCE assay protocols, kinase selectivity data for **6h**.

AUTHOR INFORMATION

Corresponding Author

*E-mail: Ian.Hardcastle@ncl.ac.uk

Funding

This work was supported by Cancer Research UK (C240/A7409, C1296/A6963 and C2115/A21421), EPSRC equipment grant (EP/F03637X/1), and the synchrotron component of the UK National Crystallography Service 2001–2010 (EP/D07746X/1).

Notes

The authors declare no competing financial interests.

ACKNOWLEDGEMENTS

We thank CCLRC for access to synchrotron diffraction facilities at SRS. The use of the EPSRC National Mass Spectroscopy Facility, Swansea University is gratefully acknowledged.

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EQUATIONS

Equation 1

$$\frac{v}{[E]} = \frac{k_{cat}[S]}{(K_m + [S])}$$

Equation 2

$$Z' = 1 - \frac{3\sigma_{c+} + 3\sigma_{c-}}{|\mu_{c+} - \mu_{c-}|}$$

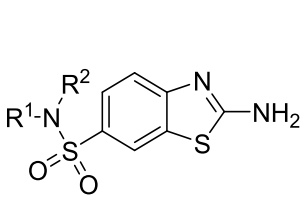
Where σ and μ represent the standard deviation and mean of the positive (c+) and negative (c-) plate controls, respectively.

TABLES

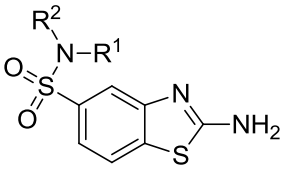
Table 1

Number	Sequence
166	5-FAM-AGR**S**PVD
168	5-FAM-EAGR**S**PVDS
170	5-FAM-HEAGR**S**PVDSL
172	5-FAM-RHEAGR**S**PVDSLS
174	5-FAM-TRHEAGR**S**PVDSLSS

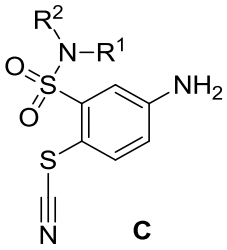
Table 2



A



B

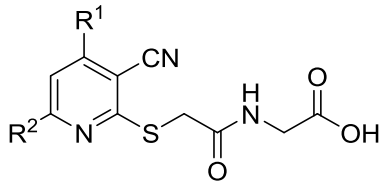


C

Compound	Structure	R^1	R^2	ERK5 IC_{50} (μM) HTS	ERK5 IC_{50} (μM) Resynthesized[a]
3a	A	-$(CH_2)_4$-		0.057	51 ± 5.0
3b	A	Et	Me	0.087	85 ± 5.0
3c	A	Me	H	0.087	-
3d	A	Et	H	0.11	-
3e	A	CH_2=$CHCH_2$-	H	0.13	-
3f	A	*s*-Bu	H	0.13	-
3g	A	-$(CH_2)_5$-		0.46	-
3h	A	*i*-Pr	H	0.60	-
3i	A	*n*-Pr	H	0.89	>120[b]
3j	A	Et	Et	0.93	-
3k	A	-$(CH_2)_2O(CH_2)_2$-		5.13	-
9a	B	-$(CH_2)_4$-		-	29 ± 1.3
9b	B	Et	Me	-	21 ± 1.5
12a	C	-$(CH_2)_4$-		-	2.3 ± 1.5
12b	C	Et	Me	-	>120[b]

a) Values are the mean of at least 3 determinations ± SD; b) n = 2

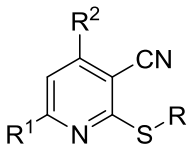
Table 3: ERK5 inhibitory activity of nicotinonitrile series **4a-k**.

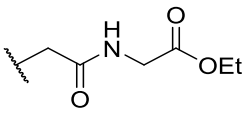
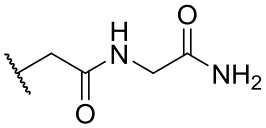
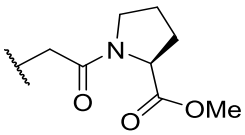
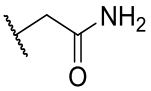
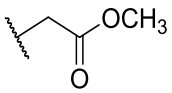
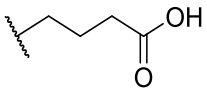
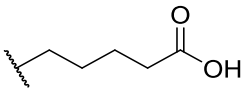
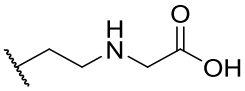
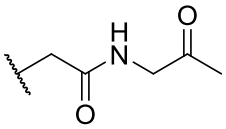
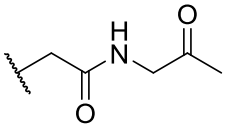


			ERK5 IC_{50} (µM)	
Compd	**R^1**	**R^2**	**HTS**	**Resynthesized**[a]
4a	4-F-Ph	Ph	1.6	4.9 ± 0.3
4b	3-F-Ph	Ph	-	>120[b]
4c	2-F-Ph	Ph	-	34.3 ± 6.4
4d	Ph	Ph	-	>120[b]
4e	4-(CF_3)-Ph	Ph	-	>120[b]
4f	2,4-di-F-Ph	Ph	-	72.9 ± 26.1
4g	4-Py	Ph	-	65.1 ± 12.4
4h	4-MeOPh	Ph	-	>120[b]
4i	Ph	4-MeOPh	-	>120[b]
4j	Ph	CH_3	-	>120[b]
4k	CH_3	CH_3	-	>120[b]

a) Values are the mean of at least 3 determinations ± SD; b) n = 2

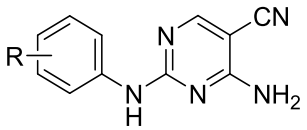
Table 4: ERK5 inhibitory activity of nicotinonitrile series **14a,15l, 17,19-21, 23, 28 and 31-32**.



Compound	R	R^1	R^2	ERK5 IC_{50} (μM)	
				HTS	Resynthesized[a]
14a	H	Ph	4-F-Ph	-	111 ± 6.5
15l		Ph	4-F-Ph	-	20.9 ± 1.6[c]
17		Ph	4-F-Ph	-	>120
19		Ph	4-F-Ph	31	29.4 ± 3.7[c]
20		Ph	4-F-Ph	-	117 ± 18
21		Ph	4-F-Ph	-	>120
23a		Ph	4-F-Ph	-	>120[d]
23b		Ph	4-F-Ph	-	>120[d]
28		Ph	4-F-Ph	-	>120
31		Ph	4-F-Ph	0.4	20.5 ± 1.3
32		Ph	4-Py	-	104.9 ± 6.5

a) Values are the mean of at least 3 determinations ± SD b) n = 1; c) n = 2; d)precipitation observed at 1.2 mM in 40% DMSO;

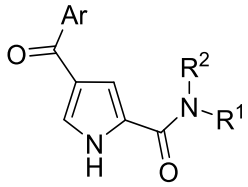
Table 5: ERK5 inhibitory activity of pyrimidine series **5a-c**.

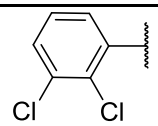
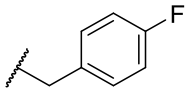
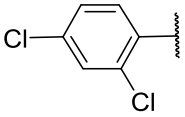
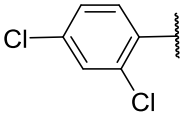
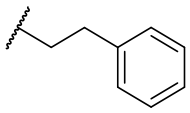
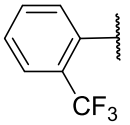
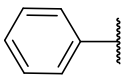
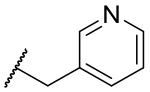


Compound	R	ERK5 IC_{50} (μM)	
		HTS	Resynthesized[a]
5a	2-CH_3	26	88 ± 3
5b	3-OCH_3	11	23 ± 7
5c	4-F	6.5	12 ± 3

a) Values are the mean of at least 3 determinations ± SD

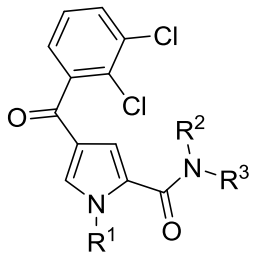
Table 6 : ERK5 inhibitory activity of pyrrole carboxamides (**6a-e**)

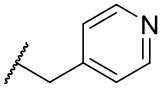
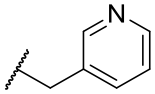
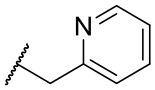
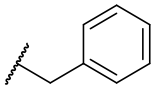
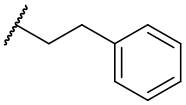
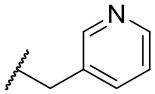
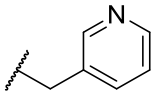


Compound	Ar	R^1	R^2	ERK5 IC_{50} (μM)	
				HTS	Resynthesized[a]
6a			H	0.66	3.7
6b		CH_3	CH_3	1.89	>120[b]
6c			H	3.50	>120[b]
6d		CH_3	H	4.32	9.6 ± 3.9
6e			H	8.0	26.0 ± 1.2

a) Values are the mean of at least 3 determinations ± SD; b) n = 2

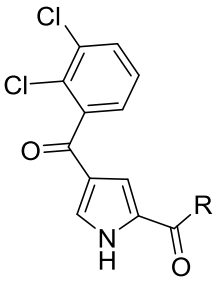
Table 7. ERK5 SAR for pyrrole carboxamides (**6f-n**)

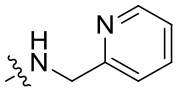
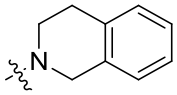
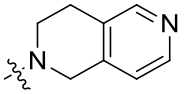
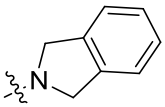
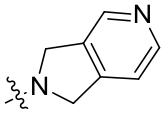


Compound	R^1	R^2	R^3	ERK5 IC_{50} (μM)	
				IMAP[a]	LANCE[a]
6f	H	H	CH_3	3.3 ± 1.0[b]	3.6 ± 1.0
6g	H	CH_3	CH_3	24 ± 27[c]	44 ± 24
6h	H	H		2.0 ± 2.0[d]	-
6i	H	H		3.8 ± 3.7[c]	1.1 ± 0.4[c]
6j	H	H		7.2 ± 0.02	3.9 ± 0.8
6k	H	H		21[e]	-
6l	H	H		>120	-
6m	CH_3	H		>120	-
6n	H	CH_3		25 ± 1.3	13 ± 1.9

a) determinations ± standard deviation (mean of n = 2 unless otherwise stated); b) IC_{50} mean of n = 4; c) IC_{50} mean of n = 6; d) IC_{50} mean of n = 10; e) IC_{50} n = 1.

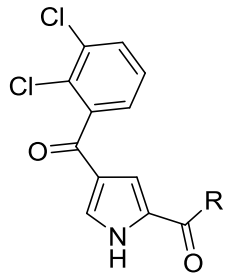
Table 8. SAR for cyclic pyrrole carboxamides (**6j, 6o-r**) against ERK5 and p38α.

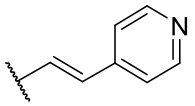
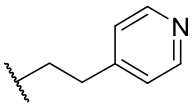
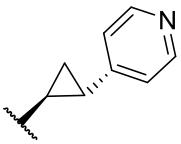
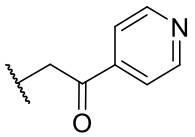


Compound ID	R	ERK5 IC50 (μM)[a]		p38α LANCE
		IMAP	LANCE	IC_{50} (μM)[a]
6j		7.2 ± 0.03		4.3 ± 1.2[b]
6o		>120	-	
6p		11 ± 2.3[b]	25 ± 1.8	28 ± 20
6q		>120	-	
6r		2.7 ± 0.4	-	> 120

a) determinations ± standard deviation (mean of n = 2 unless otherwise stated); b) IC_{50} mean of n = 4.

Table 9. ERK5 SAR for pyrroles (**39**-**42**, **44**).



Compound ID	R	ERK5 IC_{50} (μM)[a]	
		IMAP	LANCE
39	CH_3	>120	-
40		3.1 ± 0.1[b]	-
41		23 ± 4.4	24 ± 2.2
42		6.8 ± 2.0	16 ± 5.7[c]
44		>120	-

a) determinations ± standard deviation (mean of n = 2 unless otherwise stated); b) mean of n = 4; c) mean of n = 6

FIGURES

Figure 1: Crystal structure of 3-(pyrrolidin-1-ylsulfonyl)-4-thiocyanatobenzenamine **12a**.

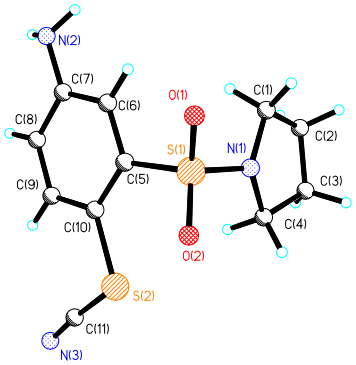


Figure 2: Crystal structure of 6-(pyrrolidine-1-sulfonyl)-benzothiazol-2-ylamine **3a**.

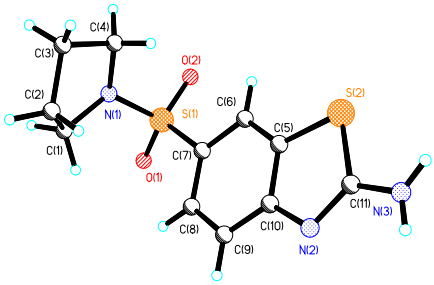
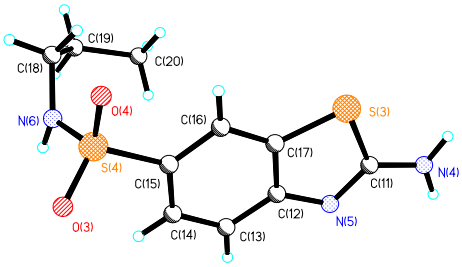
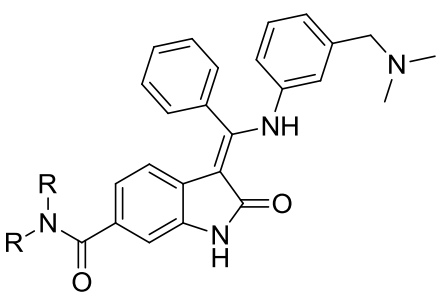


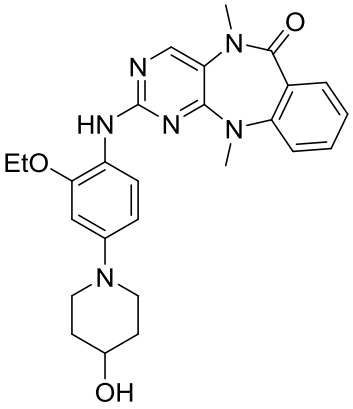
Figure 3: Crystal structure of 2-amino-*N*-propylbenzo[*d*]thiazole-6-sulfonamide **3i**.



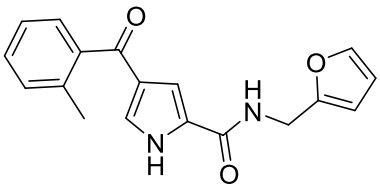
STRUCTURES



1a R = H, **1b** R = Me



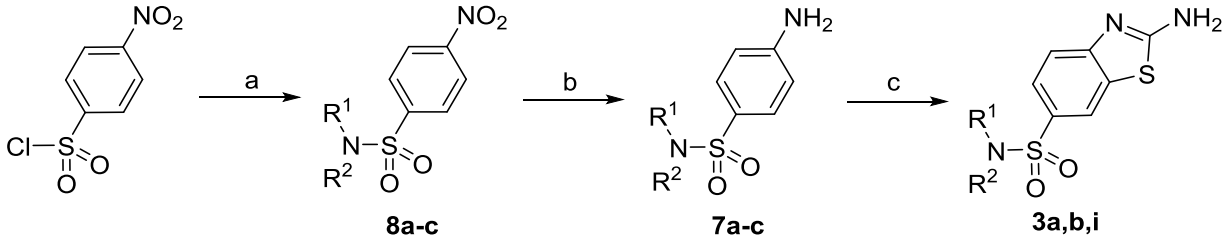
2



45

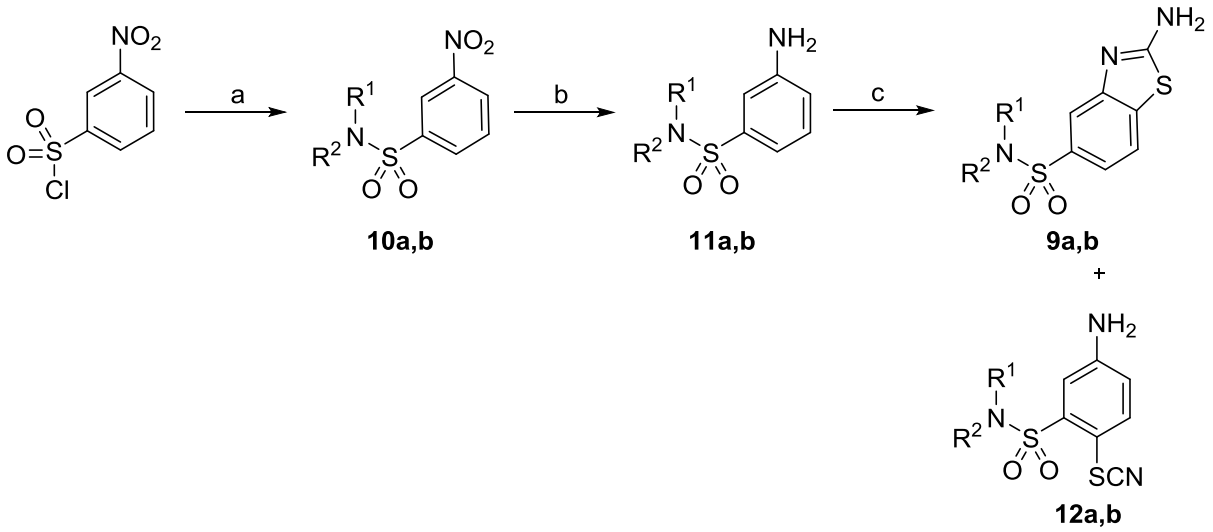
SCHEMES

Scheme 1.



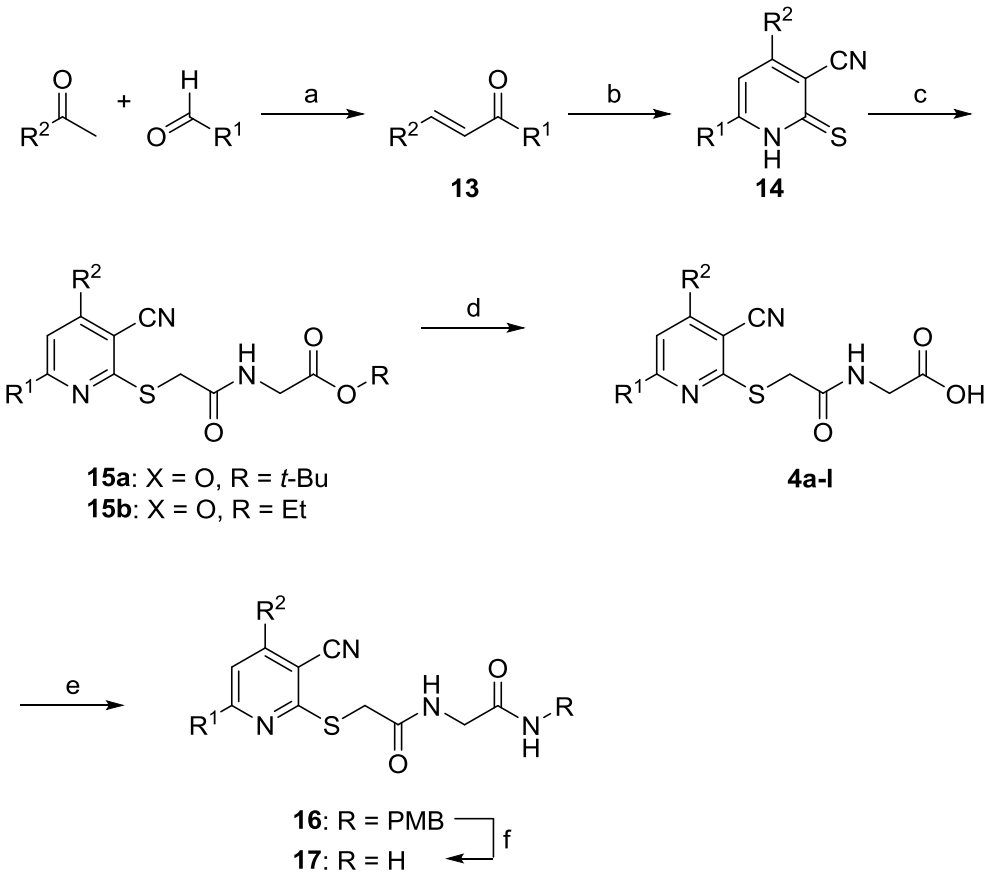
Reagents and conditions: a) pyrrolidine or *N*-methylethylamine or propylamine, Et_3N, DCM; b) Pd/C, H_2, EtOAc; c) KSCN, Cu(II)SO_4, MeOH.

Scheme 2.



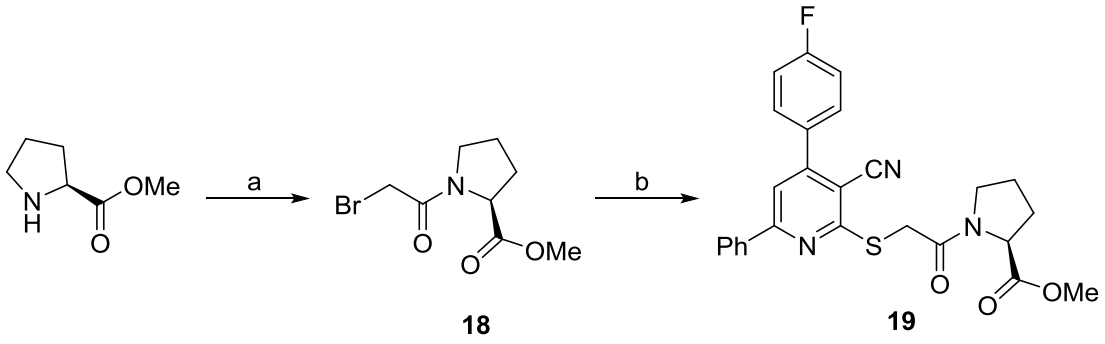
Reagents and conditions: a) pyrrolidine or *N*-methylethylamine, Et_3N, DCM; b) Pd/C, H_2, EtOAc; c) KSCN, Cu(II)SO_4, MeOH.

Scheme 3



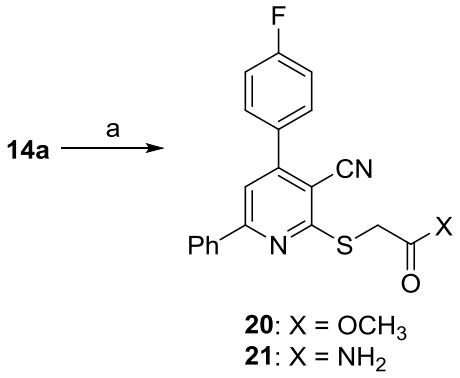
Reagents and Conditions: a) KOH, EtOH, RT; b) Method A, S_8, morpholine, EtOH, 80 ^oC 30 min then malononitrile; or Method B, 2-cyanothioacetamide, 1.6 M NaOMe in MeOH, 80 ^oC; c) *tert*-butyl or ethyl 2-(2-bromoacetamido)acetate, K_2CO_3 or KOH, DMF, 100 ^oC; d) TFA, RT; e) *p*-methoxybenzylamine, HBTU, DIPEA, DMF, 60 ^oC; f) TFA, 70 ^oC. NB: No base was required in step c after step b (Method B), as an excess of NaOMe was used in step b

Scheme 4



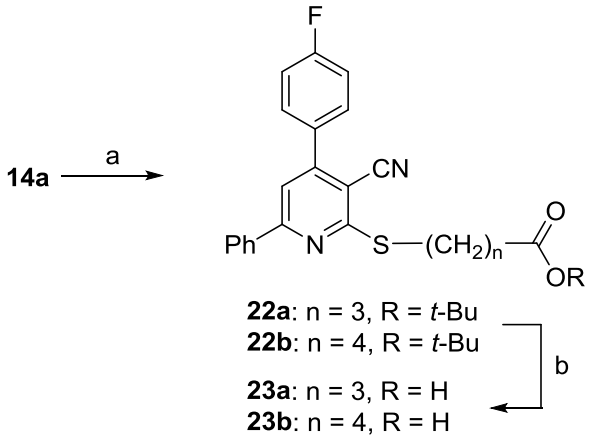
Reagents and Conditions: a) bromoacetyl chloride, $CaCO_3$, $CHCl_3$, H_2O, 0 ^oC; b) **14a**, KOH, DMF, reflux.

Scheme 5



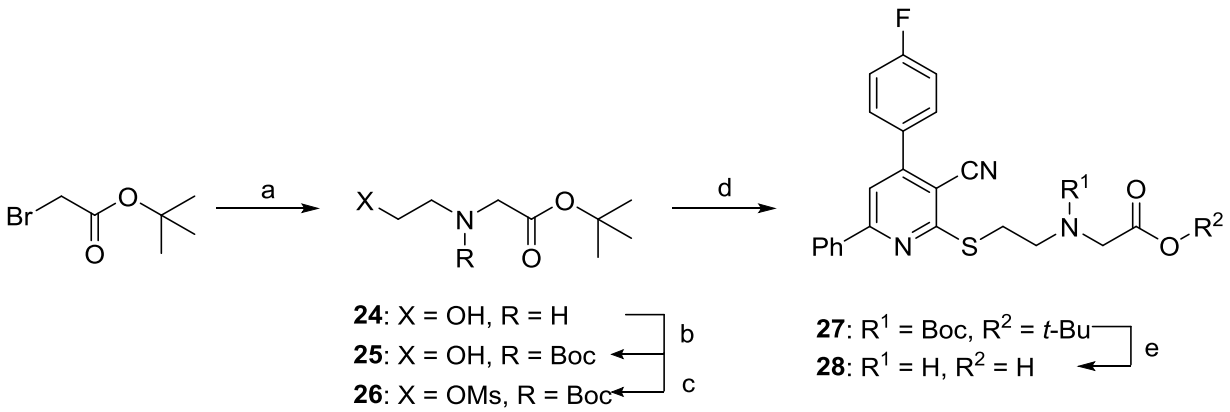
Reagents and Conditions: a) methyl bromoacetate, KOH, DMF, reflux, or chloroacetamide, $NaOAc.3H_2O$, ethanol, reflux.

Scheme 6



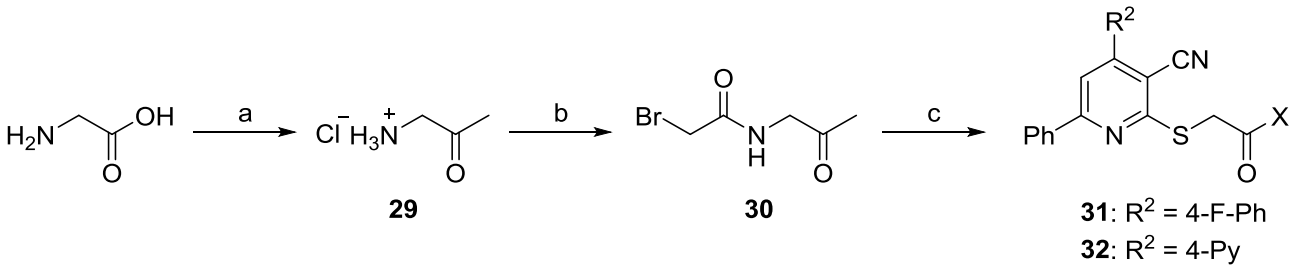
Reagents and Conditions: a) RBr, K_2CO_3, THF, 100 ^oC; b) TFA, RT.

Scheme 7



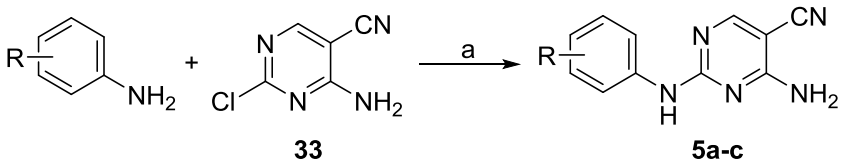
Reagents and Conditions: a) ethanolamine, RT; b) Boc_2O, Et_3N, DCM, 0 ^oC-RT; c) MsCl, Et_3N, DCM, 0 ^oC-RT; d) **14a**, DMF, 100 ^oC; e) TFA, RT.

Scheme 8



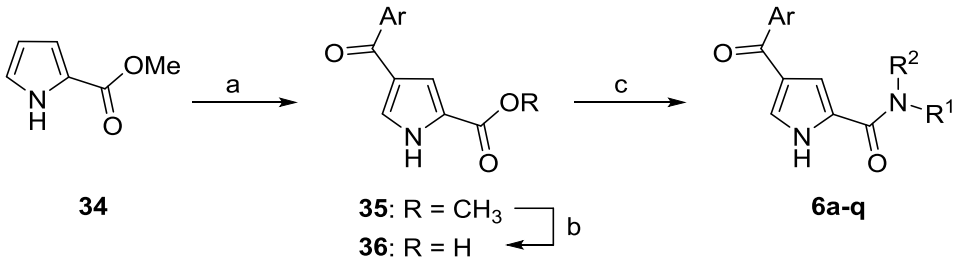
Reagents and Conditions: a)i) Ac_2O, pyridine, reflux; ii) HCl, H_2O, reflux; b) bromoacetyl chloride, $CaCO_3$, DCM, reflux; c) **14a** or **14g**, K_2CO_3, DMF, 100 °C

Scheme 9



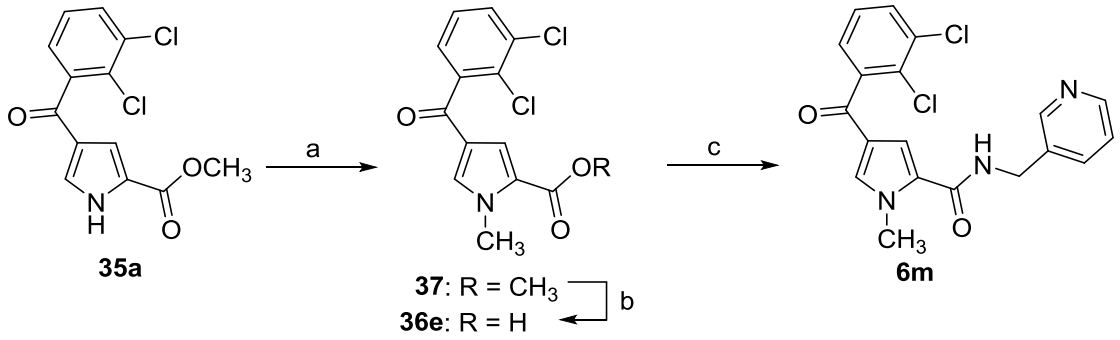
Reagents and Conditions: a) DMF, 100 °C.

Scheme 10



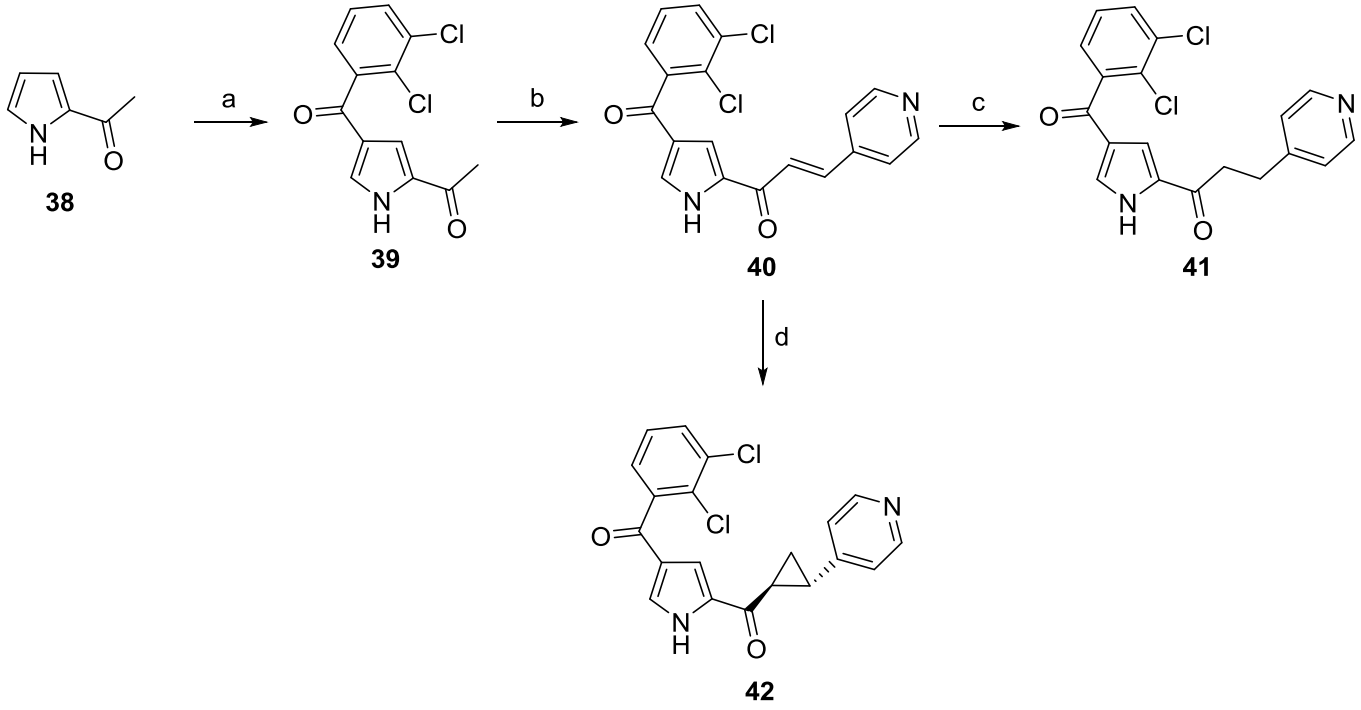
Reagents and Conditions: a) ArCOCl, $AlCl_3$, DCM, 0 °C-RT; b) LiOH, THF, H_2O, 60 °C; c) i) CDI, THF, 70 °C; ii) R^1R^2NH, 50 °C - RT.

Scheme 11



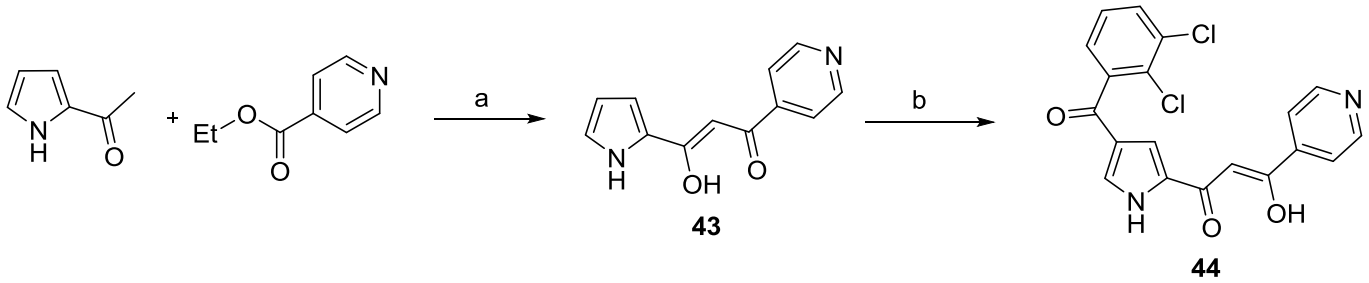
Reagents and Conditions: a) NaH, DMF, MeI; b) LiOH, THF, H_2O, 60 ^oC; c) i) CDI, THF, 70 ^oC; ii) 3-pyridylmethylamine, 50 ^oC - RT.

Scheme 12



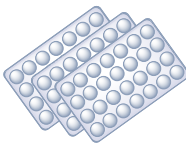
Reagents and Conditions: a) $AlCl_3$, 2,3-dichlorobenzoyl chloride, DCM, 0 ^oC-RT, 18 h.; b) Isonicotinaldehyde, KOH, EtOH, H_2O, 0 ^oC–RT, 18 h.; c) Indium powder, NH_4Cl, EtOH, H_2O, reflux, 8 h; d) $(CH_3)_2SO^+I^-$, KO^{t}Bu, DMSO, RT, 24 h.

Scheme 13



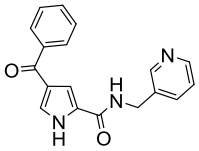
Reagents and Conditions: a) KO^{t}Bu, THF, RT, 6 h. b) $AlCl_3$, 2,3-dichlorobenzoyl chloride, DCM, 0 °C-RT, 18 h.

TOC graphic



57,617 member library

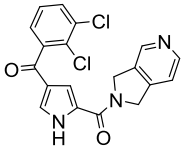
ERK5 IMAP HTS



6e

ERK5 HTS IC_{50}= 8.0 uM

ERK5 Hit Expansion



6p

ERK5 IC_{50} = 2.7 uM

p38 alpha = >120 uM

SUPPORTING INFORMATION

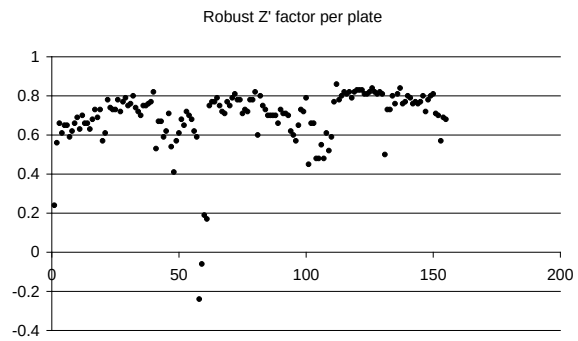
High-throughput screening and hit validation of extracellular-related kinase 5 (ERK5) inhibitors.

Stephanie M. Myers, Ruth H. Bawn, Louise C. Bisset, Timothy J. Blackburn, Betty Cottyn, Lauren Molyneux, Ai-Ching Wong, Celine Cano, William Clegg, Ross. W. Harrington, Hing Leung, Laurent Rigoreau, Sandrine Vidot, Bernard T. Golding, Roger J. Griffin, Tim Hammonds, David R. Newell, Ian R. Hardcastle*

Screening

Z' factors for each plate were calculated using Equation 2, and were typically 0.6-0.8. Plates with Z' factors below 0.4 were re-screened.

A:



B:

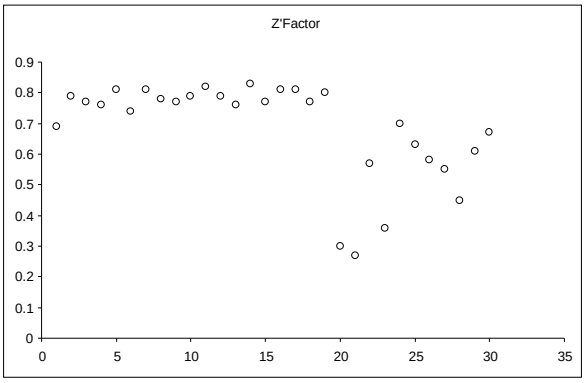


Figure S1: A) Z' factors for diversity library; b) Z' factors for kinase focussed library

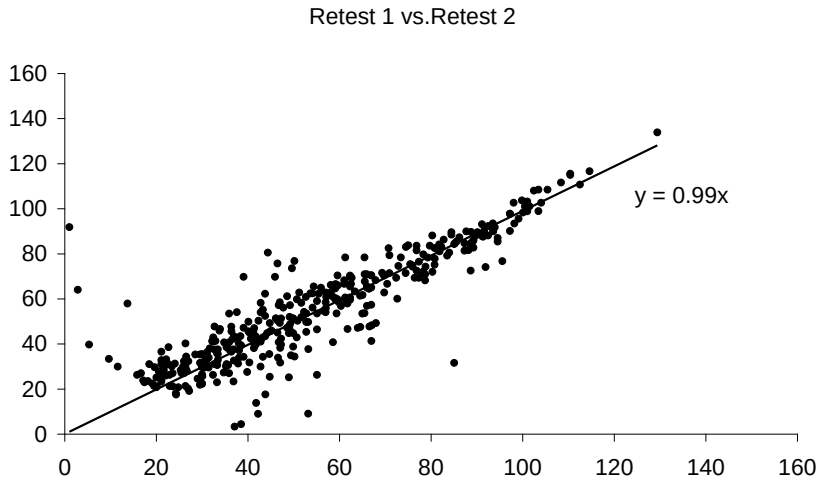


Figure S2: ERK5 retest data

ERK5 IC_{50} Determination (LANCE)

Plates were read on a Pherastar microplate reader (BMG Labtech).

5 x Assay buffer was prepared freshly: 250 mM Tris pH 7.5, 25 mM $MgCl_2$, 2.5 mM EGTA, 10 mM DTT, 0.05% Triton x 100. ERK5 (In-house preparation, co-expressed with MEK5) 2.3 uM. Substrate- Perkin Elmer, U*light*-MBP Peptide, product number TRF0109, 5 nmoles (stock concentration of 5 uM). Antibody- Perkin Elmer, Europium-anti-phospho-MBP antibody. Stock concentration of 0.625 uM. Lance Detection Buffer- 10 x stock concentration. Used at 1 x final concentration. Adenosine 5'-triphosphate disodium salt. EDTA.

Assay buffer concentrations were 50 mM Tris pH 7.5, 5 mM $MgCl_2$, 0.5 mM EGTA, 2 mM DTT, 0.01% Triton x 100. Enzyme and Substrate/ATP reaction mix was made up in equivalent of 1 x buffer. ERK5 working solution 1.44 uM, 15 nM. Made up to 2 x working stock concentration of 30 nM. Prepared in 1 x Assay Buffer. For 1 plate, added 52 μl of ERK5, 500 μl of 5 x Assay Buffer and 1948 μl of H_2O and 0.5 ml of No Protein negative control- 100 μl Assay Buffer, 400 μl H_2O. Substrate/ATP working solution: Km for ATP is 350 uM. Substrate final concentration 50 nM. 2.5 x working stock solution of ATP and Substrate mix - 875 μM ATP working solution and 125 nM Substrate. For 1 plate, added 17.5 μl of 100 mM ATP stock + 50 μl of Substrate + 400 μl of 5 x Assay Buffer + 1532.5 μl of H_2O. EDTA/Antibody Detection Reagent: Prepared a 2 x working stock of EDTA/Antibody mix, final concentrations in assay of 2 nM antibody and 5 mM EDTA. Stock concentrations of 0.625 uM and 0.5 M for Antibody and EDTA respectively. Diluted detection reagent in LANCE detection buffer at a working stock concentration of 1 x from 10 x. For 1 plate, added 84 μl of EDTA + 27 μl of Antibody + 420 μl of LANCE Detection Buffer + 3669 μl of H_2O.

Dry spotted 1 μl of compound in 20% DMSO to test wells, or 20% DMSO to blanks and controls into the assay plate using a MATRIX PlateMate® Plus. Added 5 μl of ERK5 working solution to test and control wells and 5 μl of no protein negative control solution to blanks using a Thermo Multidrop Combi or Matrix multichannel pipette. Added 4 μl of Substrate/ATP working solution to all wells using a Thermo Multidrop Combi or Matrix multichannel pipette. Incubated for 2 hours at 37 ^oC. Added 10 μl of EDTA/Antibody to all wells using a Thermo Multidrop Combi or Matrix multichannel pipette. Incubated for 2 hours at room temperature in the dark then read on the Pherastar plate reader.

p38 alpha IC_{50} Determination (LANCE)

The p38 LANCE assay protocol was carried out as described for the ERK5 LANCE assay protocol using the same quantities and concentrations unless stated below. Km for ATP is 350 μM, as was determined for ERK5. p38α/SAPK2a, active N-terminal GST-tagged recombinant full length protein (Millipore, Product # 14-251). Supplied at 10 μg/4 μl was diluted down to 10 μg/40 μl by addition of 156 μl of 50 mM Tris/HCL pH 7.5, 150 mM NaCl, 0.1 mM EGTA, 0.03% Brij-35, 50% glycerol and 0.1% 2-mercaptoethanol.

Synthesis

*Benzothiazole Series **3***

Reactions of **11a,b** with with potassium thiocyanate-copper(II) sulfate gave in each case, besides the desired 5-substituted benzothiazole **9a,b**, a significant quantity of a thiocyanatobenzene (**12a,b**). This can be rationalised by postulating the formation of the electrophilic species $^{+}$SCN from KSCN-$CuSO_4$, which attacks the aniline primarily at available *ortho* and *para* positions. The former leads, by cyclisation of an intermediate thiocyanatobenzene, to a benzothiazole, whereas the latter mode of attack is arrested at the thiocyanatobenzene.

The sequence described above is well exemplified by 3-(pyrrolidin-1-ylsulfonyl)benzenamine **11a**, which gave 3-(pyrrolidin-1-ylsulfonyl)-4-thiocyanatobenzenamine (**12a**) and (pyrrolidin-1-ylsulfonyl)benzo[*d*]thiazol-2-amine (**9a**). Structural identification by ^{1}H NMR was initially ambiguous because both compounds are 1,2,4-trisubstituted benzenes and have a similar set of coupling constants. However, the structural assignment to **12a** could be secured by crystal structure analysis (Supporting information: Figure S1). Further, the infrared spectrum of **12a** showed ν_{max} 2155 cm^{-1}, whereas the accompanying compound was silent in the absorption region for the SCN group and is therefore **9a**. The structures of the benzothiazoles **3a** and **3i** were also validated by X-ray analysis (Supporting information: Figures S2 and S3). The reactions leading to **3a-c** only afforded benzothiazoles because there was only one intermediate thiocyanate in each case, i.e. with the SCN group *ortho* to the initial amino function.

*Nicotinonitrile Series **4***

Alkylation of **14a** with *tert*-butyl 2-(2-bromoacetamido)acetate gave **15a**, leading to **4a** after deprotection using TFA. Similarly, reaction of **14a** with ethyl 2-(2-bromoacetamido)acetate gave the ethyl ester **15l**. For **4g**, 4-pyridylchalcone **13g** was prepared *via* Wittig reaction of 4-pyridine carboxaldehyde with (benzoylmethylene)triphenylphosphorane, following failure of the Claisen-Schmidt condensation (Scheme not shown).

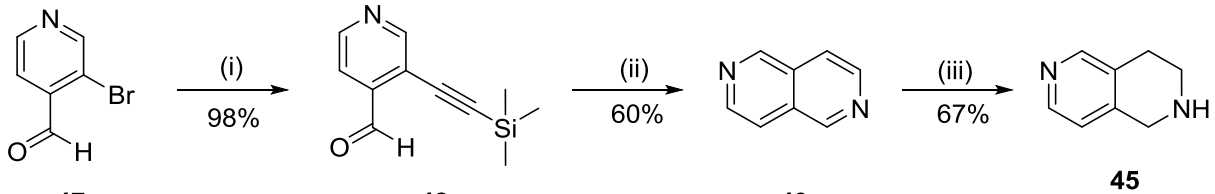
The proline derivative **19** was prepared by reaction of proline methyl ester with bromoacetyl chloride giving **18**, which was reacted with **14a** as previously described (Scheme 4).

Further variations to the thioether group were introduced *via* alkylation of **14a**. The shorter carboxamides **20** and **21** were prepared by alkylation of **14a** with chloroacetamide and methyl-2-bromoacetate, respectively (Scheme 5). Alkylation of **14a** with either *t*-butyl 4-bromobutanoate or *t*-butyl 5-bromopentanoate, followed by treatment with TFA gave the simplified alkylcarboxylic acid derivatives **23a** and **b**, respectively (Scheme 6). Similarly, the aminoacid derivative **28** resulted from the alkylation of **14a** with the protected mesylate of *N-(*2-hydroxyethyl)glycine **26** giving **27**, followed by deprotection to **28**. Mesylate **26** was obtained by reaction of *t*-butyl bromoacetate with ethanolamine giving **24**, followed by sequential Boc protection and mesylation (Scheme 7).

The acetamide derivatives **31** and **32** were prepared by the alkylation of **14a** and **14g**, respectively, with bromoacetamide **30** which was obtained by reaction of aminoacetone hydrochloride **29** with bromoacetylchloride (Scheme 8).[1]

*4-Benzoylpyrrole-2-carboxamide Series **6***

To prepare derivative **6p** pyridyl amines **45** prepared as reported (**Scheme S1**).[2] Starting from 3-bromoisonicotinaldehyde **47**, a Sonagashira reaction with ethynyltrimethylsilane afforded 3-((trimethylsilyl)ethynyl)isonicotinaldehyde **48** in 98% yield. Cyclisation of **48** in the presence of ammonia afforded 2,6-naphthyridine **49**. Selective reduction using platinum dioxide and calcium oxide in 2-methoxyethanol afforded amine **45** in 67% yield. CDI mediated amide coupling between carboxylic acid **36a** and amine **45** gave the target compound **6p** in 55% yield.



47 **48** **49** **45**

Scheme S1. (i) $PdCl_2(PPh_3)_2$, DABCO, CuI, THF, RT, 24 h. (ii) 2.0 M NH_3 in EtOH, 80 °C, 2 h. (iii) PtO_2, CaO_2, H_2, 2-methoxyethanol, RT, 16 h.

Crystal Structures

The principal aim of the crystallographic studies was a definitive identification of the compounds. The detailed molecular structures and crystallographic features require only brief comments, as bond lengths, angles and conformations are all normal and full results are provided. **12a** has only one molecule in the asymmetric unit; there are no π-π ring stacking interactions, and intermolecular NH…OS hydrogen bonds generate sheets of molecules (Figure S4). **3a** and **3i** are both benzothiazoles with the ring system substituted by an NH_2 group and by a sulfonyl group bearing either a secondary (pyrrolidine, **3a**) or a primary (*n*-propylamine, **3i**) amine substituent. **3a** has one molecule in the asymmetric unit, from which centrosymmetric π-stacked dimers are formed; centrosymmetric dimer assembly also occurs through pairs of NH…N(thiazole) intermolecular hydrogen bonds (Figure S5), while NH…OS hydrogen bonds involving just one of the two sulfonyl O atoms link these dimers together in approximately planar ribbons from which pyrrolidine substituents protrude on both sides. **3i** has two molecules with very similar conformations in the asymmetric unit, and there is no π-stacking at all in this structure; it contains the same type of hydrogen-bonded dimers as **3a** (Figure S6), though here they are formed by pairs of crystallographically independent molecules and there is no precise inversion symmetry (it is only approximate), and the availability of an extra (sulfonamide) NH group means that all O atoms act as hydrogen bond acceptors in a complex three-dimensional network.

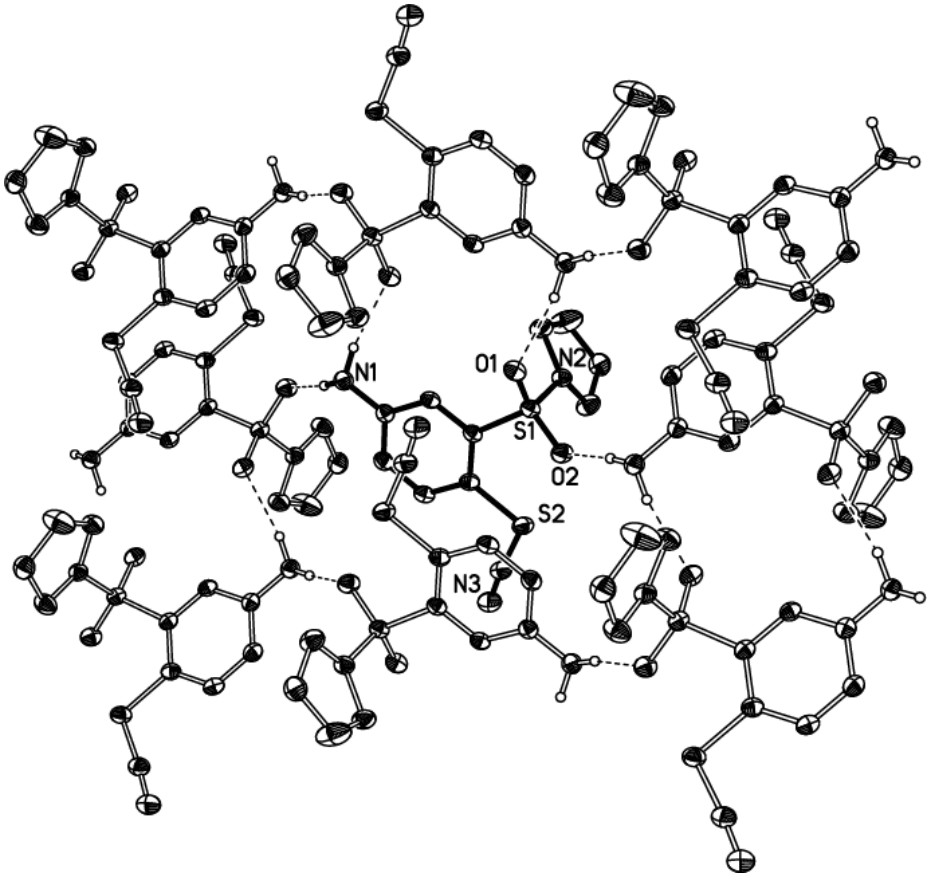


Figure S4. A hydrogen-bonded sheet of molecules of 3-(pyrrolidin-1-ylsulfonyl)-4-thiocyanatobenzenamine **12a**. C-bound H atoms have been omitted for clarity. Here and in other Figures atoms are shown as 40% probability displacement ellipsoids.

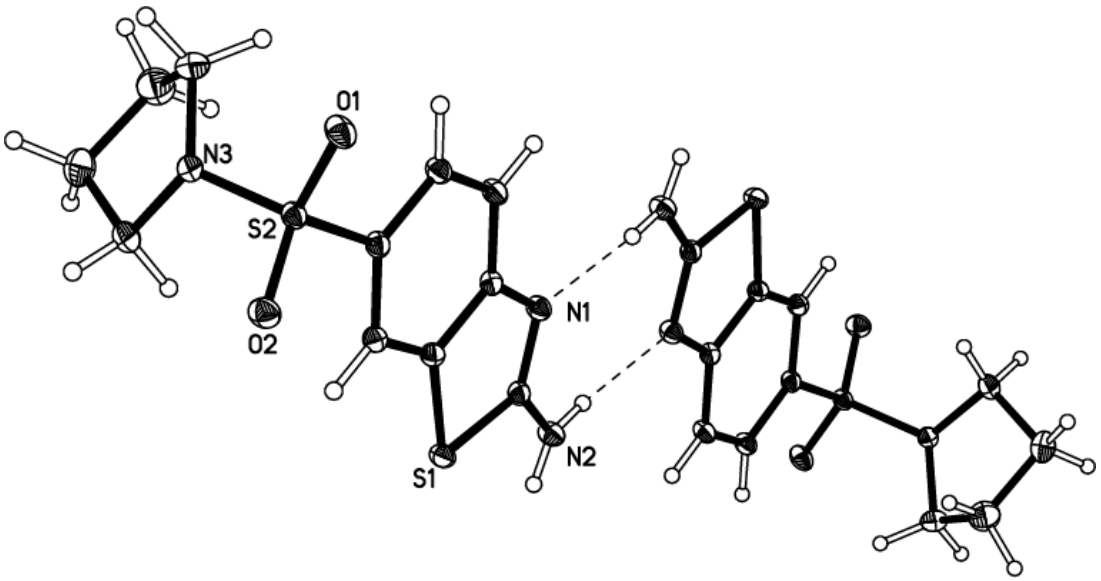


Figure S5. A hydrogen-bonded centrosymmetric dimer of 6-(pyrrolidine-1-sulfonyl)-benzothiazol-2-ylamine **3a**.

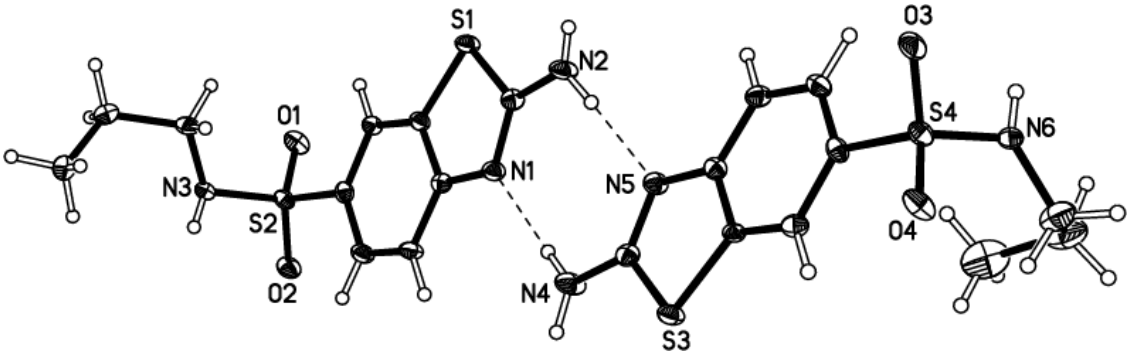


Figure S6. The approximately centrosymmetric hydrogen-bonded dimer of 2-amino-*N*-propylbenzo[*d*]thiazole-6-sulfonamide **3i**.

Experimental Section

General Methods

Reagents were purchased from fine chemicals vendors, and used as received unless otherwise stated. Solvents were purified and stored according to standard procedures. Petrol refers to that fraction in the boiling range 40-60 °C. THF refers to anhydrous tetrahydrofuran, either by distillaton from sodium benzophenone, or from commercial sources. Melting points were obtained on a Stuart Scientific SMP3 apparatus and are uncorrected. Thin layer chromatography was performed using silica gel plates (Kieselgel 60F254; 0.2 mm), and visualized with UV light or potassium permanganate. Chromatography was conducted under medium pressure in glass columns or using a Biotage SP4 instrument in prepacked columns (FLASH+ Silica columns (40-63 μm, 60 Å). Proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Spectrospin AC 300E (^{1}H at 300 MHz, ^{13}C at 75 MHz), a Jeol JNM-LA500 spectrometer (^{1}H at 500 MHz, ^{13}C at 125 MHz), or a Bruker Avance II 500 (^{1}H at 500 MHz, ^{13}C at 125 MHz) employing the solvent as internal standard. IR spectra were recorded on a Bio-Rad FTS 3000MX diamond ATR. Liquid Chromatography-Mass Spectrometry (MS) was carried out on a Micromass Platform instrument operating in positive and negative ion electrospray mode, employing a 50 x 4.6 mm C18 column (Waters Symmetry or Waters Atlantis) 5 or 12 min gradient elution with 0.05% formic acid in methanol (10-90%). Elemental analyses were performed by The School of Pharmacy, Analytical Facility, University of London, WC1N 1AX. Accurate masses were measured using a Finnigan MAR 95 XP or a Finnigan MAR 900 XLT at the EPSRC National Mass Spectrometry Service Centre (Chemistry Department, University of Wales, Swansea, Wales, SA2 8PP).

6-(Pyrrolidin-1-ylsulfonyl)benzo[*d*]thiazol-2-amine (**3a**)[3]

A suspension of 4-(pyrrolidin-1-ylsulfonyl)aniline (410 mg, 1.8 mmol), KSCN (4.43 g, 45 mmol) and anhydrous Cu(II)SO_4 (3.53 g, 23 mmol) in MeOH (9 mL) was heated at reflux for 3 h, then cooled and filtered. The filtrate was diluted with water (5 mL/mmol) and heated to reflux and EtOH (11 mL/mmol) added. The reaction mixture was cooled, filtered and concentrated *in vacuo*. The residue was diluted with water (5 mL/mmol), basified to pH 11 with aq. conc. ammonia and the product dissolved into EtOAc (5 × 10 mL/mmol). The combined organic layers were washed with aq. NH_4Cl (2 × 25 mL/mmol), followed by brine (25 mL/mmol), dried ($MgSO_4$) and concentrated *in*

vacuo. Chromatography (silica gel, 1:1 EtOAc:petrol) gave **3a** as an off white solid (480 mg, 94%): R_f = 0.40 (EtOAc); mp: 248-249 °C; λ_{max} (EtOH/nm) 229, 283; IR (cm^{-1}) 3398 (N-H), 3290 (N-H), 1524 (thiazole-C=N), 1329 (S=O), 1309 (S=O), 1145 (S=O); δ_H (300 MHz, DMSO-d_6): 1.61-1.65 (4H, m, 2 × NCH_2CH_2), 3.10-3.14 (4H, m, 2 × NCH_2), 7.45 (1H, d, *J* = 8.5 Hz, ArH), 7.60 (1H, dd, *J* = 2.0 and 8.5 Hz, ArH), 7.96 (2H, s, NH_2), 8.17 (1H, d, *J* = 2.0 Hz, ArH); δ_C (75 MHz, DMSO-d_6): 25.0 (NCH_2CH_2), 48.0 (NCH_2CH_2), 117.7 (ArC), 121.1 (ArC), 125.4 (ArC), 132.1 (ArC), 156.7 (ArC), 170.3 (ArC); LC-MS (ES+) *m/z* = 284.19 $[M+H]^+$; HRMS calcd. for $C_{11}H_{14}N_3O_2S_2$ $[M+H]^+$ 284.0522, found 284.0518.

General procedure A – Synthesis of Nitrophenylsulfonamides

To a stirred solution of the amine (1 mol equiv.) and triethylamine (1.5 equiv.) in dichloromethane (2 mL/mmol amine) at 0-5 ˚C was added the nitrobenzenesulfonyl chloride (0.5 equiv.) in small portions. The resulting yellow solution was stirred at room temperature for 3 h. The reaction mixture was diluted with dichloromethane (5 mL/mmol amine), washed with aqueous sulfuric acid (1.0 M), followed by saturated aqueous sodium bicarbonate and brine (each 5 mL/mmol amine). The organic phase was dried over $MgSO_4$, filtered and concentrated. The crude product was purified by recrystallisation from ethyl acetate/petrol.

1-((4-Nitrophenyl)sulfonyl)pyrrolidine (8a)[4]

General procedure A: *p*-nitrobenzenesulfonylchloride (0.71 g, 3.2 mmol), pyrrolidine (0.68 g, 0.80 mL, 9.6 mmol) and triethylamine (0.97 g, 1.33 mL, 9.6 mmol) in DCM (6 mL). Recrystallisation from EtOAc gave **6a** as an off white solid (800 mg, 98%): R_f = 0.48 (1:1 EtOAc:petrol); mp: 159-160 ˚C; λ_{max} (EtOH/nm) 273; IR (cm^{-1}) 1472 (N=O), 1347 (S=O), 1300 (S=O); δ_H (300 MHz, $CDCl_3$): 1.77-1.85 (4H, m, 2 × NCH_2CH_2), 3.27-3.31 (4H, m, 2 × NCH_2), 7.99-8.04 (2H, m, 2 × ArH), 8.36-8.40 (2H, m, 2 × ArH); δ_C (75 MHz, $CDCl_3$): 25.7 (NCH_2CH_2), 48.3 (NCH_2CH_2), 124.5 (ArCH), 128.8 (ArCH), 144.2 (ArC), 150.6 (ArC); LC-MS (ES+) *m/z* = 256.15 $[M+H]^+$.

***N*-Ethyl-*N*-methyl-4-nitrobenzenesulfonamide (8b)**

General procedure A. (0.35 g, 72%); mp 122-123 ˚C; δ_H (300 MHz, $CDCl_3$) 1.10 (3H, t, *J* = 7.2 Hz, CH_3CH_2), 2.74 (3H, s, CH_3N), 3.09 (2H, q, *J* = 7.2 Hz, CH_3CH_2), 7.89-7.93 (2H, m, 2 × H-Ar), 8.29-8.33 (2H, m, 2 × H-Ar); δ_C (75.5 MHz, $CDCl_3$) 11.6, 32.5, 43.6, 122.8, 126.9, 143.4, 148.8; MS (ES+) *m/z* = 243.20 $[M-H]^-$.

4-Nitro-*N*-propylbenzenesulfonamide (8c)

General procedure A. (0.41 g, 85%); mp 88-89˚C; δ_H (300 MHz, $CDCl_3$) 0.82 (3H, t, *J* = 7.5 Hz, CH_3CH_2), 1.39-1.52 (2H, m, CH_3CH_2), 2.89-2.96 (2H, m, CH_2NH), 4.61 (1H, t, *J* = 6.0 Hz, NH), 7.96-8.02 (2H, m, 2 × H-Ar), 8.28-8.33 (2H, m, 2 × H-Ar); δ_C (75.5 MHz, $CDCl_3$) 11.2, 23.5, 45.6, 124.6, 128.6, 146.9; MS (ES+) *m/z* = 243.16 $[M-H]^-$

1-(3-Nitrophenylsulfonyl)pyrrolidine (10a)

General procedure A. (1.52 g, 89%); mp 103-104 ˚C (lit mp 99-100˚C);[17] δ_H (300 MHz, $CDCl_3$) 1.74-1.80 (4H, m, 2 × NCH_2CH_2), 3.22-3.26 (4H, m, 2 × NCH_2), 7.70 (1H, dd, J = 8.0, 8.0 Hz, H-Ar), 8.08-8.12 (1H, m, H-Ar), 8.37-8.41 (1H, m,

H-Ar), 8.60 (1H, dd, *J* = 1.8, 1.8 Hz, H-Ar); δ_C (75.5 MHz, $CDCl_3$) 25.7, 48.4, 122.7, 127.1, 130.6, 133.0, 140.9, 149.0; MS (ES+) *m/z* = 256.43 $[M+H]^+$.

_N_-Ethyl-_N_-methyl-3-nitrobenzenesulfonamide (10b)

General procedure A. (0.97 g, 89%); mp 57-58 ˚C; δ_H (300 MHz, $CDCl_3$) 1.11 (3H, t, *J* = 7.0 Hz, CH_3CH_2), 2.75 (3H, s, CH_3N), 3.12 (2H, q, *J* = 7.0 Hz, CH_3CH_2), 7.69 (1H, dd, *J* = 8.0, 8.0 Hz, H-Ar), 8.06 (1H, d, *J* = 8.0 Hz, H-Ar), 8.37 (1H, d, *J* = 8.0 Hz, H-Ar), 8.56 (1H, d, *J* = 1.5 Hz, H-Ar); δ_C (75.5 MHz, $CDCl_3$) 13.4, 34.3, 45.4, 122.6, 127.1, 130.6, 132.9, 141.6, 149.0; MS (ES+) *m/z* = 243.81 $[M-H]^-$.

General procedure B – Synthesis of Aminophenylsulfonamides

To the nitrophenylsulfonamide (1 mol equiv.) in ethyl acetate (12 mL/mmol), was cautiously added palladium, 10 wt% on activated carbon (0.1 equiv.) and the reaction was stirred under H_2 for 18 h. The resulting mixture was filtered through Celite and concentrated *in* vacuo to yield the product.

4-(Pyrrolidin-1-ylsulfonyl)benzenamine (7a)

General procedure B. (0.32 g, 91%); mp 167-168 ˚C (lit mp 167.5-168˚C);[15] δ_H (300 MHz, $CDCl_3$) 1.65-1.70 (4H, m, 2 × NCH_2CH_2), 3.11-3.15 (4H, m, 2 × NCH_2), 4.04 (2H, br s, NH_2), 6.61-6.65 (2H, m, 2 × H-Ar), 7.53-7.56 (2H, m, 2 × H-Ar); δ_C (75.5 MHz, $CDCl_3$) 25.5, 48.1, 114.4, 129.8, 130.0, 150.2; MS (ES+) *m/z* = 227.25 $[M+H]^+$.

4-Amino-_N_-ethyl-_N_-methylbenzenesulfonamide (7b)

General procedure B. (0.24 g, 94%); mp 81-82 ˚C; δ_H (300 MHz, $CDCl_3$) 1.05 (3H, t, *J* = 7.2 Hz, CH_3CH_2), 2.62 (3H, s, CH_3N), 2.98 (2H, q, *J* = 7.2 Hz, CH_2CH_3), 4.06 (2H, br s, NH_2), 6.60-6.64 (2H, m, 2 × H-Ar), 7.46-7.51 (2H, m, 2 × H-Ar); δ_C (75.5 MHz, $CDCl_3$) ppm 13.3, 34.2, 45.1, 114.4, 129.8, 150.8; MS (ES+) *m/z* = 215.25 $[M+H]^+$;

4-Amino-_N_-propylbenzenesulfonamide (7c)

General procedure B. (0.28 g, 95%); mp 83-84 ˚C (lit mp 85˚C);[16] δ_H (300 MHz, $CDCl_3$) 0.80 (3H, t, *J* = 7.2 Hz, CH_3CH_2), 1.35-1.47 (2H, m, CH_3CH_2), 2.77-2.84 (2H, m, CH_2NH), 4.05 (2H, br s, NH_2), 4.23 (1H, t, *J* = 6.0 Hz, NH), 6.59-6.64 (2H, m, 2 × H-Ar), 7.54-7.59 (2H, m, 2 × H-Ar); δ_C (75.5 MHz, $CDCl_3$) 11.3, 23.3, 45.3, 114.5, 129.6, 150.7; MS (ES+) *m/z* = 215.24 $[M+H]^+$;

3-(Pyrrolidin-1-ylsulfonyl)benzenamine (11a)

General procedure B. (1.26 g, 93%); mp 157-158˚C (lit mp 155-156˚C);[17] δ_H (300 MHz, $CDCl_3$) 1.67-1.71 (4H, m, 2 × NCH_2CH_2), 3.16-3.20 (4H, m, 2 × NCH_2), 3.84 (2H, br s, NH_2), 6.79 (1H, ddd, *J* = 1.2, 2.1, 7.8 Hz, H-Ar), 7.06 (1H, dd, *J* = 1.8, 2.1 Hz, H-Ar), 7.09-7.12 (1H, m, H-Ar), 7.21 (1H, dd, *J* = 7.8, 7.8 Hz, H-Ar); δ_C (75.5 MHz, $CDCl_3$) 25.6, 48.2, 113.8, 117.6, 119.0, 130.1, 138.9, 147.5; MS (ES+) *m/z* = 227.18 $[M+H]^+$.

3-Amino-_N_-ethyl-_N_-methylbenzenesulfonamide (11b)

General procedure B. (0.80 g, 89%); mp 55-56 ˚C; δ_H (300 MHz, $CDCl_3$) 1.07 (3H, t, *J* = 7.2 Hz, CH_3CH_2), 2.67 (3H, s, CH_3N), 3.03 (2H, q, *J* = 7.2 Hz, CH_3CH_2), 3.84 (2H, br s, NH_2), 6.75-6.79 (1H, m, H-Ar), 7.00 (1H, dd, *J* = 1.9, 2.0 Hz, H-Ar), 7.04-7.07 (1H, m, H-Ar), 7.21 (1H, dd, *J* = 7.8, 7.8 Hz, H-Ar); δ_C (75.5 MHz, $CDCl_3$) 13.4, 34.3, 45.2, 113.6, 117.4, 118.9, 130.1, 147.5; MS (ES+) *m/z* = 215.25 $[M+H]^+$.

General procedure C – Benzothiazole Formation[5]

To the aminophenylsulfonamide (1 mol equiv.), KSCN (25 equiv.) and anhydrous Cu(II)SO_4 (12 equiv.), was added anhydrous methanol (5 mL/mmol amine) with stirring. After boiling at reflux for 3 h the reaction mixture was cooled and filtered. The filtrate was diluted with water (5 mL/mmol amine) and heated to reflux. After addition of ethanol (11 mL/mmol), the reaction mixture was cooled and filtered. The filtrate was concentrated and diluted with water (5 mL/mmol) and basified (pH 11) with aqueous ammonia. After extraction with ethyl acetate (5 × 10 mL/mmol), the combined organic layers were washed with saturated aqueous ammonium chloride (2 × 25 mL/mmol), brine (25 mL/mmol) and dried over $MgSO_4$. The mixture was filtered and concentrated *in vacuo.* The crude product was purified by recrystallisation from ethyl acetate-petrol or by medium pressure chromatography.

2-Amino-*N*-ethyl-*N*-methylbenzo[*d*]thiazole-6-sulfonamide (3b)

General procedure C. (0.02 g, 25%); mp 179-180˚C; δ_H (300 MHz, DMSO) 1.03 (3H, t, *J* = 7.0 Hz, CH_3CH_2), 2.63 (3H, s, CH_3N) 2.97 (2H, q, *J* = 7.0 Hz, CH_2CH_3), 7.44 (1H, d, *J* = 8.4 Hz, H-Ar), 7.56 (1H, dd, *J* =1.9, 8.4 Hz, H-Ar), 7.96 (2H, br s, NH_2), 8.13 (1H, s, H-Ar); δ_C (75.5 MHz, DMSO) 13.2, 34.3, 44.8, 117.7, 120.9, 125.2, 156.6, 170.2; MS (ES+) *m/z* = 272.15 $[M+H]^+$; HRMS $[M+H]^+$ *m/z* Calc. for $C_{10}H_{14}N_3O_2S_2$: 272.0522 Found 272.0524; υ_{max} (film)/cm^{-1} 3400.2, 3311.6; UV λ_{max} 229, 283 nm (EtOH).

2-Amino-*N*-propylbenzo[*d*]thiazole-6-sulfonamide (3i)

General procedure C. (0.28 g, 95%); mp 215-216 °C; δ_H (300 MHz, DMSO) 0.78 (3H, t, *J* = 7.0 Hz, CH_3CH_2), 1.32-1.40 (2H, m, C*H*$_2$CH$_3$), 2.63-2.70 (2H, m, CH_2NH), 7.40-7.45 (2H, m, NH, H-Ar), 7.58-7.61 (1H, m, H-Ar), 7.92 (2H, br s, NH_2), 8.10 (1H, s, H-Ar); δ_C (75MHz, DMSO) 11.4, 22.8, 44.8, 117.7, 120.3, 124.7, 131.7, 133.1, 156.1, 169.9; MS (ES+) *m/z* = 272.13 $[M+H]^+$; HRMS $[M+NH_4]^+$ *m/z* Calc. for $C_{10}H_{17}N_4O_2S_2$: 289.0787 Found 289.0784; υ_{max} (film)/cm^{-1} 3375.3, 3302.1; UV λ_{max} 228, 282 nm (EtOH).

3-(Pyrrolidin-1-ylsulfonyl)-4-thiocyanatobenzenamine (12a)

General procedure C. (0.31 g, 50%); mp 99-100 ˚C; δ_H (300 MHz, DMSO) 1.82-1.87 (4H, m, 2 × NCH_2CH_2), 3.24-3.28 (4H, m, 2 × NCH_2), 6.21 (2H, br s, NH_2), 6.88 (1H, dd, *J* = 2.7, 8.4 Hz, H-Ar), 7.19 (1H, d, *J* = 2.7 Hz, H-Ar), 7.55 (1H, d, *J* = 8.4 Hz, H-Ar); δ_C (75.5 MHz, DMSO) 25.1, 48.1, 116.1, 119.5, 121.8, 136.6, 153.4, 168.7; MS (ES+) *m/z* = 284.53 $[M+H]^+$; HRMS $[M+H]^+$ *m/z* Calc.for $C_{11}H_{14}N_3S_2O_2$: 284.0522 Found 284.0523; υ_{max} (film)/cm^{-1} 3435.2, 3356.1, 2154.5; UV λ_{max} 273 nm (EtOH).

5-Amino-*N*-ethyl-*N*-methyl-2-thiocyanatobenzenesulfonamide (12b)

General procedure C. (0.25 g, 49%); mp 59-60 ˚C; δ_H (300 MHz, DMSO) 1.10 (3H, t, *J* = 7.0 Hz, CH_3CH_2), 2.80 (3H, s, CH_3N), 3.22 (2H, q, *J* = 7.0 Hz, CH_3CH_2), 6.23 (2H, br s, NH_2), 6.87 (1H, dd, *J* = 8.5, 2.4 Hz, H-Ar), 7.19 (1H, d, *J* = 2.4 Hz, H-Ar), 7.54 (1H, d, *J* = 8.5, H-Ar); δ_C (75.5 MHz, DMSO) 13.3, 33.8, 44.5, 104.0, 112.1, 115.6, 118.4, 135.6, 140.3, 151.5; MS (ES+) *m/z* = 272.05 $[M+H]^+$; HRMS $[M+H]^+$ *m/z* Calc.for $C_{10}H_{14}N_3S_2O_2$: 272.0522 Found 272.0522; υ_{max} (film)/cm^{-1} 3466.9, 3373.5, 2149.4; UV λ_{max} 273 nm (EtOH).

5-(Pyrrolidin-1-ylsulfonyl)benzo[*d*]thiazol-2-amine (9a)

General procedure C. (0.09 g, 14%); mp 262-263 ˚C; δ_H (300 MHz, DMSO) 1.15-1.20 (4H, m, 2 × NCH_2CH_2), 3.11-3.18 (4H, m, 2 × NCH_2), 7.36 (1H, dd, *J* = 1.8, 8.1 Hz, H-Ar), 7.58 (1H, d, *J* = 1.8 Hz, H-Ar), 7.86 (2H, br s, NH_2), 7.89 (1H, d, *J* = 8.1, H-Ar); δ_C (75.5 MHz, DMSO) 25.3, 47.9, 115.6, 118.7, 135.2, 151.2; MS (ES+) *m/z* = 284.25 $[M+H]^+$; HRMS $[M+H]^+$ *m/z* Calc.for $C_{11}H_{14}N_3S_2O_2$: 284.0522 Found 284.0522; υ_{max} (film)/cm^{-1} 3346.5; UV λ_{max} 273 nm (EtOH).

2-Amino-*N*-ethyl-*N*-methylbenzo[*d*]thiazole-5-sulfonamide (9b)

General procedure C. (0.08 g, 16%); mp 232-233 ˚C; δ_H (300 MHz, DMSO) 1.03 (3H, t, *J* = 7.0 Hz, CH_3CH_2), 2.65 (3H, s, CH_3N), 3.00 (2H, q, *J* = 7.0 Hz, CH_3CH_2), 7.36 (1H, dd, *J* = 1.8, 8.4 Hz, H-Ar), 7.58 (1H, d, *J* = 1.8 Hz, H-Ar), 7.86 (2H, br s, NH_2), 7.90 (1H, d, *J* = 8.4 Hz, H-Ar); δ_C (75.5 MHz, DMSO) 13.2, 34.3, 44.9, 115.9, 119.3, 121.9, 153.4, 168.7; MS (ES+) *m/z* = 272.18 $[M+H]^+$; HRMS $[M+H]^+$ *m/z* Calc.for $C_{10}H_{14}N_3S_2O_2$: 272.0522 Found 272.0525; υ_{max} (film)/cm^{-1} 3385.1, 3249.1; UV λ_{max} 237 nm (EtOH).

General procedure D[6]

To a solution of the required 2-thioxo-1,2-dihydropyridine-3-carbonitrile (1.0 eq) and the suitable α-halogen compound (1.0 eq) in dimethylformamide (20 ml) was added potassium hydroxide (1.2 eq) at 0 ^oC. The reaction mixture was heated under reflux for 24 h, allowed to cool and diluted with water. The precipitate was collected by filtration and either recrystallization (THF) or chromatography (silica; 20-100% EtOAc/petrol) gave the desired 2-pyridyl sulfide.

2-[3-Cyano-4-(4-methoxyphenyl)-6-phenylpyridin-2-ylsulfanyl]acetylamino} acetic acid *tert*-butyl ester,15h

General procedure D: 4-(4-methoxyphenyl)-2-mercapto-6-phenylnicotinonitrile, **14h** (0.200 g, 0.6 mmol), *tert*-butyl 2-(2-bromoacetamido)acetate (0.230 g, 0.52 mmol), KOH (0.034 g, 0.6 mmol), DMF (20 mL). Yellow solid (0.057 g, 20%); m.p. 111.3˚C; λ_{max} (EtOH/nm) 276, 339; IR υ_{max}/cm^{-1} 2212, 1738, 1660; ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 1.38 (9H, s, $(CH_3)_3$), 2.07 (3H, s, O-CH_3), 3.91 (3H, s, O-CH_3), 4.11 (2H, s, CH_2S), 7.06 (2H, m, H-Ar), 7.25 (1H, br s, NH), 7.27 (2H, m, H-Ar), 7.40 (1H, m, H-Ar), 7.50 (1H, m, CH-pyridine), 7.61 (2H, m, H-Ar), 7.99 (3H, m, H-Ar and NH); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 28.3, 34.3, 42.0, 55.8, 115.1, 127.8, 129.2, 129.4, 130.2, 131.0, 82.5, 104.3, 117.2, 137.5, 154.9, 159.3, 161.9, 162.1, 168.4, 168.7; MS (ES+) *m/z* = 490.2 $[M+H]^+$.

[2-(3-Cyano-4,6-diphenylpyridin-2-ylsulfanyl)acetylamino]acetic acid *tert*-butyl ester, 15d

General procedure D: 4,6-diphenyl-2-mercaptonicotinonitrile (0.200 g, 0.68 mmol), *tert*-butyl 2-(2-bromoacetamido)acetate (0.262 g, 0.1 mmol), KOH (0.04 g, 0.68 mmol), DMF (20 mL). Yellow solid (0.073 g, 23%); mp 70.1˚C; λ_{max} (EtOH/nm) 270, 333; IR υ_{max}/cm^{-1} 2158, 1735, 1652; ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 1.51 (9H, s, $(CH_3)_3$), 3.99 (2H, d, CH_2), 4.10 (2H, s, CH_2), 7.10-7.65 (9H, m, H-Ar and CH-pyridine), 7.98 (2H, m, H-Ar); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 28.4, 42.7, 117.1, 127.9, 128.7, 129.4, 130.5, 131.1, 132.5, 60.1, 83.7, 148.5, 159.0, 167.6, 183.3, 189.0, 189.6, 192.6; MS: (ES+) *m/z* = 460.2 $[M+H]^+$.

{2-[3-Cyano-6-(4-methoxyphenyl)-4-phenylpyridin-2-ylsulfanyl]acetylamino}acetic acid *tert*-butyl ester, 15i

General procedure D: 6-(4-methoxyphenyl)-2-mercapto-4-phenylnicotinonitrile **14i** (0.100 g, 0.31 mmol), *tert*-butyl 2-(2-bromoacetamido)acetate (0.118 g, 0.47 mmol), KOH (0.034 g, 0.6 mmol), DMF (20 mL). Yellow solid (0.154 g, 61%); ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 1.39 (9H, s, $(CH_3)_3$), 3.89 (3H, s, CH_3), 3.90 (2H, m, CH_2), 4.08 (2H, s, CH_2), 7.02 (2H, d, *J* = 8.7 Hz, H-Ar), 7.27 (4H, m, NH and H-Ar), 7.52 (1H, s, CH-pyridine), 7.62 (2H, m, H-Ar), 8.04 (2H, d, *J* = 8.7 Hz, H-Ar).

[2-(3-Cyano-6-methyl-4-phenylpyridin-2-ylsulfanyl)acetylamino]acetic acid *tert*-butyl ester, 15j

General procedure D: 6-phenyl-2-mercapto-4-methylnicotinonitrile (0.100 g, 0.44 mmol), *tert*-butyl 2-(2-bromoacetamido)acetate (0.167 g, 0.66 mmol), KOH (0.024 g, 0.43 mmol), DMF (5 mL). Yellow solid (0.143 g, 82%); m.p. 126.1˚C; λ_{max} (EtOH/nm) 262; IR υ_{max}/cm^{-1} 3297, 2218, 1731, 1682; ^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm 1.40 (9H, s, $(CH_3)_3$), 3.76 (2H, d, *J* = 0.6 Hz, CH_2NH), 4.07 (2H, s, CH_2), 7.29 (1H, m, H-Ar), 7.58 (5H, m, H-Ar), 8.51 (1H, br s, NH); ^{13}C-NMR (75 MHz, DMSO-d_6) δ ppm 24.7, 28.1, 33.9, 42.3, 120.0, 128.7, 129.2, 130.3; MS (ES+) *m/z* = 398.1 $[M+H]^+$.

[2-(3-Cyano-4,6-dimethylpyridin-2-ylsulfanyl)acetylamino]acetic acid *tert*-butyl ester, 15k

General procedure D: 4,6-dimethyl-2-mercaptonicotinonitrile (0.100 g, 0.6 mmol), *tert*-butyl 2-(2-bromoacetamido)acetate (0.226 g, 9.1 mmol), KOH (0.034 g, 0.6 mmol), DMF (20 mL). Yellow solid (0.122 g, 61%); m.p. 132.4˚C; λ_{max} (EtOH/nm) 221, 266, 302; IR υ_{max}/cm^{-1} 2218, 1737, 1652;^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 1.44 (9H, s, $(CH_3)_3$), 2.48 (3H, s, CH_3), 2.59 (3H, s, CH_3), 3.92 (4H, s, CH_2), 6.89 (1H, s, CH-pyridine), 7.55 (1H, s, NH); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 20.4, 24.8, 28.4, 33.8, 42.9, 121.1, 82.5, 100.0, 105.8, 114.7, 152.8, 162.2, 168.9, 168.9; MS (ES+) *m/z* = 336.1 $[M+H]^+$; Anal. Calcd for $C_{16}H_{21}N_3O_3S$: C, 57.29; H, 6.31; N, 12.53. Found.C, 57.48; H, 6.08; N, 11.99.

2-Bromo-N-(2-oxopropyl)acetamide, 30

A mixture of glycine (10.0 g, 0.13 mol), pyridine (65 mL, 0.80 mol) and acetic anhydride (143 mL, 1.52 mol) was refluxed 6 h, then allowed to cool and poured onto ice/water (500 mL). The mixture was extracted with DCM (3 x 100 mL). The organic extracts were washed with water (3 x 100 mL), dried ($MgSO_4$), and concentrated *in vacuo* giving acetamidoacetone (4.22 g, 56%) which was used without further purification.

A mixture of conc. hydrochloric acid (6 mL), water (6 mL) and acetamidoacetone (2 g, 17.4 mmol) and the mixture was refluxed under N_2 for 6 h, then concentrated *in vacuo* giving 1-aminopropan-2-one hydrochloride **29** (1.3 g, 68%) which was used without further purification.

To a solution of 1-aminopropan-2-one hydrochloride (1.0 g, 9.0 mmol) in DCM (20 ml), was added $CaCO_3$ (5.0 eq) and bromoacetyl chloride (2.0 eq). The resulting mixture was refluxed 12 h, then allowed to cool and diluted with water, and filtered. Recrystallization (ex THF) gave **30** (0.71 g, 53%); m.p. 77.0˚C; IR υ_{max}/cm^{-1} 3074, 1724, 1643; ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 2.23 (3H, s, CH_3), 3.90 (2H, s, CH_2), 4.17 (2H, d, CH_2N), 7.19 (1H, br s, NH).

1-(2-Bromoacetyl)pyrrolidine-2-carboxylic acid methyl ester (18)

A solution of proline methyl ester (5.0 g, 30 mmol) in water (20 mL) was added to a stirred suspension of $CaCO_3$ in $CHCl_3$ at 0 ^{o}C, followed by dropwise addition of 2-bromoacetylchloride (9.5 g, 60 mmol) in $CHCl_3$ and stirring continued 16 h, then filtered. The filtrate washed with HCl (1M, 20 mL), sodium carbonate (sat., 20 mL) and water (20 mL), then dried ($MgSO_4$) and concentrated *in vacuo.* Recrystallisation (ether) gave **18** (6.62 g, 77 %); ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 1.94 (3H, m), 2.14 (2H, m), 3.62 (4H, m), 3.76 (2H, m), 4.37 (1H, m).

General procedure E[7]

A mixture of the corresponding aldehyde (1.2 eq), ketone (1.0 eq), and KOH (2.4 eq) in absolute ethanol (10 ml) was stirred at rt for 4 h, then water was added. The precipitate was collected by filtration and recrystallized (ethanol).

(*E*)-3-(4-Fluorophenyl)-1-phenylprop-2-en-1-one (13a)

General procedure E: acetophenone (0.97 mL, 8.32 mmol), 4-fluorobenzaldehyde (1.07 mL, 9.98 mmol), KOH (1.11 g, 21 mmol). Yellow solid (1.41 g, 75%); m.p. 87 ˚C (lit.[8] 52 ^{o}C) ; λ_{max} (EtOH/nm) 309; IR υ_{max}/cm^{-1} 1656; ^{1}H-NMR (500 MHz, $CDCl_3$) δ ppm; 7.14 (2H, dd, *J* = 6.6 and 8.5 Hz, H-Ar), 7.48-7.55 (3H, m, H-Ar), 7.62 (1H, ddd, *J* = 1.2, 1.5 and 7.4 Hz, H-Ar), 7.66 (2H, dd, *J* = 5.4 and 8.6 Hz, H-Ar), 7.80 (1H, d, *J* = 15.8 Hz, COC*H*), 8.03-8.05 (2H, m, H-Ar); ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm; 116.2 (d, J_{CF} = 22.0 Hz), 121.8 (d, J_{CF} = 2.2 Hz), 128.5, 128.7, 130.4 (d, J_{CF} = 8.6 Hz), 131.2 (d, J_{CF} = 3.1 Hz), 132.9, 138.2, 143.5, 164.1 (d, J_{CF} = 251.3 Hz), 190.3; MS: (ES+) *m/z* = 227.1 $[M+H]^+$.

(*E*)-3-(3-Fluorophenyl)-1-phenylprop-2-en-1-one (13b)

General procedure E: acetophenone (1.03 g, 8.60 mmol), 3-fluorobenzaldehyde (1.07 mL, 10.20 mmol), NaOH (0.82 g, 20.60 mmol). Yellow crystals (1.24 g, 64%); m.p. 86-88 ˚C (lit.[9] 87-89 ^{o}C EtOH); λ_{max} (EtOH/nm) 297, 379; IR υ_{max}/cm^{-1} 1661, 1594, 1578, 1481, 1444, 1338, 1313, 1267; ^{1}H-NMR (500 MHz, $CDCl_3$) δ ppm; 7.06 (1H, dddd, *J* = 1.0, 8.3, 9.3 and 10.8 Hz, H-2), 7.13 (1H, ddd, *J* = 0.9, 7.6 and 8.4 Hz, H-5), 7.29-7.34 (1H, m, H-4), 7.42-7.45 (2H, m, H-3' and H-5'), 7.52 (1H, ddd, *J* = 1.3, 2.0 and 7.3 Hz, H-4'), 7.56-7.59 (2H, m, COCHC*H* and H-6), 7.83 (1H, d, *J* = 16.0 Hz, COC*H*), 7.95-7.97 (2H, m, H-2' and H-6'); ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm; 116.3 (d, J_{CF} = 22.2 Hz), 123.0 (d, J_{CF} = 11.3 Hz), 124.5 (d, J_{CF} = 3.4 Hz), 124.6 (d, J_{CF} = 7.3 Hz), 128.6, 128.7, 129.8 (d, J_{CF} = 2.9 Hz), 131.8 (d, J_{CF} = 8.8 Hz), 132.9, 137.5, 138.0, 161.0 (d, J_{CF} = 253.1 Hz), 190.5; MS (ES+) *m/z* = 227.03 $[M+H]^+$.

(*E*)-3-(2-Fluorophenyl)-1-phenylprop-2-en-1-one (13c)

General procedure E: acetophenone (1.03 g, 8.60 mmol), 2-fluorobenzaldehyde (1.07 mL, 10.20 mmol), NaOH (0.82 g, 20.60 mmol). Yellow crystals (1.08 g, 56%); m.p. 49-51 ˚C (lit 47-48 °C EtOH); λ_{max} (EtOH/nm) 302, 345; IR υ_{max}/cm^{-1} 1659, 1603, 1572, 1482, 1447, 1335, 1317, 1280; ^{1}H-NMR (500 MHz, $CDCl_3$) δ ppm; 7.11-7.14 (1H, m, H-Ar), 7.35-7.42 (3H, m, H-Ar), 7.51-7.55 (3H, m, CH and H-Ar), 7.61 (1H, dd, *J* = 7.6 and 7.5 Hz, H-Ar), 7.77 (1H, d, J = 15.8 Hz, COC*H*), 8.02-8.04 (2H, m, H-Ar); MS (ES+) *m/z* = 227.04 $[M+H]^+$.

(*E*)-3-(2,4-Difluorophenyl)-1-phenylprop-2-en-1-one (13f)

General procedure E: acetophenone (0.82 mL, 7.0 mmol), 2,4-difluorobenzaldehyde (1.3 mL, 7.0 mmol), NaOH (350 mg, 8.80 mmol). Yellow crystals (1.40 g, 80%); m.p. 59-61 ˚C; λ_{max} (EtOH/nm) 311; IR υ_{max}/cm^{-1} 1659, 1604, 1589, 1497; ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 7.25 (1H, ddd, *J* = 2.5, 8.5 and 10.9 Hz, H-3), 7.41 (1H, ddd, *J* = 2.5, 9.3 and 11.5 Hz, H-5), 7.58-7.61 (2H, m, H-3' and H-5'), 7.70 (1H, ddd, *J* = 1.2, 1.3 and 7.4 Hz, H-4'), 7.78 (1H, d, *J* = 15.8 Hz, COCHC*H*), 7.98 (1H, d, *J* = 15.8 Hz, COC*H*), 8.15-8.17 (2H, m, H-2' and H-6'), 8.24 (1H, ddd, *J* = 6.8, 8.9 and 15.5 Hz, H-6); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 104.6 (dd, J_{CF} = 26.2 and 26.3 Hz), 112.6 (dd, J_{CF} = 3.5 and 21.7 Hz), 119.2 (dd, J_{CF} = 3.8 and 11.6 Hz), 123.8 (dd, J_{CF} = 2.3 and 3.7 Hz), 128.6, 128.8, 130.7 (dd, J_{CF} = 3.8 and 10.0 Hz), 133.4, 134.2 (dd, J_{CF} = 1.4 and 3.7 Hz), 137.2, 161.1 (dd, J_{CF} = 12.5 and 254.5 Hz), 163.1 (dd, J_{CF} = 13.1 and 252.1 Hz), 188.9; MS (ES+) *m/z* = 245.2 $[M+H]^+$; HRMS calcd for $C_{15}H_{10}F_2O$ $[M+H]^+$ 245.0772, found 245.0771.

(*E*)-1-Phenyl-3-(pyridin-4-yl)prop-2-en-1-one (13g)[10]

A mixture of 4-pyridine carboxaldehyde (0.56 g, 5.20 mmol) and (benzoylmethylene) triphenylphosphorane (1.97 g, 5.20 mmol) in anh. toluene (10 mL) was refluxed for 3.5 h, then allowed to cool and concentrated *in vacuo*. The residue was triturated with petrol until a solid formed, then filtered, washing with 1M HCl (25 mL). The filtrate was neutralised with 2.5M NaOH. The resulting precipitate was filtered and dried *in vacuo* giving **13g** as a yellow solid (0.85 g, 78%). m.p. 75-77 ˚C (lit. 73-74.5 °C); λ_{max} (EtOH/nm) 282; IR υ_{max}/cm^{-1} 3058, 3034, 1659, 1593, 1578, 1480, 1445, 1427; ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm; 7.41-7.7.56 (5H, m, H-Ar, COCH*CH* and CO*CH*), 7.63 (2H, d, *J* = 1.8 Hz, H-Ar), 7.95-7.98 (2H, m, H-Ar), 8.64 (2H, d, *J* = 4.5 Hz, N-CH-pyridine); MS (ES+) *m/z* = 210.0 $[M+H]^+$.

4-(4-Fluorophenyl)-2-mercapto-6-phenylnicotinonitrile (14a)[11]

A mixture of (*E*)-3-(4-fluorophenyl)-1-phenylprop-2-en-1-one (**13a**) (2.0 g, 8.85 mmol), malononitrile (0.595 g, 8.85 mmol), sulfur (0.34 g, 10.6 mmol) and morpholine (1.0 mL, 11.5 mmol) in ethanol (25 ml) was heated to reflux with stirring for 2 h, then cooled to 20 °C, acidified with hydrochloric acid, and filtered. Chromatography (silica; 50% ethyl acetate, petrol) gave **14a** as a yellow solid (0.353g, 68%); m.p. 222.6˚C; λ_{max} (EtOH/nm) 271, 330; IR υ_{max}/cm^{-1} 3066, 2214; ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 7.10-7.23 (3H, m, NH and H-Ar), 7.23-7.37 (3H, m, H-Ar), 7.46-7.59 (3H, m, CH-pyridine and H-Ar), 7.83-7.94 (2H, d, *J* = 6.3 Hz, H-Ar); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 104.5, 115.4; 116.5, 116.8, 117.8, 127.8, 130.8, 131.2, 132.4, 132.5 134.6; 137.2, 154.2, 159.6; 161.3, 162.8, 166.1; MS (ES+) *m/z* = 306 $[M+H]^+$; Anal. Calcd for $C_{18}H_{10}FN_2S$: C, 70.57; H, 3.62; N, 9.14. Found.C, 70.71; H, 3.58; N, 9.45; HPLC assay system: 87.5%

General procedure F[11]

A mixture of the required chalcone (1.0 eq.), malononitrile (1.0 eq.), sulfur (1.2 eq.) and morpholine (1.3 eq.) in ethanol (25 ml) was heated to reflux with stirring for 2 h, then cooled to 20 °C, acidified with hydrochloric acid, and filtered. The precipitate was purified by chromatography (silica; 50% ethyl acetate, petrol).

4,6-Diphenyl-2-thioxo-1,2-dihydropyridine-3-carbonitrile, 14d

General procedure F: (*E*)-3-phenyl-1-phenylprop-2-en-1-one, **13d** (5.0 g, 24 mmol), malononitrile (1.58 g, 24 mmol), sulfur (1.0 g, 28.8 mmol) and morpholine (2.7 mL, 31 mmol). Yellow solid (3.52 g, 50%); m.p. 192.4˚C; λ_{max} (EtOH/nm) 206, 269; IR υ_{max}/cm^{-1} 2215 (CN); ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 7.28 (1H, s, NH), 7.41 (3H, m, H-Ar), 7.59 (3H, m, H-Ar), 7.66 (3H, m, CH-pyridine, H-Ar), 7.99 (2H, m, H-Ar); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 115.5, 127.8, 128.9, 129.2, 129.4, 130.5, 131.1, 137.3, 155.3; MS (ES+) *m/z* = 289.1 $[M+H]^+$; HPLC 88.5%; Anal. Calcd for $C_{18}H_{12}N_2S$: C, 74.97; H, 4.19; N, 9.71. Found.C, 74.87; H, 4.01; N, 9.57.

6-(4-Methoxyphenyl)-4-phenyl-2-thioxo-1,2-dihydropyridine-3-carbonitrile, 14i

General procedure F: (*E*)-3-phenyl-1-(4-methoxyphenyl)prop-2-en-1-one, **13i** (2.0 g, 8.4 mmol), malononitrile (0.56 g, 8.4 mmol), sulfur (0.321 g, 10 mmol) and morpholine (0.95 g, 10.9 mmol). Yellow solid (1.47 g, 55%); λ_{max} (EtOH/nm) 303; IR υ_{max}/cm^{-1} 2214 (CN); ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 3.83 (3H, s, CH_3), 6.90 (2H, m, H-Ar), 7.28 (2H, m, H-Ar), 7.53 (1H, s, CH-pyridine), 7.62 (3H, m, H-Ar), 7.98 (2H, m, H-Ar).

2-[3-Cyano-4-(4-fluorophenyl)-6-phenylpyridin-2-ylsulfanyl]acetamide, 20

To a suspension of **14a** (1.0 eq) and sodium acetate trihydrate (1.1 eq) in ethanol (10 ml) was added chloroacetamide (1.0 eq). The resulting mixture was heated under reflux for 24 hours. A precipitate formed upon cooling which was collected and recrystallised from ethanol to give **20** as pale yellow needles (119 mg, 71%). m.p. 236.2˚C; λ_{max} (EtOH/nm) 230, 270 and 342; IR υ_{max}/cm^{-1} 1635 (CO), 2210 (CN), 3162 and 3360 (NH); ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 4.06 (2H, s, CH_2), 7.28 (4H, m, Hd, Hd' and NH_2), 7.54 (3H, m, Hb, Hb' and He), 7.62 (3H, m, CH-pyridine, Hc and Hc'), 8.10 (2H, d, *J* = 7.9 Hz, Ha, Ha'); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 34.4, 116.1, 116.4, 128.2, 129.2, 131.1, 131.4, 131.5, 153.2, 158.6, 160.0, 162.7, 169.1; MS (ES+) *m/z* = 364.1 $[M+H]^+$; Anal. Calcd for $C_{20}H_{14}FN_3OS$: C, 66.10; H, 3.88; N, 11.56. Found.C, 66.08; H, 3.86; N, 11.54.

General procedure G: [6]

To a mixture of the required 2-thioxo-1,2-dihydropyridine-3-carbonitrile (1.0 eq) and K_2CO_3 (1.2 eq) in THF was added the required α-halo-compound (1.0 eq). The reaction mixture was heated under reflux for 24 h, allowed to cool and diluted with water. The precipitate was collected by filtration and either recrystallization (THF) or chromatography (silica; 20-100% EtOAc, petrol) gave the desired 2-pyridyl sulfide.

2-(2-(3-Cyano-4-(4-fluorophenyl)-6-phenylpyridin-2-ylthio)acetamido)acetic acid (4a)

15a (91 mg, 0.19 mmol), was dissolved in TFA (29 µL, 0.38 mmol) and the resulting mixture was stirred at room temperature for 30 minutes, then concentrated *in vacuo* and washed (petrol) to give **4a** as a white solid (80 mg, 99%). R_f = 0.37 (EtOH); m.p. 169-172 ˚C; λmax (EtOH/nm) 270, 342; IR υmax/cm^{-1} 3260, 2215, 1730, 1622; ^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm 3.80 (2H, d, *J* = 5.4 Hz, NH-*CH*$_2$), 4.21 (2H, s, S-CH_2),7.43-7.54 (4H, m, H-Ar), 7.82-7.87 (2H, m, H-Ar), 7.94 (1H, s, CH-pyridine), 8.28-8.30 (2H, m, H-Ar), 8.64 (1H, t, *J* = 5.4 Hz, NH), 12.62 (1H, s, COOH); MS (ES+) *m/z* = 422.2 $[M+H]^+$; HRMS calcd for $C_{22}H_{16}FN_3O_3S$ $[M+H]^+$ 422.0976, found 422.0969.

***tert*-Butyl 2-(2-(3-cyano-4-(4-fluorophenyl)-6-phenylpyridin-2-ylthio)acetamido)acetate (15a)**

A 1.6M solution of NaOMe was prepared by slowly dissolving sodium metal in MeOH at rt. To **13a** (88 mg, 0.39 mmol), and 2-cyanothioacetamide (39 mg, 0.39 mmol) was added sodium methoxide (1.6M in MeOH, 0.60 mL, 0.94 mmol), The resulting solution was heated at 80 ^{o}C for 1.5 h, then allowed to cool to rt and concentrated *in vacuo*. The crude material was redissolved in DMF (1 mL/mmol) and *tert*-butyl 2-(2-bromoacetamido)acetate (149 mg, 0.59 mmol) was added. The solution was heated at 100 ^{o}C for 3-4 h, then cooled, diluted with H_2O (20 mL) and extracted with EtOAc (3 x 100 mL). Combined organic layers were washed with H_2O (3 x 100 mL) and brine (50 mL), dried (Na_2SO_4) and concentrated *in vacuo*. Chromatography (silica; 0-50% EtOAc/petrol) gave **15a** as a white solid (71 mg, 38%). m.p. 172-174 ˚C; λmax (EtOH/nm) 270, 342; IR υ_{max}/cm^{-1} 3279, 2977, 2214, 1740, 1657; ^{1}H-NMR (500 MHz, DMSO) δ ppm 1.39 (9H, s, CH3), 3.77 (2H, d, *J* = 5.9 Hz, *CH2*-NH), 4.21 (2H, s, S-CH2), 7.47 (1H, dd, *J* = 8.3 and 9.1 Hz, H-3' and H-5'), 7.54-7.55 (3H, m, H-2', H-6' and H-4'), 7.84 (2H, dd, *J* = 5.5 and 8.8 Hz, H-3 and H-5), 7.95 (1H, s, CH-pyridine), 8.29-8.31 (2H, m, H-2 and H-5), 8.65 (1H, t, *J* = 5.9 Hz, NH); MS (ES+) *m/z* = 478.2 [M+H]+; HRMS calcd for $C_{26}H_{24}FN_3O_3S$ $[M+H]^+$ 478.1595, found 478.1602.

***tert*-Butyl 2-(2-((3-cyano-4,6-dimethylpyridin-2-yl)thio)acetamido)acetate (15k)**

General Procedure G: 2-mercapto-4,6-dimethylnicotinonitrile (110 mg, 0.69 mmol), *tert*-butyl 2-(2-bromoacetamido)acetate (210 mg, 0.83 mmol), KOH (39 mg, 0.69 mmol) and DMF (2 mL/mmol). White solid (120 mg, 52%); m.p. 132-134 ˚C; λ_{max} (EtOH/nm) 266.0, 302.0; IR υ_{max}/cm^{-1} 2218, 1737, 1652; ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.44 (9H, s, CH_3), 2.47 (3H, s, CH_3), 2.59 (3H, s, CH_3), 3.92-3.93 (4H, m, NH-*CH*$_2$ and S-CH_2), 6.89 (1H, s, CH-pyridine), 7.56 (1H, br s, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm 20.4, 24.8, 28.4, 33.8, 42.9, 82.0, 105.8, 114.7, 121.1, 152.8, 162.2, 168.9, 168.9; MS (ES+) *m/z* = 336.1 $[M+H]^+$; HRMS calcd for $C_{16}H_{21}N_3O_3S$ $[M+H]^+$ 336.1376, found 336.1377.

{2-[3-Cyano-4-(4-fluorophenyl)-6-phenylpyridin-2-ylsulfanyl]acetylamino}acetic acid ethyl ester (15l)

General procedure G: 4-(4-fluorophenyl)-2-mercapto-6-phenylnicotinonitrile **14a** (0.30 g, 0.98 mmol), (2-bromoacetylamino)acetic acid ethyl ester (0.218g, 0.98 mmol), K_2CO_3 (0.16 g, 1.2 mmol), THF (20 mL), chromatography. Yellow solid (0.251 g, 57%). m.p. 171.8˚C; λ_{max} (EtOH/nm) 270, 341; IR υ_{max}/cm^{-1} 3259, 2214, 1743, 1665; ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 1.20 (3H, t, *J* = 7.2 Hz, CH_3,), 4.00 (2H, d, *J* = 5.1 Hz, *CH*$_2$NH), 3.96-4.06 (4H, m, CH_2), 7.20-7.52 (3H, m, NH and H-Ar), 7.46-7.52 (3H, m, H-Ar), 7.59-7.68 (3H, m, CH-pyridine and H-Ar), 8.08-8.11 (2H, m, H-Ar); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 14.3, 34.3, 42.1, 61.6, 116.5, 116.8, 116.9, 127.8, 129.5, 130.7, 131.2,

129.3, 154.2, 159.7, 162.2, 166.1, 168.2, 169.5; MS (ES+) *m/z* = 450.1 $[M+H]^+$; Anal. Calcd for $C_{24}H_{20}FN_3O_3S$, C, 64.13; H, 4.48; N, 9.35. Found.C, 63.89; H, 4.49; N, 9.48.

1-{2-[3-Cyano-4-(4-fluorophenyl)-6-phenylpyridin-2-ylsulfanyl]acetyl}pyrrolidine-2-carboxylic acid methyl ester (19)

General procedure G: 4-(4-fluorophenyl)-2-mercapto-6-phenylnicotinonitrile **14a** (0.30 g, 0.98 mmol), 1-(2-bromoacetyl)pyrrolidine-2-carboxylic acid methyl ester, **18** (0.281 g, 0.98 mmol), K_2CO_3 (0.16 g, 1.2 mmol), THF (20 mL), chromatography. Yellow solid (0.256 g, 55%). m.p. 212.7˚C; λ_{max} (EtOH/nm) 270, 342; IR υ_{max}/cm^{-1} 2922, 2212, 1734, 1645; ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 2.00-2.22 (4H, m, 2 x CH_2), 3.67 (3H, s, CH_3O), 3.71-3.84 (2H, m, CH_2), 4.19-4.28 (2H, m, CH_2), 4.46-4.58 (1H, m, CH), 7.14-7.25 (2H, m, H-Ar), 7.41-7.57 (4H, m, H-Ar and CH-pyridine), 7.57-7.66 (2H, m, H-Ar), 7.91-8.02 (2H, m, H-Ar); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 25.3, 29.5, 34.3, 47.7, 52.4, 59.8, 116.4, 116.7, 138.0, 129.2, 130.7, 130.8, 154.0, 172.4; MS (ES+) *m/z* = 476.1 $[M+H]^+$; Anal. Calcd for $C_{26}H_{22}FN_3O_3S$: C, 65.67; H, 4.66; N, 8.84. Found.C, 65.68; H, 4.55; N, 8.65.

[3-Cyano-4-(4-fluorophenyl)-6-phenylpyridin-2-ylsulfanyl]acetic acid methyl ester (21)

General procedure G: 4-(4-fluorophenyl)-6-phenyl-2-thioxo-1,2-dihydropyridine-3-carbonitrile (0.500 g, 1.6 mmol), methyl 2-bromoacetate (0.24 mL, 2.4 mmol), K_2CO_3 (0.45 g, 0.32 mmol), DMF (40 mL). Yellow solid (0.34 g, 55%). m.p. 185.5˚C; λ_{max} (EtOH/nm) 269 and 339; IR υ_{max}/cm^{-1} 1731 (CO), 2212 (CN); ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 3.77 (3H, s, CH_3), 4.14 (2H, s, CH_2), 7.25 (2H, m, Hd and Hd'), 7.52 (3H, m, Hb, Hb' and He), 7.63 (3H, m, CH-pyridine, Hc and Hc'), 8.08 (2H, m, Ha, Ha'); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 33.1, 52.8, 104.2, 115.4, 116.4, 116.7, 127.8, 129.3, 130.7, 130.8, 131.1, 132.6, 137.5; MS (ES+) *m/z* = 379.1 $[M+H]^+$; Anal. Calcd for $C_{21}H_{15}FN_2O_2S$: C, 66.65; H, 4.00; N, 7.40. Found.C, 66.92; H, 3.79; N, 7.42

2-[3-Cyano-4-(4-fluorophenyl)-6-phenylpyridin-2-ylsulfanyl]-N-(2-oxopropyl)acetamide (31)

General procedure G: **14a** (0.30 g, 0.98 mmol), 2-bromo-*N*-(2-oxopropyl)acetamide **30** (0.19 g, 0.98 mmol), K_2CO_3 (0.16 g, 1.2 mmol), THF (10 mL), chromatography. Yellow solid (0.137 g, 31%); m.p. 232.1˚C; λ_{max} (EtOH/nm) 270, 338; IR υ_{max}/cm^{-1} 3280, 2920, 2216, 1732, 1658; ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 1.58 (3H, s, CH_3), 2.12 (2H, s, CH_2), 4.12 (2H, s, CH_2), 7.28 (2H, d, *J* = 7.9 Hz, H-Ar), 7.30 (1H, br s, NH), 7.21-7.41 (3H, m, H-Ar), 7.50-7.70 (3H, m, CH-pyridine and H-Ar), 8.05-8.14 (2H, m, H-Ar); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 30.0, 34.3, 50.3, 116.5, 116.8, 116.9, 127.8, 129.4, 130.7, 130.9, 131.2, 159.6, 168.2; MS (ES+) *m/z* = 420.1176 $[M+H]^+$; Anal. Calcd for $C_{23}H_{18}FN_3O_2S$: C, 65.86; H, 4.33; N, 10.02. Found.C, 65.84; H, 4.31; N, 10.01.

General procedure H

The pyridine t-butyl ester (1.0 eq) was dissolved in trifluoroacetic acid (5 ml). The resulting mixture was stirred at RT for 30 minutes. The solvent was removed and the residue was treated with ethyl acetate. The precipitate was collected and washed several times with petroleum ether to give the product.

2-(2-(3-Cyano-4-(4-fluorophenyl)-6-phenylpyridin-2-ylthio)acetamido)acetic acid (4a)

General procedure H: **15a** (91 mg, 0.19 mmol), was dissolved in TFA (29 µL, 0.38 mmol) gave **4a** as a white solid (80 mg, 99%). R_f = 0.37 (EtOH); m.p. 169-172 ˚C; λmax (EtOH/nm) 270, 342; IR υmax/cm^{-1} 3260, 2215, 1730, 1622; ^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm 3.80 (2H, d, *J* = 5.4 Hz, NH-CH_2), 4.21 (2H, s, S-CH_2),7.43-7.54 (4H, m, H-Ar), 7.82-7.87 (2H, m, H-Ar), 7.94 (1H, s, CH-pyridine), 8.28-8.30 (2H, m, H-Ar), 8.64 (1H, t, *J* = 5.4 Hz, NH), 12.62 (1H, s, COOH); MS (ES+) *m/z* = 422.2 $[M+H]^+$; HRMS calcd for $C_{22}H_{16}FN_3O_3S$ $[M+H]^+$ 422.0976, found 422.0969.

2-(2-(3-Cyano-4-(3-fluorophenyl)-6-phenylpyridin-2-ylthio)acetamido)acetic acid (4b)

General procedure H: tert-butyl 2-(2-(3-cyano-4-(3-fluorophenyl)-6-phenylpyridin-2-ylthio)acetamido)acetate **15b** (35 mg, 0.067 mmol), TFA (11 µL, 0.14 mmol). White solid (28 mg, 100%); m.p. 221-224 ˚C; λ_{max} (EtOH/nm) 279.0, 348.5; IR υ_{max}/cm^{-1} 3241, 2217, 2174, 1735, 1624, 1574, 1525; ^{1}H-NMR (500 MHz, DMSO-d_6) δ ppm 3.82 (2H, d, J = 5.7 Hz, NH-CH_2), 4.22 (2H, s, S-CH_2), 7.44-7.48 (1H, m, H-Ar), 7.54-7.58 (3H, m, H-Ar), 7.61-7.71 (3H, m, H-Ar), 7.99 (1H, s, CH-pyridine), 8.31-8.33 (2H, m, H-Ar), 8.68 (1H, t, J = 5.7 Hz, NH), 12.67 (1H, s, COOH); MS (ES+) m/z = 422.2 $[M+H]^+$, 420.1 $[M-H]^-$; HRMS calcd for $C_{22}H_{16}FN_3O_3S$ $[M+H]^+$ 422.0967, found 422.0967.

2-(2-((3-Cyano-4-(2-fluorophenyl)-6-phenylpyridin-2-yl)thio)acetamido)acetic acid (4c)

General procedure H: tert-butyl 2-(2-(3-cyano-4-(2-fluorophenyl)-6-phenylpyridin-2-ylthio)acetamido)acetate **15c** (18 mg, 0.038 mmol), TFA (6 µL, 0.076 mmol). White solid (16 mg, 99%); m.p. 200-202 ˚C; λ_{max} (EtOH/nm) 340.0, 270.5; IR υ_{max}/cm^{-1} 2220,1719, 1653, 1573, 1526; ^{1}H-NMR (500 MHz, DMSO-d_6) δ ppm 3.82 (2H, d, J = 5.7 Hz, NH-CH_2), 4.23 (2H, s, S-CH_2), 7.44-7.51 (2H, m, H-Ar), 7.52-7.55 (3H, m, H-Ar and H-4'), 7.64-7.71 (2H, m, H-Ar), 8.00 (1H, s, CH-pyridine), 8.28-8.30 (2H, m, H-Ar), 8.67 (1H, t, J = 5.7 Hz, NH), 12.67 (1H, s, COOH); MS (ES+) m/z = 422.2 $[M+H]^+$, 420.1 $[M-H]^-$; HRMS calcd for $C_{22}H_{16}FN_3O_3S$ $[M+H]^+$ 422.0967, found 422.0968.

[2-(3-Cyano-4,6-diphenylpyridin-2-ylsulfanyl)acetylamino]acetic acid (4d)

General procedure H: *tert*-butyl 2-(2-((3-cyano-4,6-diphenylpyridin-2-yl)thio)acetamido)acetate, **15d** (0.200 g, 0.44 mmol), TFA (2 mL). White solid (0.155 g, 88%); m.p. 222.5˚C; λ_{max} (EtOH/nm) 269 and 339; IR υ_{max}/cm^{-1} 1645 (CO), 1724 (CO), 2214 (CN); ^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm 3.80 (2H, d, *J* = 6.8 Hz, CH_2), 4.20 (2H, s, CH_2), 7.54 (3H, m, Ha, Ha' and He'), 7.60 (3H, m, Hd, Hd' and He), 7.93 (2H, m, Hb and Hb'), 7.93 (1H, s, CH-pyridine), 8.29 (2H, m, Hc and Hc'), 8.64 (1H, t, *J* = 6.8 Hz, NH), 12.55 (1H, br s, COOH); ^{13}C-NMR (75 MHz, DMSO-d_6) δ ppm 34.2, 41.6, 128.1, 129.2, 130.4, 131.1, 136.2, 137.1 115.9, 116.5, 116.7, 154.7, 158.6, 162.4, 167.6, 171.0; MS (ES+) *m/z* = 404.1 $[M+H]^+$; Anal. Calcd for $C_{22}H_{17}N_3O_3S$: C, 65.49; H, 4.25; N, 10.42. Found.C, 65.44; H, 3.68; N, 6.23.

2-(2-((3-Cyano-6-phenyl-4-(4-(trifluoromethyl)phenyl)pyridin-2-yl)thio)acetamido)acetic acid (4e)

General procedure H: *tert*-butyl 2-(2-((3-cyano-6-phenyl-4-(4-(trifluoromethyl)phenyl)pyridin-2-yl)thio)acetamido)acetate **15e** (100 mg, 0.19 mmol), TFA (29 µL, 0.38 mmol). White solid (80 mg, 90%); m.p. 243-244 ˚C; λ_{max} (EtOH/nm) 345.0, 268.0; IR υ_{max}/cm^{-1} 3285, 3069, 2214, 1738, 1667; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 3.82

(2H, d, *J* = 5.7 Hz, NH-*CH*$_2$), 4.23 (2H, s, S-CH$_2$), 7.54-7.56 (3H, m, H-Ar and H-4'), 7.99-8.01 (5H, m, H-Ar and CH-pyridine) 8.30-8.32 (2H, m, H-Ar), 8.67 (1H, t, *J* = 5.7 Hz, NH), COOH not visualised; ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 33.8, 41.3, 102.7, 115.4, 116.0, 124.0 (q, J_{CF} = 273.4 Hz), 125.7 (q, J_{CF} = 3.6 Hz), 127.8, 128.9, 129.8, 130.2 (q, J_{CF} = 32.2 Hz), 130.9, 136.4, 139.7, 152.7, 158.3, 162.1, 167.1, 171.0; MS (ES+) *m/z* = 472.2 $[M+H]^+$; HRMS calcd for $C_{23}H_{16}F_3N_3O_3S$ $[M+H]^+$ 472.0937, found 472.0932.

2-(2-((3-Cyano-4-(2,4-difluorophenyl)-6-phenylpyridin-2-yl)thio)acetamido)acetic acid (4f)

General procedure H: *tert*-butyl 2-(2-((3-cyano-4-(2,4-difluorophenyl)-6-phenylpyridin-2-yl)thio)acetamido)acetate **15f** (450 mg, 0.91 mmol), TFA (139 µL, 1.82 mmol). White solid (400 mg, 100%); m.p. 226-227 ˚C; λ_{max} (EtOH/nm) 339.5, 270.5; IR υ_{max}/cm^{-1} 3259, 3079, 2221, 1728, 1619; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 3.75 (2H, d, *J* = 5.8 Hz, NH-*CH*$_2$), 4.23 (2H, s, S-CH$_2$), 7.30 (1H, ddd, *J* = 2.1, 8.4 and 10.6 Hz, H-5), 7.53-7.60 (4H, m, H-Ar, H-4' and H-3) 7.77 (1H, ddd, *J* = 6.6, 8.7 and 15.1 Hz, H-6), 7.99 (1H, s, CH-pyridine), 8.27-8.29 (2H, m, H-Ar), 8.60 (1H, t, *J* = 5.8 Hz, NH), 12.62 (1H, s, COOH); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 33.7, 41.1, 104.1, 104.8 (dd, J_{CF} = 26.8 and 26.9 Hz), 112.4 (dd, J_{CF} = 3.4 and 21.7 Hz), 115.0, 117.2, 120.1 (dd, J_{CF} = 4.0 and 15.0 Hz) 127.8, 128.9, 131.0, 132.8 (dd, J_{CF} = 3.8 and 10.3 Hz), 136.3, 148.1, 158.3, 159.1 (dd, J_{CF} = 12.7 and 250.3 Hz), 161.6, 163.4 (dd, J_{CF} = 12.3 and 250.7 Hz), 167.2, 171.0; MS (ES+) *m/z* = 440.2 $[M+H]^+$; HRMS calcd for $C_{22}H_{15}F_2N_3O_3S$ $[M+H]^+$ 440.0875, found 440.0874.

2-(2-((3-Cyano-6-phenyl-[4,4'-bipyridin]-2-yl)thio)acetamido)acetic acid (4g)

General procedure H: *tert*-butyl 2-(2-(3-cyano-6-phenyl-4-4'-bipyridin-2-ylthio)acetamido)acetate **15g** (80 mg, 0.17 mmol), TFA (26 µL, 0.34 mmol). White solid (67 mg, 98%); m.p. 268-271 ^{o}C; λ_{max} (EtOH/nm) 348.5, 276.5, 249.0; IR υ_{max}/cm^{-1} 3267, 2212, 1726, 1665, 1570, 1520; ^{1}H-NMR (500 MHz, DMSO-d_6) δ ppm 3.82 (2H, d, *J* = 5.7 Hz, NH-*CH*$_2$), 4.23 (2H, s, S-CH$_2$), 7.54-7.57 (3H, m, H-Ar and H-4'), 7.78 (2H, d, *J* = 6.1 Hz, CH-pyridine), 8.02 (1H, s, CH-pyridine), 8.30-8.32 (2H, m, H-Ar), 8.66 (1H, t, *J* = 5.7 Hz, NH), 8.83 (2H, d *J* = 6.1 Hz, N-CH-pyridine), 12.64 (1H, s, COOH); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 33.7, 41.1, 102.5, 115.2, 116.0, 123.1, 127.8, 128.9, 131.0, 136.3, 143.1, 150.2, 151.6, 158.4, 162.2, 167.2, 171.0; MS (ES+) *m/z* = 405.2 $[M+H]^+$; HRMS calcd for $C_{21}H_{16}N_4O_3S$ $[M+H]^+$ 405.1016, found 405.1015.

{2-[3-Cyano-4-(4-methoxyphenyl)-6-phenylpyridin-2-ylsulfanyl]acetylamino}acetic acid (4h)

General procedure H: *tert*-butyl 2-(2-((3-cyano-4-(4-methoxyphenyl)-6-phenylpyridin-2-yl)thio)acetamido)acetate, **15h** (0.200 g, 0.41 mmol), TFA (2 mL). White solid (0.165 g, 93%); m.p. 221.3˚C; λ_{max} (EtOH/nm) 275; IR υ_{max}/cm^{-1} 1659 (CO), 1742 (CO), 2214 (CN); ^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm 3.80 (2H, d, *J* = 6.8 Hz, CH$_2$), 3.85 (3H, s, CH$_3$), 4.19 (2H, s, CH$_2$), 7.16 (2H, d, *J* = 9.6 Hz, Ha and Ha'), 7.53 (3H, m, Hd, Hd' and He), 7.76 (2H, d, *J* = 9.6 Hz, Hb and Hb'), 7.89 (1H, s, CH-pyridine), 8.28 (2H, m, Hc and Hc'), 8.63 (1H, t, *J* = 6.8 Hz, NH), 12.55 (1H, br s, COOH); ^{13}C-NMR (75 MHz, DMSO-d_6) δ ppm 34.2, 41.6, 55.9 114.9, 128.1, 130.6, 131.0, 137.2, 147.6, 103.3 116.0, 116.4, 128.2, 154.3, 158.4, 161.4, 162.4, 167.7, 171.1; MS (ES+) *m/z* = 434.1 $[M+H]^+$; Anal. Calcd for $C_{23}H_{19}N_3O_4S$: C, 63.73; H, 4.42; N, 9.69. Found.C, 63.58; H, 4.49; N, 9.61.

{2-[3-Cyano-6-(4-methoxy-phenyl)-4-phenyl-pyridin-2-ylsulfanyl]-acetylamino}-acetic acid (4i)

General procedure G: **14i** (0.100 g, 0.31 mmol), *tert*-butyl 2-(2-bromoacetamido)acetate (0.118 g, 0.47 mmol), KOH (0.018 mg, 0.31 mmol), DMF (10 mL). The crude material was used directly in the next step.

General procedure H: **15i** (0.154 g, 0.31 mmol), TFA (2 mL). White solid (0.082 g, 61%); m.p. 224.7˚C; λ_{max} (EtOH/nm) 272; IR υ_{max}/cm^{-1} 1659 (CO), 1730 (CO), 2214 (CN), 2932 (COOH), 3265 (NH); ^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm 3.84 (2H, d, *J* = 1.5 Hz, CH_2N), 3.85 (3H, s, O-CH_3), 4.18 (2H, s, CH_2), 7.07 (2H, d, *J* = 8.7 Hz, Hd and Hd'), 7.59 (3H, m, Hb, Hb' and He), 7.72 (2H, m, Ha and Ha'), 7.85 (1H, s, CH-pyridine), 8.28 (2H, d, *J* = 8.7 Hz, Hc and Hc'), 8.62 (1H, t, *J* = 1.5 Hz, NH), 12.60 (1H, br s, COOH); ^{13}C-NMR (75 MHz, DMSO-d_6) δ ppm 14.4, 55.8, 60.0, 114.8, 128.9, 129.2, 129.5, 129.9, 130.3, 115.7, 136.3, 154.5, 162.1, 162.2, 167.7, 171.1; MS (ES+) *m/z* = 434.1 [M+H]$^+$; Anal. Calcd for $C_{23}H_{19}N_3O_4S$: C, 63.73; H, 4.42; N, 9.69. Found.C, 61.15; H, 4.62; N,6.93.

[2-(3-Cyano-6-methyl-4-phenylpyridin-2-ylsulfanyl)acetylamino]acetic acid (4j)

General procedure G: 6-methyl-4-phenyl-2-thioxo-1,2-dihydropyridine-3-carbonitrile (0.100 g, 0.44 mmol), *tert*-butyl 2-(2-bromoacetamido)acetate (0.167 g, 0.66 mmol), KOH (0.024 g, 0.43 mmol), DMF (5 mL). The crude material was used directly in the next step.

General procedure H: **15j** (0.100 g, 0.25 mmol), TFA (2 mL). White solid (0.051 g, 60%); m.p. 217.4˚C; λ_{max} (EtOH/nm) 261; IR υ_{max}/cm^{-1} 1626 (CO), 1715 (CO), 2215 (CN), 3294 (NH); ^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm 2.57 (3H, s, CH_3), 3.79 (2H, m, *CH_2*NH), 4.07 (2H, s, CH_2), 7.28 (1H, s, CH-pyridine), 7.57 (5H, m, H-Ar), 8.49 (1H, br s, NH), 12.56 (1H, br s, COOH); ^{13}C-NMR (75 MHz, DMSO-d_6) δ ppm 23.1, 32.3, 40.0, 114.2, 118.4, 127.1, 127.7, 128.7, 134.4, 152.2, 160.2, 160.8, 166.2, 169.5; MS (ES+) *m/z* = 342.1 [M+H]$^+$.

[2-(3-Cyano-4,6-dimethylpyridin-2-ylsulfanyl)acetylamino]acetic acid (4k)

General procedure H: *tert*-butyl 2-(2-((3-cyano-4,6-dimethylpyridin-2-yl)thio)acetamido)acetate **15k** (0.050 g, 1.5 mmol), TFA (10 mL). White solid (0.035 g, 81%); m.p. 219.0˚C; λ_{max} (EtOH/nm) 222, 265 and 304; IR υ_{max}/cm^{-1} 1632 (CO), 1717 (CO), 2214 (CN); ^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm 2.41 (6H, s, CH_3), 3.76 (2H, d, *J* = 4.2Hz, *CH_2*NH), 4.02 (2H, s, CH_2), 7.11 (1H, s, CH-pyridine), 8.45 (1H, t, *J* = 4.2 Hz, NH), 12.58 (1H, br s, COOH); ^{13}C-NMR (75 MHz, DMSO-d_6) δ ppm 19.9, 24.5, 33.7, 44.5, 120.9, 115.2, 152.7, 161.8, 167.8, 171.0, 173.6, 193.2; MS (ES+) *m/z* = 280.0748 [M+H]$^+$; Anal. Calcd for $C_{12}H_{13}N_3O_3S$: C, 51.60; H, 4.69; N, 15.04. Found.C, 47.10; H, 2.83; N, 14.27.

4-(3-Cyano-4-(4-fluorophenyl)-6-phenylpyridin-2-ylthio)butanoic acid (23a)

General procedure H: *tert*-butyl 4-(3-cyano-4-(4-fluorophenyl)-6-phenylpyridin-2-ylthio)butanoate **22a** (100 mg, 0.22 mmol), TFA (34 µL, 0.44 mmol). White solid (64 mg, 72%); m.p. 160-164 ˚C; λ_{max} (EtOH/nm) 272.0, 346.0; IR υ_{max}/cm^{-1} 2924, 2217, 1711, 1596, 1568, 1525, 1509; ^{1}H-NMR (500 MHz, DMSO-d_6) δ ppm 1.95 (2H, quint, *J* = 7.1 Hz, CH_2-*CH_2*-CH_2), 2.38 (2H, t, *J* = 7.1 Hz, S-CH_2), 3.41 (2H, t, *J* = 7.1 Hz, CH_2-CO), 7.40 (2H, dd, *J* = 8.8 and 8.9 Hz, H-3 and H-5), 7.49-7.50 (3H, m, H-Ar), 7.78 (2H, dd, *J* = 5.4 and 8.8 Hz, H-2 and H-6), 7.89 (1H, s, CH-pyridine), 8.24-8.26 (2H, m, H-

Ar), 12.12 (1H, s, COOH); MS (ES+) *m/z* = 393.2 $[M+H]^+$; HRMS calcd for $C_{22}H_{17}FN_2O_2S$ $[M+H]^+$ 393.1068, found 393.1069.

5-(3-Cyano-4-(4-fluorophenyl)-6-phenylpyridin-2-ylthio)pentanoic acid (23b)

General procedure H: *tert*-butyl 5-(3-cyano-4-(4-fluorophenyl)-6-phenylpyridin-2-ylthio)pentanoate **22b** (120 mg, 0.26 mmol), TFA (40 µL, 0.52 mmol). White solid (58 mg, 54%); m.p. 205-206 ˚C; λ_{max} (EtOH/nm) 270.0, 345.5; IR υ_{max}/cm^{-1} 2898, 2210, 1695; ^{1}H-NMR (500 MHz, DMSO-d_6) δ ppm 1.69-1.83 (4H, m, CH_2-*CH_2*-*CH_2*-CH_2), 2.30 (2H, t, *J* = 7.4 Hz, S-CH_2), 3.42 (2H, t, *J* = 7.0 Hz, CH_2-CO), 7.44 (2H, dd, *J* = 8.7 and 8.8 Hz, H-Ar), 7.55-7.57 (3H, m, H-Ar), 7.84 (2H, dd, *J* = 5.5 and 8.8 Hz, H-Ar and H-4'), 7.91 (1H, s, CH-pyridine), 8.27-8.30 (2H, m, H-Ar), 12.05 (1H, s, COOH); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 23.8, 28.3, 29.6, 33.2, 103.1, 115.7, 115.8 (d, J_{CF} = 21.8 Hz), 116.0, 127.5, 129.0, 130.8, 131.2 (d, J_{CF} = 8.9 Hz), 132.1 (d, J_{CF} = 2.7 Hz), 136.7, 153.2, 157.9, 162.7, 163.2 (d, J_{CF} = 248.3 Hz), 174.3; MS (ES+) *m/z* = 405.2 $[M-H]^-$; HRMS calcd for $C_{23}H_{19}FN_2O_2S$ $[M-H]^-$ 405.41362, found 405.40444.

General Procedure I

To the relevant chalcone **13** (1 eq.) and 2-cyanothioacetamide (1 eq.) was added a freshly prepared solution of NaOMe (1.6M in MeOH, 2.4 eq.). The resulting solution was heated at 80 ^{o}C for 1.5 h, then cooled to RT and concentrated *in vacuo*. The crude material was redissolved in DMF (1 mL/mmol) and the relevant bromoacetamide or bromoalkyl (1.5 eq.) was added. The solution was heated at 100 ^{o}C for 3-4 h, then cooled and diluted with H_2O (20 mL) and the product extracted into EtOAc (3 x 100 mL). Combined organic layers were washed with H_2O (3 x 100 mL and brine (50 mL) dried over Na_2SO_4 and concentrated in vacuo. Chromatography gave the products.

***tert*-Butyl 2-(2-(3-cyano-4-(3-fluorophenyl)-6-phenylpyridin-2-ylthio)acetamido)acetate (15b)**

General Procedure I: **13b** (88 mg, 0.39 mmol), 8.5% w/v sodium methoxide in MeOH (0.60 mL, 0.94 mmol), 2-cyanothioacetamide (39 mg, 0.39 mmol) followed by *tert*-butyl 2-(2-bromoacetamido)acetate (149 mg, 0.59 mmol). White solid (86 mg, 46%); m.p. 173-174 ˚C; λ_{max} (EtOH/nm) 270.0, 339.5; IR υ_{max}/cm^{-1} 2980, 2932, 2218, 1724, 1649, 1570, 1526, 1483, 1437, 1366; ^{1}H-NMR (500 MHz, $CDCl_3$) δ ppm 1.31 (9H, s, CH_3), 3.84 (2H, d, *J* = 5.1 Hz, NH-*CH_2*), 4.03 (2H, s, S-CH_2), 7.07 (1H, br s, NH), 7.17-7.20 (1H, m, H-Ar), 7.26 (1H, ddd, *J* = 2.2, 2.3 and 9.1 Hz, H-Ar), 7.36-7.38 (1H, ddd, *J* = 1.0, 2.2 and 7.7 Hz, H-Ar), 7.43-7.49 (4H, m, H-Ar), 7.53 (1H, s, CH-pyridine), 7.99-8.01 (2H, m, H-Ar); MS (ES+) *m/z* = 478.1 $[M+H]^+$.

***tert*-Butyl 2-(2-(3-cyano-4-(2-fluorophenyl)-6-phenylpyridin-2-ylthio)acetamido)acetate (15c)**

General Procedure I: **13c** (54 mg, 0.23 mmol), 8.5% w/v sodium methoxide in MeOH (0.35 mL, 0.55 mmol), 2-cyanothioacetamide (35 mg, 0.23 mmol) followed by *tert*-butyl 2-(2-bromoacetamido)acetate (88 mg, 0.35 mmol). White solid (22 mg, 20%); m.p. 164-168 ˚C; λ_{max} (EtOH/nm) 270.0, 341.5; IR υ_{max}/cm^{-1} 2972, 2216, 1730, 1653, 1616, 1573, 1525, 1485; ^{1}H-NMR (500 MHz, $CDCl_3$) δ ppm 1.31 (9H, s, CH_3), 3.84 (2H, d, *J* = 5.1 Hz, NH-*CH_2*), 4.03 (2H, s, S-CH_2), 7.09 (1H, br s, NH), 7.20-7.27 (2H, m, H-Ar), 7.40-7.49 (5H, m, H-Ar), 7.56 (1H, s, CH-pyridine), 7.98-8.00 (2H, m, H-Ar); MS (ES+) *m/z* = 478.1 $[M+H]^+$; HRMS calcd for $C_{26}H_{24}FN_3O_3S$ $[M+H]^+$ 478.1595, found 478.1590.

***tert*-Butyl 2-(2-((3-cyano-6-phenyl-4-(4-(trifluoromethyl)phenyl)pyridin-2-yl)thio)acetamido)acetate (15e)**

General Procedure I: **13e** (320 mg, 1.14 mmol), 8.5% w/v sodium methoxide in MeOH (1.75 mL, 2.74 mmol), 2-cyanothioacetamide (170 mg, 1.71 mmol) and *tert*-butyl 2-(2-bromoacetamido)acetate (860 mg, 3.42 mmol). White solid (170 mg, 28%); m.p. 203-205 ˚C; λ_{max} (EtOH/nm) 269.0, 340.5; IR υ_{max}/cm^{-1} 3294, 2982, 2212, 1743, 1660, 1572, 1525; ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.30 (9H, s, CH_3), 3.83 (2H, d, *J* = 5.1 Hz, NH-*CH*$_2$), 4.03 (2H, s, S-CH_2), 7.03 (1H, t, *J* = 5.1 Hz, NH), 7.43-7.45 (3H, m, H-Ar), 7.53 (1H, s, CH-pyridine), 7.67 (2H, d, *J* = 8.2 Hz, H-Ar), 7.75 (2H, d, *J* = 8.2 Hz, H-Ar), 7.98-8.00 (2H, m, H-Ar); ^{13}C-NMR (125 MHz, $CDCl_3$) δ ppm 28.0, 34.0, 42.3, 82.3, 103.9, 114.9, 116.5, 126.2 (q, J_{CF} = 3.9 Hz), 127.5, 128.9, 129.2, 131.1, 136.5, 139.4, 153.3, 159.5, 162.0, 167.8, 168.4, *C*-F_3, C-*CF*$_3$ and quaternary carbons are not visualised; MS (ES+) *m/z* = 528.3 $[M+H]^+$; HRMS calcd for $C_{27}H_{24}F_3N_3O_3S$ $[M+H]^+$ 528.1563, found 528.1560.

***tert*-Butyl 2-(2-((3-cyano-4-(2,4-difluorophenyl)-6-phenylpyridin-2-yl)thio)acetamido)acetate (15f)**

General Procedure I: (*E*)-3-(2,4-difluorophenyl)-1-phenylprop-2-en-1-one **13f** (590 mg, 2.43 mmol), 8.5% w/v sodium methoxide in MeOH (3.70 mL, 5.83 mmol), 2-cyanothioacetamide (370 mg, 3.65 mmol) and *tert*-butyl 2-(2-bromoacetamido)acetate (1.83 g, 7.29 mmol). White solid (490 mg, 41%); m.p. 156-158 ˚C; λ_{max} (EtOH/nm) 274.0, 328.0, 380.5; IR υ_{max}/cm^{-1} 3326, 2973, 2924, 2218, 1729, 1655, 1618; ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.30 (9H, s, CH_3), 3.83 (2H, d, *J* = 5.5 Hz, NH-*CH*$_2$), 4.02 (2H, s, S-CH_2), 6.94-7.02 (2H, m, H-Ar), 7.05 (1H, t, *J* = 5.5 Hz, NH), 7.40-7.46 (4H, m, H-5 and H-Ar), 7.51 (1H, d, *J* = 1.5 Hz, H-Ar,), 7.97-7.98 (2H, m, H-Ar); ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm 27.9, 33.9, 42.3, 82.3, 105.2 (dd, J_{CF} = 25.4 and 25.5 Hz), 105.3, 112.4 (dd, J_{CF} = 3.9 and 21.8 Hz), 114.7, 117.6 (d, J_{CF} = 1.9 Hz), 120.0 (dd, J_{CF} = 3.9 and 14.6 Hz), 127.5, 129.2, 131.0, 131.7 (dd, J_{CF} = 3.8 and 10.0 Hz), 136.5, 148.5, 159.3, 159.7 (dd, J_{CF} = 12.3 and 253.5 Hz), 161.5, 164.2 (dd, J_{CF} = 11.9 and 253.6 Hz), 167.9, 168.4; MS (ES+) *m/z* = 496.3 $[M+H]^+$; HRMS calcd for $C_{26}H_{23}F_2N_3O_3S$ $[M+H]^+$ 496.1501, found 496.1498.

***tert*-Butyl 2-(2-(3-cyano-6-phenyl-4-4'-bipyridin-2-ylthio)acetamido)acetate (15g)**

General Procedure I: **13g** (140 mg, 0.67 mmol), 8.5% w/v sodium methoxide in MeOH (1.0 mL, 1.68 mmol), 2-cyanothioacetamide (67 mg, 0.67 mmol) followed by *tert*-butyl 2-(2-bromoacetamido)acetate (0.25 g, 1.01 mmol). White solid (114 mg, 39%); m.p. 166-168 ˚C; λ_{max} (EtOH/nm) 221.0, 249.0, 276.5, 347.5; IR υ_{max}/cm^{-1} 2938, 2863, 2212, 1665; ^{1}H-NMR (500 MHz, $CDCl_3$) δ ppm 1.40 (9H, s, CH_3), 3.94 (2H, d, *J* = 5.2 Hz, NH-*CH*$_2$), 4.15 (2H, s, S-CH_2), 7.03 (1H, br s, NH), 7.56-7.57 (3H, m, H-Ar), 7.65 (1H, s, CH-pyridine), 7.90 (2H, d, *J* = 4.6 Hz, H-2 and H-6), 8.10 (2H, dd, *J* = 2.5 and 6.1 Hz, H-Ar), 8.95 (2H, d, *J* = 4.6 Hz, H-3 and H-5); ^{13}C-NMR (125 MHz, $CDCl_3$) δ ppm 28.0, 34.0, 42.3, 82.5, 103.2, 114.3, 115.8, 121.2, 124.5, 127.7, 129.4, 131.6, 136.1, 146.7, 150.1 , 160.2, 162.7, 167.4, 168.5; MS (ES+) *m/z* = 461.3 $[M+H]^+$; HRMS calcd for $C_{25}H_{24}N_4O_3S$ $[M+H]^+$ 461.1642, found 461.1636.

***tert*-Butyl 4-(3-cyano-4-(4-fluorophenyl)-6-phenylpyridin-2-ylthio)butanoate (22a)**

General Procedure I: **13a** (75 mg, 0.33 mmol), 8.5% w/v sodium methoxide in MeOH (0.50 mL, 0.80 mmol), 2-cyanothioacetamide (33 mg, 0.33 mmol) followed by *tert*-butyl bromobutyrate (112 mg, 0.50 mmol). White solid (50

mg, 34%); m.p. 104-105 ˚C; λ_{max} (EtOH/nm) 271.0; IR υ_{max}/cm^{-1} 2978, 2930, 2212, 1721, 1603, 1570; ^{1}H-NMR (500 MHz, $CDCl_3$) δ ppm 1.46 (9H, s, CH_3), 2.15 (2H, quint, *J* = 7.3 Hz, CH_2-*CH_2*-CH_2), 2.48 (2H, t, *J* = 7.3 Hz, S-CH_2), 3.48 (2H, t, *J* = 7.3 Hz, CH_2-CO), 7.24 (2H, dd, *J* = 8.4 and 8.6 Hz, H-3 and H-5), 7.51-7.55 (4H, m, CH-pyridine, H-3', H-4' and H-5'), 7.61-7.66 (2H, m, H-2 and H-6), 8.09-8.11 (2H, m, H-2' and H-6'); MS (ES+) *m/z* = 449.1 $[M+H]^+$.

***tert*-Butyl 5-(3-cyano-4-(4-fluorophenyl)-6-phenylpyridin-2-ylthio)pentanoate (22b)**

General Procedure I: **13a** (118 mg, 0.52 mmol), 8.5% w/v sodium methoxide in MeOH (0.80 mL, 1.25 mmol), 2-cyanothioacetamide (52 mg, 0.52 mmol) followed by *tert*-butyl bromovalerate (185 mg, 0.78 mmol). White solid (140 mg, 58%); m.p. 120-121˚C; λ_{max} (EtOH/nm) 272.0, 347.5; IR υ_{max}/cm^{-1} 2984, 2936, 2214, 1707, 1595, 1566; ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 1.44 (9H, s, CH_3), 1.80-1.92 (4H, m, CH_2-*CH_2*-*CH_2*-CH_2), 2.30 (2H, t, *J* = 7.3 Hz, S-CH_2), 3.43 (2H, t, *J* = 6.9 Hz, CH_2-CO), 7.25 (2H, dd, *J* = 8.4 and 8.6 Hz, H-3 and H-5), 7.50 (1H, s, CH-pyridine), 7.51-7.56 (3H, m, H-3', H-4' and H-5'), 7.61-7.65 (2H, m, H-2 and H-6), 8.09-8.11 (2H, m, H-2' and H-6'); ^{13}C-NMR (125 MHz, $CDCl_3$) δ ppm 24.4, 28.1, 28.7, 30.3, 35.1, 80.3, 103.7, 115.4, 115.8, 116.2 (d, J_{CF} = 23.0 Hz), 127.3, 129.0, 130.4 (d, J_{CF} = 8.7 Hz), 130.7, 132.3 (d, J_{CF} = 3.4 Hz), 137.4, 153.4, 158.6, 164.0 (d, J_{CF} = 254.6 Hz), 164.3, 172.7; MS (ES+) *m/z* = 463.1 $[M+H]^+$; HRMS calcd for $C_{27}H_{27}FN_2O_2S$ $[M+H]^+$ 463.1850, found 463.1848.

2-((3-Cyano-4-(4-fluorophenyl)-6-phenylpyridin-2-yl)thio)-N-(2-oxopropyl)acetamide (31)

General Procedure I: 4-fluorochalcone **13a** (15 mg, 0.067 mmol), 8.5% w/v sodium methoxide in MeOH (0.10 mL, 0.16 mmol), 2-cyanothioacetamide (7 mg, 0.067 mmol) and 2-bromo-*N*-(2-oxopropyl)acetamide **30** (20 mg, 0.10 mmol). White solid (10 mg, 36%); m.p. 231-233 ˚C; λ_{max} (EtOH/nm) 270.0, 338.0; IR υ_{max}/cm^{-1} 3281, 2920, 2216, 1732, 1659; ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 2.02 (3H, s, CH_3), 4.03 (2H, s, S-CH_2), 4.04 (2H, d, *J* = 5.9 Hz, NH-*CH_2*), 7.16-7.20 (2H, m, H-Ar), 7.26 (1H, br s, NH), 7.44-7.45 (3H, m, H-Ar), 7.52 (1H, s, CH-pyridine), 7.56-7.59 (2H, m, H-Ar), 7.99-8.00 (2H, m, H-Ar); MS (ES+) *m/z* = 420.3 $[M+H]^+$; HRMS calcd for $C_{23}H_{18}FN_3O_2S$ $[M+H]^+$ 420.1177, found 420.1180.

2-((3-Cyano-6-phenyl-[4,4'-bipyridin]-2-yl)thio)-N-(2-oxopropyl)acetamide (32)

General Procedure I: 4-pyridylchalcone (48 mg, 0.23 mmol), 8.5% w/v sodium methoxide in MeOH (0.35 mL, 0.56 mmol), 2-cyanothioacetamide (46 mg, 0.23 mmol) and 2-bromo-*N*-(2-oxopropyl)acetamide **30** (67 mg, 0.35 mmol). Purification *via* column chromatography (silica; 0-15% MeOH/DCM). White solid (72 mg, 78%); m.p. 238-240 ˚C; λ_{max} (EtOH/nm) 345.0, 277.0, 249.0; IR υ_{max}/cm^{-1} 2913, 2213, 1730, 1657, 1570, 1517; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 2.03 (3H, s, CH_3), 3.98 (2H, d, *J* = 5.5 Hz, NH-*CH_2*), 4.25 (2H, s, S-CH_2), 7.55-7.56 (3H, m, H-Ar and H-4'), 7.78 (2H, d, *J* = 6.9 Hz, CH-pyridine), 8.03 (1H, s, CH-pyridine), 8.30-8.32 (2H, m, H-Ar), 8.61 (1H, t, *J* = 5.5 Hz, NH), 8.83 (2H, d, *J* = 6.9 Hz, N-CH-pyridine); ^{13}C NMR (125 MHz, DMSO-*d_6)* δ ppm 26.9, 33.7, 49.5, 102.5, 115.2, 116.0, 123.1, 127.8, 128.9, 131.0, 136.3, 143.1, 150.2, 151.6, 158.4, 162.2, 167.2, 204.3; MS (ES+) *m/z* = 403.3 $[M+H]^+$; HRMS calcd for $C_{22}H_{18}N_4O_2S$ $[M+H]^+$ 403.1223, found 403.1218.

***tert*-Butyl 2-(2-hydroxyethylamino)acetate (24)**

To a solution of ethanolamine (3.0 mL, 49.7 mmol) in THF (0.5 mL/mmol) was added *tert*- butyl bromoacetate (2 mL, 13.5 mmol) dropwise. The resulting solution was stirred at RT for 72 h. The mixture was concentrated *in vacuo* and the residue redissolved in DCM (50 mL). The organic phase was washed with an aqueous solution of saturated $NaHCO_3$ (50 mL) and brine (50 mL), then dried over Na_2SO_4 and concentrated *in vacuo* to give **24** as a yellow oil (1.96 g, 82%). IR υ_{max}/cm^{-1} 3399, 2970, 1730, 1638; ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 1.49 (9H, s, CH_3), 2.82 (2H, t, *J* = 5.0 Hz, *CH*$_2$-NH), 3.36 (2H, s*, CH*$_2$-CO), 3.65 (2H, t, *J* = 5.0 Hz, *CH*$_2$-OH), NH and OH not visualised; MS (ES+) *m/z* = 176.2 $[M+H]^+$.

tert-Butyl 2-((_tert_-butoxycarbonyl)(2-((3-cyano-4-(4-fluorophenyl)-6-phenylpyridin-2-yl)thio)ethyl)amino)acetate (27)

To a solution of *tert*-butyl 2-(2-hydroxyethylamino)acetate **24** (0.53 g, 3.03 mmol) in DCM (3 mL/mmol) was added Boc anhydride (0.80 g, 3.64 mmol), Et_3N (0.51 mL, 3.64 mmol) and DMAP (10 mol%). The resulting solution was stirred at RT for 3 h. The mixture was diluted with water (15 mL) and the product extracted with DCM (2 x 15 mL). Combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The resulting solid was redissolved in DCM (3 mL/mmol). Mesyl chloride (0.28 mL, 3.64 mmol) and triethylamine (0.63 mL, 4.55 mmol) were added at 0 ^oC. The resulting solution was warmed to RT and stirred for 16 h. The reaction mixture was quenched with an aqueous solution of $NaHCO_3$ and extracted with EtOAc (3 x 50 mL). Combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The resulting oil was redissolved in DMF (4 mL) and 4-(4-fluorophenyl)-6-phenyl-2-thioxo-1,2-dihydropyridine-3-carbonitrile **14a** (90 mg, 0.29 mmol) and KOH (17 mg, 0.32 mmol) were added. The resulting solution was heated to 100 ^oC for 3 h, cooled, diluted with water and extracted with DCM (3 x 20 mL). Combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification *via* column chromatography (silica; 0-50% EtOAc/petrol) gave **27** as a white solid (20 mg, 12%). m.p. 152-154 $^\circ C$; λ_{max} (EtOH/nm) 338.0, 270.0; IR υ_{max}/cm^{-1} 3282, 2926, 2215, 1653, 1526, 1508; ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.25 (9H, s, CH_3), 1.34 (9H, s, CH_3), 3.38 (2H, t, *J* = 7.0 Hz, S-CH_2), 3.57 (2H, t, *J* = 7.0 Hz, S-CH_2-*CH*$_2$), 4.59 (2H, s, NH-*CH*$_2$), 7.17-7.20 (2H, m, dd, *J* = 8.6 and 8.7 Hz, H-Ar), 7.39-7.42 (3H, m, H-Ar), 7.57-7.80 (3H, m, H-Ar and CH-pyridine), 8.02-8.09 (2H, m, H-Ar); MS (ES+) *m/z* = 564.7 $[M+H]^+$.

2-((2-((3-Cyano-4-(4-fluorophenyl)-6-phenylpyridin-2-yl)thio)ethyl)amino)acetic acid (28)

General procedure H: *tert*-butyl 2-((*tert*-butoxycarbonyl)(2-((3-cyano-4-(4-fluorophenyl)-6-phenylpyridin-2-yl)thio)ethyl)amino)acetate **27** (15 mg, 0.027 mmol), TFA (5 µL, 0.054 mmol). White solid (10 mg, 91%); m.p. 193-194 $^\circ C$; λ_{max} (EtOH/nm) 339.0, 269.0; IR υ_{max}/cm^{-1} 3285, 3073, 2926, 2215, 1653, 1508; ^{1}H NMR (500 MHz, MeOD) δ ppm 3.17 (2H, t, *J* = 7.1 Hz, CH_2), 3.31 (2H, s, CH_2), 3.63 (2H, t, *J* = 7.1 Hz, CH_2), 7.45-7.51 (2H, m, H-Ar), 7.54-7.62 (3H, m, H-Ar), 7.83-7.88 (2H, m, H-Ar), 7.97 (1H, s, CH-pyridine), 8.30-8.39 (2H, m, H-Ar); MS (ES+) *m/z* = 408.3 $[M+H]^+$; HRMS calcd for $C_{22}H_{18}FN_3O_2S$ $[M-H]^-$ 406.1031, found 406.1026.

2-(3-Cyano-4-(4-fluorophenyl)-6-phenylpyridin-2-ylthio)-*N*-(2-(4 methoxybenzylamino)-2-oxoethyl)acetamide, 16

To a solution of 2-(2-(3-cyano-4-(4-fluorophenyl)-6-phenylpyridin-2-ylthio)acetamido)acetic acid **4a** (180 mg, 0.43 mmol) in DMF (20 mL) was added HBTU (200 mg, 0.52 mmol), DIPEA (50 µL, 0.52 mmol) and *p*-methoxybenzylamine (0.56 mL, 4.3 mmol). The resulting mixture was heated at 60 °C for 4.5 h. The mixture was cooled and water (10 mL) added, which resulted in precipitation of the product. Filtration gave **16** as a white solid (110 mg, 47%). m.p. 216-220 ˚C; λ_{max} (EtOH/nm) 269.5, 339.0; IR υ_{max}/cm^{-1} 3281, 3069, 2212, 1632, 1547, 1508; ^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm 3.71 (3H, s, O-CH_3), 3.77 (2H, d, *J* = 3.9 Hz, NH-*CH_2*-CO), 4.17 (2H, d, *J* = 3.9 Hz, NH-*CH_2*-Ar), 4.21 (2H, s, S-CH_2), 6.84 (2H, d, *J* = 8.9 Hz, H-Ar), 7.14 (2H, d, *J* = 8.9 Hz, H-Ar), 7.43-7.53 (5H, m, H-Ar), 7.80-7.83 (2H, m, H-Ar), 7.93 (1H, s, CH-pyridine), 8.21-8.30 (3H, m, NH and H-Ar), 8.63 (1H, t, *J* = 5.9 Hz, NH); MS (ES+) *m/z* = 541.6 $[M+H]^+$; HRMS calcd for $C_{30}H_{25}FN_4O_3S$ $[M+H]^+$ 541.1704, found 541.1700.

***N*-(2-Amino-2-oxoethyl)-2-((3-cyano-4-(4-fluorophenyl)-6-phenylpyridin-2-yl)thio)acetamide, 17**

General procedure H: 2-((3-cyano-4-(4-fluorophenyl)-6-phenylpyridin-2-yl)thio)-*N*-(2-((4- methoxybenzyl)amino)-2-oxoethyl)acetamide **16** (50 mg, 0.093 mmol),TFA (5 mL/mmol) gave **17** as a white solid (11 mg, 52%). m.p. 231-232 ˚C; λ_{max} (EtOH/nm) 338.0, 269.5; IR υ_{max}/cm^{-1} 3285, 3073, 2926, 2215, 1653, 1508; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 3.57 (2H, d, *J* = 5.5 Hz, NH-*CH_2*), 4.09 (2H, s, S-CH_2), 7.24 (1H, s, NH_2), 7.35 (2H, dd, *J* = 8.6 and 8.7 Hz, H-Ar), 7.42-7.43 (3H, m, H-Ar and H-4'), 7.72 (2H, dd, *J* = 5.4 and 8.6 Hz, H-Ar), 7.83 (1H, s, CH-pyridine), 8.17-8.19 (2H, m, H-Ar), 8.40 (1H, t, *J* = 5.5 Hz, NH); MS (ES+) *m/z* = 421.3 $[M+H]^+$; HRMS calcd for $C_{22}H_{17}FN_4O_2S$ $[M+H]^+$ 421.1129, found 421.1130.

General procedure J

A mixture of 2-amino-6-chloro-pyrimidine-3-carbonitrile **33** (1.0 eq), and the corresponding aniline (1.0 eq) in DMF (2 ml) was heated to 100 °C for 24 hours, then allowed to cool to rt, poured into water (20 mL) water and extracted with dichloromethane (3 x 20 mL). The combined organic layers were washed with water (3 x 20 mL)and brine (20 mL),dried (Na_2SO_4) and concentrated *in vacuo.* Recrystallization (ethanol) gave the product.

4-Amino-2-(2-tolylamino)pyrimidine-5-carbonitrile (5a)[12,13]

General procedure J: 4-amino-2-chloropyrimidine-5-carbonitrile (0.200 g, 1.29 mmol), *o*-toluidine (0.089 mL, 1.29 mmol) and DMF (2 mL) gave **5a** as a white solid (0.145 g, 50%); m.p. 186.7˚C; λ_{max} (EtOH/nm) 260 and 302; IR υ_{max}/cm^{-1} 2208 (CN); 3163 (NH); ^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm 2.17 (3H, s, CH_3), 7.09 (6H, m, NH_2 and H-Ar), 8.26 (1H, s, H-6), 9.00 (s, 1H, NH); ^{13}C-NMR (75 MHz, DMSO-d_6) δ ppm 18.2, 80.4, 117.2, 125.6, 126.3, 126.7, 130.6, 133.6, 137.5, 161.8, 162.4, 163.6; MS (ES+) *m/z* = 226.1 $[M+H]^+$.

4-Amino-2-(3-methoxyphenylamino)pyrimidine-5-carbonitrile (5b)

General procedure J: 4-amino-2-chloropyrimidine-5-carbonitrile (0.200 g, 1.29 mmol), 3-methoxyaniline (0.100 mL, 1.29 mmol) and DMF (2 mL). Yellow solid (0.121 g, 39%); m.p. 196.0˚C; λ_{max} (EtOH/nm) 313; IR υ_{max}/cm^{-1} 2214 (CN); 3435 (NH); ^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm 3.72 (3H, s, O-CH_3), 6.56 (1H, d, *J* = 7.8 Hz, Hc), 7.15 (1H, t, *J* = 7.8 Hz, Hb), 7.30 (1H, d, *J* = 7.8 Hz, Ha), 7.36-7.48 (3H, m, Hd and NH_2), 8.34 (1H, s, H-6), 9.64 (1H, s, NH); ^{13}C-NMR (75

MHz, DMSO-d_6) δ ppm 55.5, 80.9, 106.6, 108.4, 113.0, 117.0, 129.5, 141.1, 160.0, 160.6, 162.2, 163.4; MS (ES+) *m/z* = 242.1 $[M+H]^+$; $C_{12}H_{11}N_5O$ requires C, 59.74; H, 4.60; N, 29.03; found C, 59.80; H, 4.40; N, 28.99

4-Amino-2-(4-fluorophenylamino)pyrimidine-5-carbonitrile (5c)

General procedure J: 4-amino-2-chloropyrimidine-5-carbonitrile (0.200 g, 1.29 mmol), 4-fluoroaniline (0.123 mL, 1.29 mmol) and DMF (2 mL). Yellow solid (0.129 g, 44%); m.p. 264.6˚C; λ_{max} (EtOH/nm) 310; IR υ_{max}/cm^{-1} 2214 (CN); 3329 (NH); ^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm 7.10 (2H, t, *J* = 8.4 Hz, Ha and Hd), 7.48 (2H, br s, NH_2), 7.76 (2H, br s, Hb and Hc), 8.35 (1H, s, H-6), 9.73 (1H, s, NH); ^{13}C-NMR (75 MHz, DMSO-d_6) δ ppm 80.9, 115.0, 115.5, 117.0, 122.2, 122.4, 136.4, 156.6, 160.6, 162.2, 163.5; MS (ES+) *m/z* = 230.1 $[M+H]^+$; $C_{11}H_8FN_5$ requires C, 57.64; H, 3.52; N, 30.55; found C, 57.81; H, 3.42; N, 30.35

General procedure K:

Methyl pyrrole-2-carboxylate (1.01 g, 8.04 mmol) was added to a stirred solution of aluminium trichloride (1.54 g, 16.1 mmol) and the benzoylchloride derivative (3.35 g, 16.1 mmol) in DCM (20 mL) at 0 ^{o}C, and the resulting mixture was stirred for 4h at rt. The reaction was quenched by careful addition of water, diluted by addition of an equal amount of EtOAc and water, and extracted with EtOAc (3 x). The combined organic layers were washed with $NaHCO_3$ (sat., aq.), water and brine before being dried (Na_2SO_4), and concentrated *in vacuo*. Chromatography (SiO_2; 40-80% EtOAc, petroleum ether) and precipitation (EtOAc/MeOH/petroleum ether) gave **35a** as a yellow solid (1.24 g, 52%)

Methyl 4-(2,3-dichlorobenzoyl)-1*H*-pyrrole-2-carboxylate (35a)

General procedure K: methyl pyrrole-2-carboxylate (1.01 g, 8.04 mmol), aluminium trichloride (1.54 g, 16.1 mmol), 2,3-dichlorobenzoylchloride derivative (3.35 g, 16.1 mmol) in DCM (20 mL) at 0 ^{o}C, gave **35a** as a yellow solid (1.24 g, 52%); mp 189.9-190.7 °C. λ_{max} (EtOH/nm) 285. IR υ_{max}/cm^{-1} 3285, 3113, 2953, 1687, 1656. ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 3.80 (3H, s, OCH_3), 7.02 (1H, s, pyrrole-CH), 7.44-7.50 (3H, m, pyrrole-CH, ClCC*H*CHCH, ClCCHC*H*CH), 7.79 (1H, dd, *J* = 1.5, 7.5 Hz, ClCCHCHC*H*), 12.87 (1H, br s, pyrrole-NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 51.6, 115.1, 124.3, 124.8, 126.9, 127.5, 128.6, 130.4, 131.5, 132.3, 141.3, 160.3, 187.2. MS (ES^+) *m/z* = 299.48 $[M+H]^+$

Methyl 4-(2,4-dichlorobenzoyl)-1*H*-pyrrole-2-carboxylate (35b)

General procedure K: methyl pyrrole-2-carboxylate (3.0 g, 24 mmol), aluminium chloride (5.52 g, 60 mmol) and 2,4-dichlorobenzoylchloride (10.1 g, 48 mmol) **35b** was obtained as a yellow solid (4.54 g, 64%). mp 173-174 °C, λ_{max} (EtOH/nm) 284. IR υ_{max}/cm^{-1} 3190, 3114, 1705, 1628, 1582, 1555, 1481, 1447, 1393. ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 3.79 (s, 3H, OCH_3), 7.02 (s, 1H, PyrH), 7.46 (s, 1H, PyrH), 7.48-7.57 (m, 2H, 2 x ArH), 7.74 (*app.* d, 1H, *J* = 1.2 Hz, ArH), 12.84 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 51.0, 114.7, 123.9, 124.7, 127.0, 129.1, 129.6, 130.4, 134.6, 137.7, 159.9, 186.7. HRMS (ES^+) calcd for $[M+NH_4]^+$, 315.0298, found, 315.0296 (^{35}Cl). Anal. calcd for $C_{13}H_9Cl_2NO_3$: C, 52.37%, H, 3.04%, N, 4.70%. Found: C, 52.25%, H, 3.09%, N, 4.71%

Methyl 4-(2-(trifluoromethyl)benzoyl)-1*H*-pyrrole-2-carboxylate (35c)

General procedure K: methyl pyrrole-2-carboxylate (3.0 g, 24 mmol), aluminium chloride (5.8 g, 60 mmol) and 2-trifluoromethylbenzoylchloride (10.0 g, 48 mmol) **35c** was obtained as a yellow solid (1.43 g, 20%). mp 141-142 °C, λ_{max} (EtOH/nm) 279, 232. IR υ_{max}/cm^{-1} 3273, 1707, 1640, 1553, 1439, 1385, 1308, 1274.; ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 3.78 (s, 3H, OCH_3), 6.98 (s, 1H, =CH), 7.39 (s, 1H, =CH), 7.58 (d, 1H, *J* = 6.6 Hz, ArH), 7.66-7.81 (m, 2H, 2 x ArH), 7.86 (d, 1H, *J* = 7.5 Hz, ArH), 12.83 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 50.8, 114.5, 119.8 (q, J_{C-F} = 272 Hz, CF_3), 123.8, 125.1, 125.7 (q, J_{C-F} = 31 Hz, *C*CF_3), 126.1 (q, J_{C-F} = 4.7 Hz, ArH), 127.6, 129.2, 129.6, 131.8, 138.6, 160.0, 188.3. HRMS (ES$^+$) calcd for $C_{14}H_{10}F_3NO_3$ $[M+H]^+$, 298.0686, found, 298.0688. Anal. calcd for $C_{14}H_{10}F_3NO_3$ requires C, 56.57%, H, 3.39%, N, 4.71%. Found: C, 56.64%, H, 3.59%, N, 4.89%

4-Benzoyl-1*H*-pyrrole-2-carboxylate (35d)

General procedure K: methyl pyrrole-2-carboxylate (3.0 g, 24 mmol), aluminium chloride (5.52 g, 60 mmol) and benzoylchloride (6.7 g, 48 mmol) **35d** was obtained as a yellow solid (1.83 g, 90%); mp 148-149 °C, λ_{max} (EtOH/nm) 285 and 238. IR ν_{max} (cm^{-1}): 3293, 1715, 1620, 1596, 1555, 1446, 1384. ^{1}H NMR (300 MHz, DMSO-d_6) δ_H ppm 3.81 (s, 3H, OCH_3), 7.15 (s, 1H, =CH), 7.48-7.67 (m, 4H, 3 x ArH and =CH), 7.73-7.83 (m, 2H, 2 x ArH), 12.76 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C ppm 30.1, 115.5, 123.2, 124.2, 128.0, 128.7, 131.3, 138.6, 160.1, 188.6. HRMS (ES$^+$) calcd for $C_{13}H_{11}NO_3$ $[M+H]^+$, 230.0812, found, 230.0811. Anal. calcd for $C_{13}H_{11}NO_3$: C, 68.11%, H, 4.84%, N, 6.11%. Found: C, 68.33%, H, 4.78%, N, 6.21%

1-(4-(2,3-Dichlorobenzoyl)-1*H*-pyrrol-2-yl)ethanone (39)

General procedure K: 2-acetyl pyrrole (600 mg, 5.40 mmol), 2,3-dichlorobenzoyl chloride (2.30 g, 11.0 mmol) and aluminium chloride (1.32 g, 13.7 mmol) in DCM (10 mL). Chromatography (silica gel, 3:2 EtOAc/petrol) gave **39** as a pale pink solid (850 mg, 56%); R_f = 0.65 (2:3 EtOAc/petrol); mp: 144-146 ˚C; λ_{max} (EtOH/nm) 290; IR (cm^{-1}) 3348 (N-H), 1655 (C=O), 1519 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.23 (3H, s, CH_3), 7.23 (1H, d, *J* = 1.4 Hz, pyrrole-H), 7.32-7.34 (2H, m, ArH and pyrrole-H), 7.37 (1H, dd, *J* = 7.7 and 7.8 Hz, ArH), 7.68 (1H, dd, *J* = 1.7 and 7.8 Hz, ArH), 12.5 (1H, s br, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 25.8 (CH_3), 116.5, 124.8, 126.9, 127.5, 128.6, 131.4, 131.5, 132.3, 133.6, 141.4, 187.4 (C=O), 188.4 (C=O); HRMS calcd. for $C_{13}H_{10}{}^{35}Cl_2NO_2$ $[M+H]^+$ 282.0083, found 282.0083.

(*Z*)-3-(4-(2,3-Dichlorobenzoyl)-1*H*-pyrrol-2-yl)-3-hydroxy-1-(pyridin-4-yl)prop-2-en-1-one (44)

General procedure K: **43** (200 mg, 0.71 mmol), aluminium chloride (400 mg, 2.50 mmol) and 2,3-dichlorobenzoyl chloride (135 mg, 1.40 mmol) in DCM (10 mL). Chromatography (silica, 4:1 EtOAc/petrol) gave **44** as a pale yellow solid (150 mg, 55 %); mp: 243-245 ˚C; λ_{max} (EtOH/nm) 358, 294; IR (cm^{-1}) 3125, 1619, 1598; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.36 (1H, s, CHCO), 7.49-7.54 (2H, m, 2 × ArH), 7.64 (1H, s, pyrrole-H), 7.82 (1H, dd, *J* = 1.5 and 7.0 Hz, ArH), 7.84 (1H, s, pyrrole-H), 7.99 (2H, d, *J* = 6.0 Hz, 2 × pyridyl-H), 8.79 (2H, d, *J* = 6.0 Hz, 2 × pyridyl-H), 12.95 (1H, s br, NH), 15.90 (1H, s br, OH); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 95.2, 117.2, 120.1, 125.7, 127.1, 127.6, 128.7, 131.6, 131.8, 132.4, 132.5, 140.5, 141.3, 150.5, 173.4, 182.9, 187.4; HRMS calcd. for $C_{19}H_{13}{}^{35}Cl_2N_2O_3$ $[M+H]^+$ 387.0298, found 387.0296.

General procedure L:

Lithium hydroxide (6.4 g, 268 mmol) was added to a stirred solution of the appropriate methyl pyrrole-2-carboxylate (2.0 g, 6.7 mmol) in THF (50 mL) and H_2O (80 mL). The resulting mixture was stirred at 65 °C for 20 h, then HCl (1M) was added to pH 7, and extracted with EtOAc (3 x 10 mL). The combined organics were washed with H_2O (50 mL) and brine (50 mL), dried (Na_2SO_4), and concentrated *in vacuo*. Chromatography (SiO_2; 50-80% EtOAc/petroleum ether) and precipitation (EtOAc/MeOH/petroleum ether) gave the product.

4-(2,3-Dichlorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (36a)

General procedure L: Lithium hydroxide (6.4 g, 268 mmol), **35a** (2.0 g, 6.7 mmol) in THF (50 mL) and H_2O (80 mL). gave **36a** as a slightly pink solid (88%); mp 249-250 °C, λ_{max} (EtOH/nm) 285, 238. IR υ_{max}/cm^{-1} 3300 (OH), 1672 (CO), 1638, 1550, 1443, 1384. ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 6.97 (*app.* t, 1H, *J* = 2.0 Hz, PyrH), 7.34-7.40 (m, 1H, PyrH), 7.40-7.52 (m, 2H, 2 x ArH), 7.76 (dd, 1H, *J* = 7.2 and 2.4 Hz, ArH), 12.66 (s, 1H, NH), 12.90 (br s, 1H, COOH). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 114.3, 124.4, 125.3, 126.5, 127.3, 128.1, 129.2, 131.0, 132.1, 141.3, 160.8, 186.7. HRMS (ES^+) calcd for $C_{12}H_7{}^{35}Cl_2NO_3$ $[M+NH_4]^+$, 301.0141, found, 301.0139. Anal. calcd for $C_{12}H_7Cl_2NO_3$: C, 50.73%, H, 2.48%, N, 4.93%. Found: C, 50.59%, H, 2.26%, N, 4.78%.

4-(2,4-Dichlorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (36b)

General procedure L: **35b** (1.0 g, 3.35 mmol) gave **36b** as slightly pink solid (785 mg, 82%); mp 213-214 °C, λ_{max} (EtOH/nm) 284, 233. IR υ_{max}/cm^{-1} 3299 (NH), 1674(CO), 1641, 1584, 1553, 1499, 1439, 1385, 1279, 1223, 1101, 880, 861, 756 (Cl). ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 3.42 (OH), 6.97 (s, 1H, PyrH), 7.38 (s, 1H, PyrH), 7.46-7.57 (m, 2H, 2 x ArH), 7.74 (s, 1H, ArH), 12.64 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 114.2, 124.6, 125.4, 127.0, 129.07, 129.13, 129.6, 130.4, 134.5, 137.9, 160.9, 186.8. HRMS (ES^+) calcd for $C_{12}H_7{}^{35}Cl_2NO_3$ $[M+H]^+$, 282.9798, found, 282.9797 (^{35}Cl). Anal. calcd for $C_{12}H_7{}^{35}Cl_2NO_3$: C, 50.73%, H, 2.48%, N, 4.93%. Found: C, 50.88%, H, 2.33%, N, 4.79%

4-(2-(Trifluoromethyl)benzoyl)-1*H*-pyrrole-2-carboxylic acid (36c)

General procedure L: **35c** (1.0 g, 3.36 mmol) gave **36c** as a slightly pink solid (762 mg, 80%); mp 221-222 °C, λ_{max} (EtOH/nm) 279, 232. IR υ_{max}/cm^{-1} 3317 (NH), 1639 (CO), 1557, 1443, 1388, 1313, 1281, 1227. ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 6.93 (*app.* t, 1H, *J* = 1.8 Hz, =CH), 7.26-7.36 (m, 1H, =CH), 7.57 (d, 1H, *J* = 6.9 Hz, ArH), 7.66-7.81 (m, 2H, 2 x ArH), 7.86 (d, 1H, *J* = 6.6 Hz, ArH), 12.63 (s, 1H, NH), 12.90 (br s, 1H, COOH). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 114.0, 123.1 (q, $J_{C\text{-}F}$ = 272 Hz, CF_3), 124.7, 124.8, 125.4 (q, $J_{C\text{-}F}$ = 31.4 Hz, *C*CF_3), 125.8 (q, $J_{C\text{-}F}$ = 4.6 Hz, ArH), 127.4, 128.5, 129.3, 131.5, 138.4, 160.6, 188.1. HRMS (ES^+) calcd for $C_{13}H_8F_3NO_3$ $[M+H]^+$, 284.0529, found, 284.0527.

4-Benzoyl-1H-pyrrole-2-carboxylic acid (36d)

General procedure L: **35d** (1.0 g, 3.36 mmol) gave **36d** was obtained as a slightly pink solid (811 mg, 86%); mp 225-226 °C, λ_{max} (EtOH/nm) 285, 237. IR υ_{max}/cm^{-1} 3333, 1667, 1624, 1549, 1426, 1382. ^{1}H NMR (300 MHz, DMSO-d_6) δ_H

ppm 7.11 (s, 1H, =CH), 7.44-7.67 (m, 4H, 3 x ArH and =CH), 7.72-7.84 (m, 2H, 2 x ArH), 12.55 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C ppm 115.1, 124.1, 124.6, 128.1, 128.4, 131.4, 138.8, 161.1, 188.8. HRMS (ES^+) calcd for $C_{12}H_9NO_3$ $[M+H]^+$, 216.0655, found, 216.0655. Anal. calcd for $C_{12}H_9NO_3$: C, 66.97%, H, 4.22%, N, 6.51%. Found: C, 66.81%, H, 4.17%, N, 6.33%.

4-(2,3-Dichlorobenzoyl)-1-methyl -1*H*-pyrrole-2-carboxylic acid (36e)

To a solution of **35a** (100 mg, 0.34 mmol) in DMF (3 mL) was added sodium hydride (12 mg, 0.51 mmol) with stirring at 0 °C, followed by methyl iodide (72 mg, 0.51 mmol). The mixture was allowed to warm to rt and stirring continued 18h, then evaporated. Precipitation from EtOAc, petrol gave the ester. The crude material was treated according to General Procedure L to give **36e** (97 mg, 72%). ^{1}H NMR (300 MHz, DMSO-d_6) δ_H ppm 3.90 (s, 3H, CH_3) 7.03 (d, J = 1.5 Hz, 1H, PrH), 7.40-7.51 (m, 2H, PrH and ArH), 7.58-7.62 (m, 1H, ArH), 7.75-7.85 (m, 1H, ArH). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C ppm 36.6, 116.8, 121.8, 125.1, 126.5, 127.3, 128.1, 131.0, 132.0, 134.9, 141.2, 160.9, 186.3.

General procedure M:

A solution of carbonyldiimidazole (2.0 eq.) and the required carboxylic acid (1.0 eq.) in THF (5 mL/mmol) was heated to 80 °C for 4 h. The appropriate amine (2.5 eq.) was added and the mixture heated at 50 °C for 3 h then at RT for 18 h. The product was extracted into EtOAc (50 mL/mmol), washed with water (50 mL/mmol), brine (50 mL/mmol) and dried over Na_2SO_4. The solvent was removed under vacuum and the crude product purified as specified.

4-(2,3-Dichlorobenzoyl)-*N*-(4-fluorobenzyl)-1*H*-pyrrole-2-carboxamide (6a)

General procedure M: CDI (55 mg, 0.34 mmol) and **36a** (100 mg, 0.35 mmol), THF (3 mL), 4-fluorobenzylamine (70 mg, 0.56 mmol). Chromatography (SiO_2; 30% EtOAc, petrol) gave **6a** as a white solid (60 mg, 44%); mp 222-223 °C, λ_{max} (EtOH/nm) 288, 237. IR υ_{max}/cm^{-1} 3364 (OH), 3172 (NH), 3119 (NH), 2922, 2851, 2372, 1616 (CO), 1568, 1505, 1391, 1288, 1223, 1150 (CF), 851, 797, 744, 696 (Cl). HPLC (Method **A**): 99.7% R_t = 18.6 min. ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 4.40 (s, 2H, CH_2), 7.10-7.24 (m, 3H, 2 x ArH and =CH), 7.27-7.37 (m, 3H, 2 x ArH and =CH), 7.40-7.52 (m, 2H, 2 x ArH), 7.77 (dd, 1H, *J* = 7.5 and 1.8 Hz, ArH), 8.90 (*app.* t, 1H, *J* = 5.7 Hz, NH), 12.46 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 41.1 (CH_2), 110.2 (=CH), 114.6 (d, J_{C-F} = 21 Hz, 2 x ArH), 124.1 (C), 126.5 (ArH), 127.3 (C), 128.0 (C-Cl), 128.1 (=CH and ArH), 128.9 (d, J_{C-F} = 8 Hz, 2 x ArH), 130.9 (ArH), 132.0 (C, Ar), 135.3 (CH_2*C*), 141.6 (C-Cl), 159.5 (CON), 160.9 (d, J_{C-F} = 245 Hz, C-F), 187.0 (CO). HRMS (ES^+) calcd for $C_{19}H_{13}{}^{35}Cl_2FN_2O_2$ $[M+NH_4]^+$, 408.0676, found, 408.0672. Anal. calcd for $C_{19}H_{13}Cl_2FN_2O_2$: C, 58.33%, H, 3.35%, N, 7.16%. Found: C, 58.33%, H, 3.45%, N, 7.18%.

4-(2,4-Dichlorobenzoyl)-*N*,*N*-dimethyl-1*H*-pyrrole-2-carboxamide (6b)

General procedure M: CDI (114 mg, 0.7 mmol), **36b** (100 mg, 0.35 mmol),THF (4 mL), dimethylamine (2M in THF; 0.44 mL, 0.88 mmol). Chromatography (SiO_2; 5-10% MeOH/EtOAc) and precipitation (EtOAc/MeOH/petroleum ether) gave **6b** as a white solid (80 mg, 73%); mp 208-209 °C, λ_{max} (EtOH/nm) 287. IR υ_{max}/cm^{-1} 3151, 1640, 1586, 1553, 1484, 1377, 1341. ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 3.07 (br s, 3H, CH_3), 3.17 (br s, 3H, CH_3), 6.90 (s, 1H, PyrH),

7.21 (s, 1H, PyrH), 7.47-7.56 (m, 2H, 2 x ArH), 7.74 (s, 1H, ArH), 12.29 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C ppm 36.7, 111.2, 124.1, 126.9, 127.0, 127.9, 129.0, 129.6, 130.5, 134.4, 138.0, 160.9, 186.8. HRMS (ES^+) calcd for $C_{14}H_{12}{}^{35}Cl_2N_2O_2$ $[M+H]^+$, 311.0349, found, 311.0345. Anal. calcd for $C_{14}H_{12}Cl_2N_2O_2$ requires C, 54.04%, H, 3.89%, N, 9.00%. Found: C, 53.94%, H, 3.68%, N, 8.98%

4-(2,4-Dichlorobenzoyl)-*N*-phenethyl-1*H*-pyrrole-2-carboxamide (6c)

General procedure M: CDI (114 mg, 0.7 mmol), **36b** (100 mg, 0.35 mmol), THF (4 mL), phenylethylamine (107 mg, 0.88 mmol). Chromatography (SiO_2; 50% EtOAc, petrol) and precipitation (EtOAc/MeOH/petroleum ether) gave **6c** as a white solid (80 mg, 59%); mp 226-227 °C, λ_{max} (EtOH/nm) 288, 239. IR υ_{max}/cm^{-1} 3356 (NH), 1643 (CO), 1602, 1570, 1533, 1493, 1288. ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 2.81 (t, 2H, J = 7.1 Hz, CH_2), 3.40-3.51 (m, 2H, CH_2), 7.15 (s, 1H, PyrH), 7.15-7.34 (m, 6H, PyrH and 5 x ArH), 7.45-7.59 (m, 2H, 2 x ArH), 7.75 (s, 1H, ArH), 8.35-8.46 (m, 1H, NH), 12.35 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C ppm 34.8, 39.8, 109.8, 124.2, 125.6, 126.9, 127.7, 127.9, 128.1, 128.4, 129.0, 129.5, 130.4, 134.3, 138.2, 139.1, 159.5, 186.9. HRMS (ES^+) calcd for $C_{20}H_{16}{}^{35}Cl_2N_2O_2$ $[M+H]^+$, 387.0662, found, 387.0666. $C_{20}H_{16}Cl_2N_2O_2$ requires C, 62.03%, H, 4.16%, N, 7.23%. Found: C, 61.88%, H, 3.98%, N, 7.13%

***N*-Methyl-4-(2-(trifluoromethyl)benzoyl)-1*H*-pyrrole-2-carboxamide (6d)**

General procedure M: CDI (113 mg, 0.7 mmol), **36c** (100 mg, 0.35 mmol), THF (4 mL), methylamine (2M in THF, 0.44 mL, 0.88 mmol). Chromatography (SiO_2; 40-95% EtOAc, petrol) and precipitation (EtOAc/MeOH/petroleum ether) gave **6d** as a white solid (71 mg, 68%); mp 223-224 °C, λ_{max} (EtOH/nm) 284, 235. IR υ_{max}/cm^{-1} 3381 (NH), 3178 (NH), 3119, 1621 (CO), 1574, 1535, 1487, 1394, 1314, 1288, 1240. ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 2.72 (d, 3H, J = 4.5 Hz, CH_3), 7.10 (s, 1H, =CH), 7.14 (s, 1H, =CH), 7.56 (d, 1H, J = 6.9 Hz, ArH), 7.67-7.81 (m, 2H, 2 x ArH), 7.85 (d, 1H, J = 8.1 Hz, ArH), 8.28 (*app.* d, 1H, J = 4.5 Hz, NH), 12.34 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 24.8, 109.3, 123.2 (q, $J_{C\text{-}F}$ = 272 Hz, CF_3), 124.4, 125.4 (q, $J_{C\text{-}F}$ = 32 Hz, *C*CF_3), 125.6 (q, $J_{C\text{-}F}$ = 4.7 Hz, ArH), 127.1, 127.4, 128.0, 129.1, 131.4, 138.7, 159.8, 188.2. HRMS (ES^+) calcd for $C_{14}H_{11}F_3N_2O_2$ $[M+H]^+$, 297.0845, found, 297.0843.

4-Benzoyl-*N*-pyridin-3-ylmethyl-1*H*-pyrrole-2-carboxamide (6e)

General procedure M: CDI (113 mg, 0.7 mmol), **36d** (75 mg, 0.35 mmol), THF (4 mL), 3-pyridylmethylamine (61 mg, 0.56 mmol) **6e** was obtained as a white solid (104 mg, 73%); mp 214-215 °C, λ_{max} (C_2H_5OH/nm) 289 and 242. IR υ_{max}/cm^{-1} 3340, 3181, 3055, 1668), 1532, 1423. ^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm 4.47 (d, 2H, J = 5.7 Hz, CH_2), 7.30-7.40 (m, 2H, ArH and =CH), 7.43 (s, 1H, =CH), 7.47-7.66 (m, 3H, 3 x ArH), 7.67-7.83 (m, 3H, 3 x ArH), 8.46 (d, 1H, J = 3.9 Hz, ArH), 8.55 (br s, 1H, ArH), 8.93 (*app.* t, 1H, J = 5.6 Hz, NH), 12.35 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C ppm 39.6, 111.1, 122.9, 123.8, 127.1, 127.3, 128.0, 131.1, 134.6, 134.7, 139.0, 147.7, 148.5, 159.9, 188.9. HRMS (ES^+) calcd for $C_{18}H_{15}N_3O_2$ $[M+H]^+$, 306.1237, found, 306.1233. Anal. calcd for $C_{18}H_{15}N_3O_2$: C, 70.81%, H, 4.95%, N, 13.76%. Found: C, 70.20%, H, 4.78%, N, 13.41%.

4-(2,3-Dichlorobenzoyl)-*N*-methyl-1*H*-pyrrole-2-carboxamide (6f)

General procedure M: CDI (55 mg, 0.34 mmol) and **36a** (100 mg, 0.35 mmol), THF (3 mL), methylamine (2M in THF, 0.44 mL, 0.88 mmol) **6f** was obtained as a white solid (72 mg, 69%); mp 254-255 °C, λ_{max} (C_2H_5OH/nm) 287 and 235. IR υ_{max}/cm^{-1} 3362, 3187, 3130, 1619, 1580, 1537, 1491. ^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm 2.73 (*app.* d, 3H, *J* = 3.6 Hz, CH_3), 7.11 (s, 1H, =CH), 7.24 (s, 1H, =CH), 7.40-7.52 (m, 2H, 2 x ArH), 7.77 (dd, 1H, *J* = 7.5 and 1.2 Hz, ArH), 8.29 (*app.* d, 1H, *J* = 3.3 Hz, NH), 12.39 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C ppm 24.8, 109.4, 123.8, 126.2, 127.0, 127.5, 127.8, 128.2, 130.6, 131.7, 141.3, 159.7, 186.7. HRMS (ES^+) calcd for $C_{13}H_{10}{}^{35}Cl_2N_2O_2$ $[M+H]^+$, 297.0192, found, 297.0194. Anal. calcd for $C_{13}H_{10}Cl_2N_2O_2$: C, 52.55%, H, 3.39%, N, 9.43%. Found: C, 52.25%, H, 3.10%, N, 9.20%.

4-(2,3-Dichlorobenzoyl)-*N*,*N*-dimethyl-1*H*-pyrrole-2-carboxamide (6g)

General procedure M: CDI (55 mg, 0.34 mmol) and **36a** (100 mg, 0.35 mmol), THF (3 mL), dimethylamine (2M in THF, 0.44 mL, 0.88 mmol) **6g** was obtained as a slightly brown solid (105 mg, 96%); mp 223-224 °C, λ_{max} (C_2H_5OH/nm) 286 and 233. IR υ_{max}/cm^{-1} 3200, 3117, 2922, 2851, 1647, 1599, 1541, 1505, 1406. ^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm 3.04 (br s, 3H, CH_3), 3.02 (br s, 3H, CH_3), 6.92 (s, 1H, =CH), 7.21 (dd, 1H, *J* = 3.3 and 1.2 Hz, =CH), 7.41-7.52 (m, 2H, 2 x ArH), 7.77 (dd, 1H, *J* = 6.9 and 1.2 Hz, ArH), 12.32 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C ppm 36.7, 36.8, 111.2, 123.9, 126.5, 127.1, 127.3, 128.0, 130.9, 132.0, 141.4, 160.9, 186.7. HRMS (ES^+) calcd for $C_{14}H_{12}{}^{35}Cl_2N_2O_2$ $[M+H]^+$, 311.0349, found, 311.0347 (^{35}Cl).

4-(2,3-Dichlorobenzoyl)-*N*-(pyridin-4-ylmethyl)-1*H*-pyrrole-2-carboxamide (6h)

General procedure M: CDI (114 mg, 0.7 mmol), **36a** (100 mg, 0.35 mmol),THF (4 mL), 4-pyridylmethylamine (61 mg, 0.56 mmol) **6h** was obtained as a white solid (128 mg, 97%); mp 244-245 °C, λ_{max} (C_2H_5OH/nm) 285 and 236. IR υ_{max}/cm^{-1} 3337, 3121, 3057, 2920, 2850, 1618, 1564, 1527, 1491, 1410. ^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm 3.59 (d, 2H, *J* = 5.7 Hz, CH_2), 6.39 (s, 1H, =CH), 6.42 (d, 2H, *J* = 5.7 Hz, 2 x ArH), 6.46 (s, 1H, =CH), 6.54-6.66 (m, 2H, 2 x ArH), 6.91 (dd, 1H, *J* = 7.5 and 2.1 Hz, ArH), 7.64 (d, 2H, *J* = 7.5 Hz, ArH), 8.12 (*app.* t, 1H, *J* = 5.9 Hz, NH), 11.63 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C ppm 40.9, 110.4, 121.8, 124.1, 126.5, 127.3, 127.9, 128.1, 130.8, 132.0, 141.5, 148.0, 149.1, 159.8, 186.9. HRMS (ES^+) calcd for $C_{18}H_{13}{}^{35}Cl_2N_2O_2$ $[M+H]^+$, 374.0458, found, 374.0459.

4-(2,3-Dichlorobenzoyl)-*N*-(pyridin-3-ylmethyl)-1*H*-pyrrole-2-carboxamide (6i)

General procedure M: CDI (114 mg, 0.7 mmol), **36a** (100 mg, 0.35 mmol),THF (4 mL), 3-pyridylmethylamine (61 mg, 0.56 mmol) **6i** was obtained as a white solid (79 mg, 60%); mp 178-179 °C, λ_{max} (C_2H_5OH/nm) 287 and 237. IR υ_{max}/cm^{-1} 3435, 3168, 1624, 1575, 1541, 1489, 1392, 1293. ^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm 4.47 (d, 2H, *J* = 2.7 Hz, CH_2), 7.20 (s, 1H, =CH), 7.30 (s, 1H, =CH), 7.32-7.39 (m, 1H, ArH), 7.40-7.52 (m, 2H, 2 x ArH), 7.69 (d, 1H, *J* = 7.8 Hz, ArH), 7.76 (dd, 1H, *J* = 7.5 and 1.8 Hz, ArH), 8.45 (d, 1H, *J* = 4.8 Hz, ArH), 8.52 (s, 1H, ArH), 8.93 (*app.* t, 1H, *J* = 5.7 Hz, NH), 12.45 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C ppm 41.2, 110.3, 123.0, 124.1, 126.5, 127.3, 128.0, 128.1, 130.9, 132.0, 134.5, 134.7, 141.5, 147.7, 148.5, 159.7, 187.0. HRMS (ES^+) calcd for $C_{18}H_{13}{}^{35}Cl_2N_2O_2$ $[M+H]^+$, 374.0458, found, 374.0456.

4-(2,3-Dichlorobenzoyl)-*N*-(pyridin-2-ylmethyl)-1*H*-pyrrole-2-carboxamide (6j)

General procedure M: CDI (114 mg, 0.7 mmol), **36a** (100 mg, 0.35 mmol),THF (4 mL), 2-pyridylmethylamine (61 mg, 0.56 mmol) **6j** was obtained as a white solid (111 mg, 84%); mp 228-229 °C, λ_{max} (C_2H_5OH/nm) 286 and 236. IR (Diamond ATR) υ_{max}/cm^{-1} 3372, 3165, 3121, 1621, 1570, 1526, 1494, 1291, 1211, 904, 745, 698. HPLC (Method **A**): 99.9% R_t = 10.9 min; (Method **B**): 99.8% R_t = 14.0 min. ^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm 4.52 (d, 2H, *J* = 5.7 Hz, CH_2), 7.20-7.35 (m, 4H, 2 x =CH and 2 x ArH), 7.40-7.54 (m, 2H, 2 x ArH), 7.70-7.82 (m, 2H, 2 x ArH), 8.50 (d, 1H, *J* = 3.9 Hz, ArH), 8.96 (*app.* t, 1H, *J* = 5.4 Hz, NH), 12.44 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C ppm 43.9 (CH_2), 110.4 (=CH), 120.7 (ArH), 121.6 (ArH), 124.1 (C), 126.5 (ArH), 127.3 (C), 128.0 (ArH), 128.1 (=CH), 128.2 (ArH), 130.8 (ArH), 132.0 (C, Ar), 136.2 (ArH), 141.6 (C-Cl), 148.4 (ArH), 158.3 (C, Ar), 159.7 (CON), 186.9 (CO). HRMS (ES^+) calcd for $C_{18}H_{13}{}^{35}Cl_2N_2O_2$ $[M+H]^+$, 374.0458, found, 374.0459 (^{35}Cl). Anal. calcd for $C_{18}H_{13}Cl_2N_2O_2$ C, 57.77%, H, 3.50%, N, 11.23%. Found: C, 57.75%, H, 3.23%, N, 11.20%.

***N*-Benzyl-4-(2,3-dichlorobenzoyl)-1*H*-pyrrole-2-carboxamide (6k)**

General procedure M: CDI (114 mg, 0.7 mmol), **36a** (100 mg, 0.35 mmol),THF (4 mL), benzylamine (60 mg, 0.56 mmol), **6k** was obtained as a white solid (62 mg, 46%); mp 223-224 °C, λ_{max} (C_2H_5OH/nm) 286 and 237. IR υ_{max}/cm^{-1} 3347, 3161, 3123, 2922, 1620, 1570, 1533, 1491, 1410. ^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm 4.43 (d, 2H, *J* = 6.0 Hz, CH_2), 7.17-7.37 (m, 7H, 2 x =CH and 5 x ArH), 7.40-7.51 (m, 2H, 2 x ArH), 7.76 (dd, 1H, *J* = 7.5 and 2.1 Hz, ArH), 8.88 (*app.* t, 1H, *J* = 6.0 Hz, NH), 12.43 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C ppm 42.1, 110.4, 124.4, 126.6, 126.7, 127.2, 127.5, 128.1, 128.2, 128.3, 128.5, 131.1, 132.2, 139.4, 141.9, 159.8, 187.2. HRMS (ES^+) calcd for $C_{19}H_{14}{}^{35}Cl_2N_2O_2$ $[M+H]^+$, 373.0505, found, 373.0502.

4-(2,3-Dichlorobenzoyl)-*N*-phenethyl-1*H*-pyrrole-2-carboxamide (6l)

General procedure M: CDI (114 mg, 0.7 mmol), **36a** (100 mg, 0.35 mmol),THF (4 mL), phenethylamine (68 mg, 0.56 mmol), **6l** was obtained as a white solid (56 mg, 41%); mp 232-233 °C, λ_{max} (C_2H_5OH/nm) 288 and 236. IR υ_{max}/cm^{-1} 3404, 3332, 3125, 2924, 2854, 1655, 1618, 1572, 1533, 1492, 1433, 1392, 1288, 1242, 1192, 1141, 747, 696. ^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm 2.80 (t, 2H, *J* = 7.4 Hz, CH_2), 3.44 (*app.* q, 2H, *J* = 6.7 Hz, NHCH_2), 7.13 (s, 1H, =CH), 7.15-7.33 (m, 6H, =CH and 5 x ArH), 7.39-7.52 (m, 2H, 2 x ArH), 7.77 (dd, 1H, *J* = 7.8 and 1.8 Hz, ArH), 8.42 (*app.* t, 1H, *J* = 5.6 Hz, NH), 12.37 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C ppm 34.8, 39.8, 109.8, 124.0, 125.6, 126.5, 127.3, 127.8, 127.9, 128.0, 128.2, 128.4, 130.8, 132.0, 139.1, 141.6, 159.5, 186.9. HRMS (ES^+) calcd for $C_{20}H_{16}{}^{35}Cl_2N_2O_2$ $[M+H]^+$, 387.0662, found, 387.0662.

4-(2,3-Dichlorobenzoyl)-1-methyl-*N*-(pyridin-3-ylmethyl)-1*H*-pyrrole-2-carboxamide (6m)

General procedure M: CDI (88 mg, 0.54 mmol), **36e** (80 mg, 0.27 mmol),THF (4 mL), 3-pyridylmethylamine (73 mg, 0.68 mmol), **6m** was obtained as a white solid (63 mg, 61%); mp 183-184 °C, λ_{max} (C_2H_5OH/nm) 284 and 240. IR υ_{max}/cm^{-1} 3106, 1641, 1518, 1281. ^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm 3.86 (s, 3H, CH_3), 4.41 (d, 2H, *J* = 5.7 Hz, CH_2),

7.24 (s, 1H, =CH), 7.35 (dd, 1H, *J* = 7.2 and 5.1 Hz, ArH), 7.39-7.57 (m, 3H, =CH and 2 x ArH), 7.69 (d, 1H, *J* = 7.8 Hz, ArH), 7.77 (d, 1H, *J* = 7.5 Hz, ArH), 8.45 (d, 1H, *J* = 4.5 Hz, ArH), 8.52 (s, 1H, ArH), 8.91 (*app.* t, 1H, *J* = 5.3 Hz, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C ppm 36.5, 112.5, 121.5, 122.9, 126.4, 127.3, 127.5, 128.0, 130.8, 132.0, 133.8, 134.55, 134.62, 141.4, 147.6, 148.5, 160.3, 186.3. HRMS (ES$^+$) calcd for $C_{19}H_{15}{}^{35}Cl_2N_3O_2$ $[M+H]^+$, 388.0614, found, 388.0611. Anal. calcd for $C_{19}H_{15}Cl_2N_3O_2$: C, 58.78%, H, 3.89%, N, 10.82%. Found: C, 58.49%, H, 3.71%, N, 10.41%

4-(2,3-Dichlorobenzoyl)-*N*-methyl-*N*-(pyridin-3-ylmethyl)-1*H*-pyrrole-2-carboxamide (6n)

General procedure M: CDI (114 mg, 0.7 mmol), **36a** (100 mg, 0.35 mmol),THF (4 mL), *N*-methyl-1-(pyridin-3-yl)methanamine (68 mg, 0.56 mmol), **6n** was obtained as a white solid (68 mg, 50%); mp 175-176 °C, λ_{max} (C_2H_5OH/nm) 286 and 235. IR υ_{max}/cm^{-1} 3225, 3061, 1649, 1597, 1548, 1481, 1449, 1410. ^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm 3.21 (br s, 3H, CH_3), 4.74 (br s, 2H, CH_2), 7.25 (s, 1H, =CH), 7.32-7.51 (m, 3H, 2 x ArH and =CH), 7.68 (d, 1H, *J* = 7.5 Hz, ArH), 7.75 (dd, 1H, *J* = 7.2 and 2.1 Hz, ArH), 8.50 (dd, 1H, *J* = 4.7 and 1.7 Hz, ArH), 8.52 (s, 1H, ArH), 12.43 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C ppm 35.6, 49.4, 111.5, 123.2, 124.0, 126.5, 126.6, 127.3, 128.0, 128.3, 130.9, 132.0, 132.6, 134.6, 141.3, 148.1, 148.4, 161.3, 186.7. HRMS (ES$^+$) calcd for $C_{19}H_{15}{}^{35}Cl_2N_3O_2$ $[M+H]^+$, 388.0614, found, 388.0613. Anal. calcd for $C_{19}H_{15}Cl_2N_3O_2$: C, 58.78%, H, 3.89%, N, 10.82%. Found: C, 58.77%, H, 4.01%, N, 10.61%

(4-(2,3-Dichlorobenzoyl)-1*H*-pyrrol-2-yl)(3,4-dihydro-2,6-naphthyridin-2(1*H*)-yl)methanone (6o)

General procedure M: **36a** (50 mg, 0.18 mmol), CDI (60 mg, 0.35 mmol), 1,2,3,4-tetrahydro-2,6-naphthyridine (60 mg, 0.45 mmol) in THF (3 mL). Chromatography (KP-NH silica, 1:9 MeOH/EtOAc) gave **6o** as a white solid (40 mg, 55%); mp: 225-227 ˚C; λ_{max} (EtOH/nm) 287; IR (cm^{-1}) 3202, 1604, 1551; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 2.95 (2H, s, NCH_2CH_2), 3.95 (2H, s, NCH_2CH_2), 4.90 (2H, s, NCH_2), 7.04 (1H, s, pyrrole-H), 7.27 (1H, s, pyrrole-H), 7.32 (1H, d, *J* = 5.1 Hz, pyridyl-H), 7.46-7.51 (2H, m, 2 × ArH), 7.79 (1H, dd, *J* = 2.0 and 7.4 Hz, ArH), 8.37 (1H, d, *J* = 5.1 Hz, pyridyl-H), 8.43 (1H, s, pyridyl-H), 12.45 (1H, s br, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 121.3, 124.2, 127.0, 127.6, 128.5, 130.5, 131.4, 132.3, 141.6, 142.0, 147.0, 161.3 (C=O), 187.2 (C=O); HRMS calcd. for $C_{20}H_{16}{}^{35}Cl_2N_3O_2$ $[M+H]^+$ 400.0614, found 400.0618.

(4-(2,3-Dichlorobenzoyl)-1*H*-pyrrol-2-yl)(isoindolin-2-yl)methanone (6p)

General procedure M: **36a** (50 mg, 0.18 mmol), CDI (60 mg, 0.35 mmol), isoindoline (55 mg, 0.05 mL, 0.45 mmol) in THF (3 mL). Chromatography (silica gel, 1:1 EtOAc/petrol) gave **6p** as a white solid (54 mg, 78%); mp: 290-292 ˚C; λ_{max} (EtOH/nm) 285; IR (cm^{-1}) 3191, 1606, 1585; ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.89 (2H, s, NCH_2), 5.19 (2H, s, NCH_2), 7.22-7.23 (1H, m, pyrrole-H), 7.26 (1H, s br, pyrrole-H), 7.33-7.35 (2H, m, 2 × ArH), 7.41-7.45 (2H, m, 2 × ArH), 7.47-7.52 (2H, m, 2 × ArH), 7.80 (1H, dd, *J* = 2.0 and 7.4 Hz, ArH), 12.47 (1H, s br, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 53.0, 53.3, 111.8, 122.8, 123.1, 124.8, 127.0, 127.4, 127.6, 127.8, 128.6, 129.7, 131.4, 132.3, 135.2, 137.1, 141.5, 159.2, 187.3; HRMS calcd. for $C_{20}H_{15}{}^{35}Cl_2N_2O_2$ $[M+H]^+$ 385.0505, found 385.0505.

(4-(2,3-Dichlorobenzoyl)-1*H*-pyrrol-2-yl)(3,4-dihydroisoquinolin-2(1*H*)-yl)methanone (6q)

General procedure M: **36a** (50 mg, 0.18 mmol), CDI (60 mg, 0.35 mmol), 1,2,3,4-tetrahydroisoquinoline (60 mg, 0.06 mL, 0.45 mmol) in THF (3 mL). Chromatography (silica gel, 1:1 EtOAc/petrol) gave **6q** as a white solid (65 mg, 91%); mp: 192-194 ˚C; λ_{max} (EtOH/nm) 286; IR (cm^{-1}) 3202, 1604, 1548; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 2.93 (2H, s, NCH_2CH_2), 3.91 (2H, s, NCH_2CH_2), 4.84 (2H, s, NCH_2), 7.01 (1H, s, pyrrole-H), 7.20-7.28 (5H, m, pyrrole-H, 4 × ArH), 7.47-7.49 (2H, m, 2 × ArH), 7.79 (1H, dd, *J* = 2.4 and 7.1 Hz, ArH), 12.38 (1H, s br, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 124.2, 126.2, 126.4, 126.6, 127.0, 127.2, 127.6, 128.3, 128.5, 129.3, 131.4, 132.3, 133.3, 134.8, 141.6, 187.3 (C=O); HRMS calcd. for $C_{21}H_{17}{}^{35}Cl_2N_2O_2$ $[M+H]^+$ 399.0662, found 399.0662.

(4-(2,3-Dichlorobenzoyl)-1*H*-pyrrol-2-yl)(1*H*-pyrrolo[3,4-*c*]pyridin-2(3*H*)-yl)methanone (6r)

General procedure M: **36a** (55 mg, 0.2 mmol), CDI (65 mg, 0.4 mmol), 2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine hydrochloride (60 mg, 0.5 mmol) in THF (3 mL). Chromatography (KP-NH silica, 1:9 MeOH/EtOAc) gave **6r** as a white solid (43 mg, 56%); mp: 258-260 ˚C; λ_{max} (EtOH/nm) 286; IR (cm^{-1}) 3181, 1633, 1577; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 4.94 (2H, d, *J* = 10.2 Hz, NCH_2), 5.18 (2H, d, *J* = 7.8 Hz, NCH_2), 7.22 (1H, s, pyrrole-H), 7.24 (1H, s, pyrrole-H), 7.31 (1H, dd, *J* = 1.6 and 7.6 Hz, ArH), 7.35 (1H, dd, *J* = 7.6 and 7.7 Hz, ArH), 7.41-7.43 (1H, m, pyridyl-H), 7.60 (1H, dd, *J* = 1.6 and 7.7 Hz, ArH), 8.40 (1H, d, *J* = 5.0 Hz, pyridyl-H), 8.53 (1H, s, pyridyl-H), 13.24 (1H, s br, pyrrole-NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 51.4, 52.7, 111.9, 118.5, 124.9, 127.6, 128.5, 129.8, 131.4, 131.9, 132.2, 141.5, 144.6, 148.0, 159.2, 187.3; HRMS calcd. for $C_{19}H_{14}{}^{35}Cl_2N_3O_2$ $[M+H]^+$ 386.0458, found 386.0460.

(*E*)-1-(4-(2,3-Dichlorobenzoyl)-1*H*-pyrrol-2-yl)-3-(pyridin-4-yl)prop-2-en-1-one (40)

A mixture **39** (200 mg, 0.70 mmol) and 4-pyridine carboxaldehyde (75 mg, 0.07 mL, 0.70 mmol) in EtOH (1 mL) was cooled to 0 ^{o}C, potassium hydroxide (40%, 285 mg in 0.7 mL H_2O) was added dropwise and the resulting solution stirred at rt for 18 h. Upon addition of H_2O (40 mL) a precipitate formed which was collected by filtration. Recrystallisation (EtOH) gave **40** as a beige solid (245 mg, 95%); mp: 262-265 ˚C; λ_{max} (EtOH/nm) 324, 286, 219; IR (cm^{-1}) 3225, 1649, 1588; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.49-7.52 (2H, m, 2 × ArH), 7.59 (1H, s, pyrrole-H), 7.68 (1H, d, *J* = 16.0 Hz, alkene-H), 7.82 (1H, dd, *J* = 2.4 and 7.0 Hz, ArH), 7.87 (2H, d, *J* = 6.0 Hz, 2 × pyridyl-H), 7.92 (1H, s, pyrrole-H), 8.07 (1H, d, *J* = 16.0 Hz, alkene-H), 8.67 (2H, d, *J* = 6.0 Hz, 2 × pyridyl-H), 12.92 (1H, s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 117.8, 122.6, 125.3, 126.5, 127.1, 127.6, 128.7, 131.6, 132.4, 132.8, 134.4, 139.5. 141.4, 141.7, 149.5, 150.3, 178.6, 187.5; HRMS calcd. for $C_{19}H_{11}{}^{35}Cl_2N_2O_2$ $[M-H]^-$ 369.0203, found 369.0201.

1-(4-(2,3-Dichlorobenzoyl)-1*H*-pyrrol-2-yl)-3-(pyridin-4-yl)propan-1-one (41)

To ammonium chloride (205 mg, 3.85 mmol), in ethanol (0.30 mL) and water (0.30 mL) was added **40** (75 mg, 0.20 mmol) followed by indium powder (35 mg, 0.30 mmol). The mixture was heated at reflux for 8 h, cooled to RT and diluted with H_2O (10 mL). The solid was extracted into EtOAc (2 × 25 mL), washed with brine (25 mL) and dried (Na_2SO_4) and evaporated. Chromatography(C18 silica, 70% MeCN, 0.1% formic acid, water) gave **41** as an off-white solid (20 mg, 27%); mp: 249-251 ˚C; λ_{max} (EtOH/nm) 294, 237; IR (cm^{-1}) 1640, 1551; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.93 (2H, t, *J* = 7.5 Hz, CH_2CH_2), 3.26 (2H, t, *J* = 7.5 Hz, CH_2CH_2), 7.31 (2H, d, *J* = 6.0 Hz, 2 × pyridyl-H), 7.42 (1H, s, pyrrole-H), 7.44 (1H, dd, *J* = 1.7 and 7.7 Hz, ArH), 7.46 (1H, s br, pyrrole-H), 7.49 (1H, dd, *J* = 7.7 and 7.9 Hz, ArH), 7.79

(1H, dd, *J* = 1.7 and 7.9 Hz, ArH), 8.45 (2H, d, *J* = 6.0 Hz, 2 × pyridyl-H), 12.66 (1H, s br, NH); ^{13}C NMR (125 MHz, CD_3OD) δ 30.4, 38.7, 117.3, 125.8, 126.8, 127.9, 129.2, 129.9, 132.6, 132.8, 134.7, 134.8, 142.9, 149.7, 153.6, 190.3, 191.0; HRMS calcd. for $C_{19}H_{15}{}^{35}Cl_2N_2O_2$ $[M+H]^+$ 373.0505, found 373.0509.

(*E*)-(4-(2,3-Dichlorobenzoyl)-1*H*-pyrrol-2-yl)(2-(pyridin-4-yl)cyclopropyl)methanone (42)

A solution of (*E*)-1-(4-(2,3-dichlorobenzoyl)-1*H*-pyrrol-2-yl)-3-(pyridine-4-yl)prop-2-en-1-one **40** (100 mg, 0.26 mmol) in DMSO (1 mL) was added to a mixture of trimethylsulfoxonium iodide (70 mg, 0.32 mmol) and potassium *tert*-butoxide (35 mg, 0.32 mmol). The resulting solution was stirred at rt for 24 h then further trimethylsulfoxonium iodide (70 mg, 0.32 mmol) and potassium *tert*-butoxide (35 mg, 0.32 mmol) was added, and stirring continued for a further 24 h until complete by LCMS. The mixture was treated with brine (10 mL) and the product extracted with EtOAc (2 × 10 mL), washed with water (10 mL), brine (10 mL), dried(Na_2SO_4) and evaporated. HPLC (C18 silica 1:1 0.1% Formic acid, MeCN) gave **42** as a white solid (30 mg, 24%); mp: 125-127 ˚C; λ_{max} (EtOH/nm) 293, 234; IR (cm^{-1}) 3119, 1636, 1548; ^{1}H NMR (500 MHz, CD_3OD) δ ppm 1.41 (1H, ddd, *J* = 4.2, 6.4 and 8.4 Hz, C*H*$_2$), 1.63 (1H, ddd, *J* = 4.2, 5.4 and 9.2 Hz, C*H*$_2$), 2.44 (1H, ddd, *J* = 4.2, 6.4 and 9.2 Hz, cyclopropane-CH), 2.87 (1H, ddd, *J* = 4.2, 5.4 and 8.4 Hz, cyclopropane-CH), 7.10 (2H, d, *J* = 6.2 Hz, 2 × pyridyl-H), 7.18 (1H, dd, *J* = 1.6 and 7.6 Hz, ArH), 7.23 (1H, dd, *J* = 7.6 and 7.8 Hz, ArH), 7.27 (1H, d, *J* = 1.5 Hz, pyrrole-H), 7.31 (1H, d, *J* = 1.5 Hz, pyrrole-H), 7.50 (1H, dd, *J* = 1.6 and 7.8 Hz, ArH), 8.22 (2H, d, *J* = 6.2 Hz, 2 × pyridyl-H); ^{13}C NMR (125 MHz, CD_3OD) δ ppm 19.9, 28.3, 30.0, 117.8, 123.0, 127.0, 128.0, 129.2, 132.7, 132.8, 134.8, 142.9, 150.0, 152.9, 189.4, 190.4; HRMS calcd. for $C_{20}H_{15}{}^{35}Cl_2N_2O_2$ $[M+H]^+$ 385.0505, found 385.0506.

1-(Pyridin-4-yl)-3-(1*H*-pyrrol-2-yl)propane-1,3-dione (43)

To a stirred solution of potassium *tert*-butoxide (370 mg, 3.3 mmol) in THF (5 mL) was added 2-acetyl pyrrole (165 mg, 1.5 mmol) followed by ethyl isonicotinate (500 mg, 3.3 mmol). The resulting mixture was stirred at RT for 6 h. The mixture was acidified to pH 4 with aq. HCl (1.0 M) and water (50 mL) added, upon which the product precipitated. The product was collected by filtration and recrystallised from EtOH to give **43** as a yellow solid (230 mg, 33%); mp: 190-191 ˚C; λ_{max} (EtOH/nm) 359, 283; IR (cm^{-1}) 3044, 1595; ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.34 (1H, s, pyrrole-H), 7.17 (1H, s, CHCO), 7.27 (1H, s, pyrrole-H), 7.41 (1H, s, pyrrole-H), 7.94 (2H, d, *J* = 6.0 Hz, 2 × pyridyl-H), 8.78 (2H, d, *J* = 6.0 Hz, 2 × pyridyl-H), 12.18 (1H, br s, NH), 16.30 (1H, br s, OH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 94.9, 111.0, 117.7, 119.9, 127.3, 129.9, 141.0, 150.5, 172.2, 182.1; HRMS calcd. for $C_{12}H_{11}N_2O_2$ $[M+H]^+$ 215.0815, found 215.0817.

Kinase Selectivity for 6h

Kinase selectivity screening was conducted by Millipore.

Kinase	%inhibition @ 10 uM
Abl(h)	8
Aurora-A(h)	11
CDK2/cyclinA(h)	5

c-RAF(h)	-27
cSRC(h)	0
DAPK1(h)	-6
EGFR(h)	-14
GSK3α(h)	14
IKKα(h)	-14
IR(h)	-4
IRAK4(h)	7
JAK3(h)	-5
JNK1α1(h)	27
MAPK1(h)	45
MAPKAP-K2(h)	11
MEK1(h)	-9
PKBβ(h)	4
PKCα(h)	-6
SAPK2a(h)	76
SGK(h)	5

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X-ray Crystallography

Table S1. Crystal data and structure refinement for irh34 (**12a**).

Identification code	irh34	
CCDC reference	1410001	
Chemical formula (moiety)	$C_{11}H_{13}N_3O_2S_2$	
Chemical formula (total)	$C_{11}H_{13}N_3O_2S_2$	
Formula weight	283.36	
Temperature	120(2) K	
Radiation, wavelength	synchrotron, 0.6946 Å	
Crystal system, space group	monoclinic, $P2_1/n$	
Unit cell parameters	a = 7.826(3) Å	α = 90°
	b = 9.969(4) Å	β = 98.843(4)°
	c = 16.338(6) Å	γ = 90°
Cell volume	1259.5(8) $Å^3$	
Z	4	
Calculated density	1.494 g/cm^3	
Absorption coefficient μ	0.344 mm^{-1}	
F(000)	592	
Crystal colour and size	colourless, 0.040 × 0.040 × 0.010 mm^3	
Reflections for cell refinement	959 (θ range 2.3 to 28.5°)	
Data collection method	Bruker APEX2 CCD diffractometer	
	thin-slice ω scans	
θ range for data collection	3.3 to 29.7°	
Index ranges	h −11 to 11, k −14 to 14, l −22 to 22	
Completeness to θ = 24.6°	99.2 %	
Reflections collected	12427	
Independent reflections	3627 (R_{int} = 0.0336)	
Reflections with $F^2>2\sigma$	2752	
Absorption correction	multi-scan	
Min. and max. transmission	0.983 and 0.996	
Structure solution	direct methods	
Refinement method	Full-matrix least-squares on F^2	
Weighting parameters a, b	0.0717, 0.4971	
Data / restraints / parameters	3627 / 0 / 171	
Final R indices [$F^2>2\sigma$]	R1 = 0.0449, wR2 = 0.1180	
R indices (all data)	R1 = 0.0643, wR2 = 0.1295	
Goodness-of-fit on F^2	1.034	
Largest and mean shift/su	0.001 and 0.000	
Largest diff. peak and hole	0.48 and −0.50 e $Å^{-3}$	

Table S2. Atomic coordinates and equivalent isotropic displacement parameters ($Å^2$) for irh34. U_{eq} is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U_{eq}
S1	0.45275(6)	0.22259(5)	0.61587(3)	0.02745(13)
S2	0.30115(7)	0.53007(5)	0.58625(3)	0.03477(15)
O1	0.58167(18)	0.12389(15)	0.60562(9)	0.0329(3)
O2	0.50063(19)	0.33638(15)	0.66871(9)	0.0347(3)
N1	0.3157(2)	0.14337(19)	0.30508(11)	0.0324(4)
N2	0.2995(2)	0.14560(17)	0.65177(11)	0.0301(4)
N3	0.0974(3)	0.7440(2)	0.50956(14)	0.0483(5)
C1	0.3714(2)	0.28568(19)	0.51623(12)	0.0266(4)
C2	0.3757(2)	0.19692(19)	0.45052(12)	0.0271(4)
C3	0.3048(2)	0.2314(2)	0.36934(12)	0.0276(4)
C4	0.2301(3)	0.3586(2)	0.35621(13)	0.0313(4)
C5	0.2293(3)	0.4478(2)	0.42095(13)	0.0313(4)
C6	0.2988(2)	0.4141(2)	0.50191(12)	0.0287(4)
C7	0.1571(3)	0.2202(2)	0.68066(17)	0.0434(5)
C8	0.0229(3)	0.1145(3)	0.68861(16)	0.0423(5)
C9	0.0617(4)	−0.0015(3)	0.6380(3)	0.0702(10)
C10	0.2360(3)	0.0155(2)	0.61470(16)	0.0402(5)
C11	0.1779(3)	0.6545(2)	0.53731(14)	0.0363(5)

Table S3. Bond lengths [Å] and angles [°] for irh34.

S1–O1	1.4374(16)	S1–O2	1.4399(16)
S1–N2	1.6086(17)	S1–C1	1.770(2)
S2–C6	1.797(2)	S2–C11	1.694(2)
N1–H1A	0.86(3)	N1–H1B	0.91(3)
N1–C3	1.381(3)	N2–C7	1.477(3)
N2–C10	1.485(3)	N3–C11	1.145(3)
C1–C2	1.396(3)	C1–C6	1.405(3)
C2–H2	0.950	C2–C3	1.400(3)
C3–C4	1.399(3)	C4–H4	0.950
C4–C5	1.383(3)	C5–H5	0.950
C5–C6	1.392(3)	C7–H7A	0.990
C7–H7B	0.990	C7–C8	1.507(3)
C8–H8A	0.990	C8–H8B	0.990
C8–C9	1.480(4)	C9–H9A	0.990
C9–H9B	0.990	C9–C10	1.482(3)
C10–H10A	0.990	C10–H10B	0.990

O1–S1–O2	119.11(9)	O1–S1–N2	106.97(9)
O1–S1–C1	107.21(9)	O2–S1–N2	107.29(9)
O2–S1–C1	107.17(9)	N2–S1–C1	108.78(9)
C6–S2–C11	99.85(10)	H1A–N1–H1B	118(3)
H1A–N1–C3	112.8(19)	H1B–N1–C3	110.9(17)
S1–N2–C7	121.21(15)	S1–N2–C10	119.32(14)
C7–N2–C10	110.40(17)	S1–C1–C2	115.75(14)
S1–C1–C6	123.77(15)	C2–C1–C6	120.42(18)
C1–C2–H2	119.3	C1–C2–C3	121.35(18)
H2–C2–C3	119.3	N1–C3–C2	120.17(19)
N1–C3–C4	122.19(18)	C2–C3–C4	117.60(18)
C3–C4–H4	119.4	C3–C4–C5	121.11(19)
H4–C4–C5	119.4	C4–C5–H5	119.2
C4–C5–C6	121.65(19)	H5–C5–C6	119.2
S2–C6–C1	120.14(15)	S2–C6–C5	122.00(16)
C1–C6–C5	117.85(18)	N2–C7–H7A	110.9
N2–C7–H7B	110.9	N2–C7–C8	104.23(19)
H7A–C7–H7B	108.9	H7A–C7–C8	110.9
H7B–C7–C8	110.9	C7–C8–H8A	110.3
C7–C8–H8B	110.3	C7–C8–C9	107.03(19)
H8A–C8–H8B	108.6	H8A–C8–C9	110.3
H8B–C8–C9	110.3	C8–C9–H9A	109.8
C8–C9–H9B	109.8	C8–C9–C10	109.3(2)
H9A–C9–H9B	108.3	H9A–C9–C10	109.8
H9B–C9–C10	109.8	N2–C10–C9	105.00(19)
N2–C10–H10A	110.7	N2–C10–H10B	110.7
C9–C10–H10A	110.7	C9–C10–H10B	110.7
H10A–C10–H10B	108.8	S2–C11–N3	174.9(2)

Table S4. Torsion angles [°] for irh34.

O1–S1–N2–C7	171.32(17)	O1–S1–N2–C10	−44.80(18)
O2–S1–N2–C7	42.44(19)	O2–S1–N2–C10	−173.68(16)
C1–S1–N2–C7	−73.18(19)	C1–S1–N2–C10	70.69(18)
O1–S1–C1–C2	29.00(17)	O1–S1–C1–C6	−153.84(16)
O2–S1–C1–C2	157.96(15)	O2–S1–C1–C6	−24.88(19)
N2–S1–C1–C2	−86.35(16)	N2–S1–C1–C6	90.82(18)
S1–C1–C2–C3	175.70(14)	C6–C1–C2–C3	−1.6(3)
C1–C2–C3–N1	177.92(18)	C1–C2–C3–C4	0.4(3)
N1–C3–C4–C5	−176.34(19)	C2–C3–C4–C5	1.1(3)
C3–C4–C5–C6	−1.5(3)	C4–C5–C6–S2	179.10(16)
C4–C5–C6–C1	0.3(3)	S1–C1–C6–S2	5.4(2)
S1–C1–C6–C5	−175.86(15)	C2–C1–C6–S2	−177.61(14)
C2–C1–C6–C5	1.2(3)	C11–S2–C6–C1	−172.97(16)
C11–S2–C6–C5	8.29(19)	S1–N2–C7–C8	166.45(16)
C10–N2–C7–C8	19.7(3)	N2–C7–C8–C9	−19.5(3)
C7–C8–C9–C10	12.8(4)	C8–C9–C10–N2	−0.7(4)
S1–N2–C10–C9	−159.6(2)	C7–N2–C10–C9	−12.1(3)

Table S5. Anisotropic displacement parameters (Å^2) for irh34. The anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2}U^{11} + ... + 2hka^*b^*U^{12}]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
S1	0.0243(2)	0.0263(2)	0.0300(2)	0.00216(17)	−0.00146(17)	−0.00299(17)
S2	0.0385(3)	0.0260(3)	0.0381(3)	−0.00274(19)	0.0004(2)	−0.00121(19)
O1	0.0262(7)	0.0331(7)	0.0378(8)	0.0058(6)	0.0001(6)	0.0018(6)
O2	0.0345(8)	0.0305(8)	0.0355(8)	−0.0018(6)	−0.0061(6)	−0.0051(6)
N1	0.0348(9)	0.0311(9)	0.0300(9)	0.0012(7)	0.0005(7)	0.0015(7)
N2	0.0286(8)	0.0277(8)	0.0343(9)	0.0004(7)	0.0059(7)	−0.0040(6)
N3	0.0579(14)	0.0351(10)	0.0518(13)	0.0006(9)	0.0084(10)	0.0077(9)
C1	0.0235(8)	0.0259(9)	0.0292(9)	0.0033(7)	0.0002(7)	−0.0040(7)
C2	0.0241(9)	0.0244(9)	0.0321(10)	0.0036(7)	0.0019(7)	−0.0020(7)
C3	0.0239(9)	0.0296(10)	0.0291(9)	0.0011(7)	0.0034(7)	−0.0042(7)
C4	0.0308(10)	0.0310(10)	0.0309(10)	0.0042(8)	0.0014(8)	−0.0006(8)
C5	0.0307(10)	0.0251(9)	0.0372(11)	0.0050(7)	0.0023(8)	0.0005(7)
C6	0.0258(9)	0.0250(9)	0.0346(10)	0.0001(7)	0.0032(7)	−0.0035(7)
C7	0.0390(12)	0.0391(12)	0.0555(14)	−0.0104(10)	0.0185(11)	−0.0042(9)
C8	0.0373(12)	0.0462(13)	0.0454(13)	0.0002(10)	0.0132(10)	−0.0029(10)
C9	0.0514(17)	0.0477(16)	0.123(3)	−0.0266(17)	0.0494(19)	−0.0202(13)
C10	0.0398(12)	0.0319(11)	0.0519(13)	−0.0072(9)	0.0164(10)	−0.0112(9)
C11	0.0383(11)	0.0282(10)	0.0429(12)	−0.0022(8)	0.0072(9)	−0.0026(8)

Table S6. Hydrogen coordinates and isotropic displacement parameters (Å^2) for irh34.

	x	y	z	U
H1A	0.237(4)	0.157(3)	0.2631(18)	0.047(8)
H1B	0.334(3)	0.058(3)	0.3243(17)	0.044(7)
H2	0.4279	0.1114	0.4611	0.033
H4	0.1791	0.3842	0.3019	0.038
H5	0.1802	0.5343	0.4099	0.038
H7A	0.1104	0.2897	0.6400	0.052
H7B	0.1968	0.2637	0.7347	0.052
H8A	0.0282	0.0876	0.7473	0.051
H8B	–0.0944	0.1495	0.6683	0.051
H9A	–0.0258	–0.0077	0.5875	0.084
H9B	0.0575	–0.0855	0.6700	0.084
H10A	0.2295	0.0176	0.5537	0.048
H10B	0.3134	–0.0587	0.6372	0.048

Table S7. Hydrogen bonds for irh34 [Å and °].

D–H...A	d(D–H)	d(H...A)	d(D...A)	<(DHA)
N1–H1A...O2a	0.86(3)	2.22(3)	3.065(3)	168(3)
N1–H1B...O1b	0.91(3)	2.19(3)	3.084(3)	166(2)

Symmetry operations for equivalent atoms
a x–1/2,–y+1/2,z–1/2 b –x+1,–y,–z+1

Table S8. Crystal data and structure refinement for irh33 (**3a**).

Identification code	irh33	
CCDC reference	1410003	
Chemical formula (moiety)	$C_{11}H_{13}N_3O_2S_2$	
Chemical formula (total)	$C_{11}H_{13}N_3O_2S_2$	
Formula weight	283.36	
Temperature	120(2) K	
Radiation, wavelength	synchrotron, 0.6946 Å	
Crystal system, space group	triclinic, $P\bar{1}$	
Unit cell parameters	a = 6.3088(11) Å	α = 92.623(2)°
	b = 9.4321(16) Å	β = 106.331(2)°
	c = 11.166(2) Å	γ = 105.543(2)°
Cell volume	609.02(18) Å^3	
Z	2	
Calculated density	1.545 g/cm^3	
Absorption coefficient μ	0.356 mm^{-1}	
F(000)	296	
Crystal colour and size	colourless, 0.100 × 0.060 × 0.040 mm^3	
Reflections for cell refinement	956 (θ range 3.2 to 29.7°)	
Data collection method	Bruker APEX2 CCD diffractometer	
	thin-slice ω scans	
θ range for data collection	3.1 to 29.7°	
Index ranges	h −8 to 8, k −13 to 13, l −15 to 15	
Completeness to θ = 24.6°	95.1 %	
Reflections collected	5928	
Independent reflections	3259 (R_{int} = 0.0154)	
Reflections with $F^2>2\sigma$	2992	
Absorption correction	multi-scan	
Min. and max. transmission	0.905 and 0.985	
Structure solution	direct methods	
Refinement method	Full-matrix least-squares on F^2	
Weighting parameters a, b	0.0614, 0.2169	
Data / restraints / parameters	3259 / 0 / 171	
Final R indices [$F^2>2\sigma$]	R1 = 0.0350, wR2 = 0.0964	
R indices (all data)	R1 = 0.0374, wR2 = 0.0989	
Goodness-of-fit on F^2	1.061	
Largest and mean shift/su	0.001 and 0.000	
Largest diff. peak and hole	0.47 and −0.37 e Å^{-3}	

Table S9. Atomic coordinates and equivalent isotropic displacement parameters ($Å^2$) for irh33. U_{eq} is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U_{eq}
S1	1.26080(5)	0.76007(3)	0.82726(3)	0.01752(10)
S2	0.71598(5)	0.17379(3)	0.72181(3)	0.01532(10)
O1	0.58683(18)	0.08027(11)	0.79114(9)	0.0215(2)
O2	0.92256(17)	0.14878(11)	0.70740(9)	0.0210(2)
N1	0.95529(19)	0.80431(12)	0.93174(10)	0.0166(2)
N2	1.2484(2)	1.01893(12)	0.93190(11)	0.0184(2)
N3	0.54162(19)	0.15819(13)	0.58099(10)	0.0185(2)
C1	1.1430(2)	0.87383(13)	0.90441(11)	0.0155(2)
C2	1.0334(2)	0.60795(13)	0.82872(11)	0.0155(2)
C3	0.9865(2)	0.46098(13)	0.77987(11)	0.0165(2)
C4	0.7895(2)	0.35855(13)	0.79122(11)	0.0154(2)
C5	0.6441(2)	0.40232(14)	0.85089(12)	0.0172(2)
C6	0.6944(2)	0.54971(14)	0.89994(12)	0.0175(2)
C7	0.8898(2)	0.65449(13)	0.88922(11)	0.0150(2)
C8	0.3007(2)	0.16057(18)	0.56295(14)	0.0271(3)
C9	0.2750(3)	0.2817(2)	0.47955(17)	0.0346(4)
C10	0.4228(3)	0.26626(19)	0.39552(15)	0.0297(3)
C11	0.6329(2)	0.23542(17)	0.48523(13)	0.0231(3)

Table S10. Bond lengths [Å] and angles [°] for irh33.

S1–C1	1.7744(12)	S1–C2	1.7405(13)
S2–O1	1.4399(10)	S2–O2	1.4350(10)
S2–N3	1.6197(12)	S2–C4	1.7589(13)
N1–C1	1.3157(16)	N1–C7	1.3815(15)
N2–H2A	0.82(2)	N2–H2B	0.85(2)
N2–C1	1.3299(17)	N3–C8	1.4823(18)
N3–C11	1.4826(17)	C2–C3	1.3881(16)
C2–C7	1.4140(16)	C3–H3	0.950
C3–C4	1.3964(17)	C4–C5	1.4061(17)
C5–H5	0.950	C5–C6	1.3890(17)
C6–H6	0.950	C6–C7	1.3969(17)
C8–H8A	0.990	C8–H8B	0.990
C8–C9	1.520(2)	C9–H9A	0.990
C9–H9B	0.990	C9–C10	1.525(2)
C10–H10A	0.990	C10–H10B	0.990
C10–C11	1.527(2)	C11–H11A	0.990
C11–H11B	0.990		

C1–S1–C2	88.63(6)	O1–S2–O2	120.23(6)
O1–S2–N3	106.50(6)	O1–S2–C4	107.91(6)
O2–S2–N3	106.39(6)	O2–S2–C4	107.34(6)
N3–S2–C4	107.95(6)	C1–N1–C7	110.37(10)
H2A–N2–H2B	122(2)	H2A–N2–C1	119.2(14)
H2B–N2–C1	118.3(15)	S2–N3–C8	119.93(9)
S2–N3–C11	118.53(9)	C8–N3–C11	110.42(11)
S1–C1–N1	115.66(9)	S1–C1–N2	119.28(10)
N1–C1–N2	125.05(12)	S1–C2–C3	128.51(9)
S1–C2–C7	109.51(9)	C3–C2–C7	121.98(11)
C2–C3–H3	121.1	C2–C3–C4	117.78(11)
H3–C3–C4	121.1	S2–C4–C3	118.79(9)
S2–C4–C5	119.90(9)	C3–C4–C5	121.23(11)
C4–C5–H5	119.9	C4–C5–C6	120.23(11)
H5–C5–C6	119.9	C5–C6–H6	120.2
C5–C6–C7	119.65(11)	H6–C6–C7	120.2
N1–C7–C2	115.81(11)	N1–C7–C6	125.05(11)
C2–C7–C6	119.13(11)	N3–C8–H8A	111.0
N3–C8–H8B	111.0	N3–C8–C9	103.62(12)
H8A–C8–H8B	109.0	H8A–C8–C9	111.0
H8B–C8–C9	111.0	C8–C9–H9A	111.2
C8–C9–H9B	111.2	C8–C9–C10	102.67(13)
H9A–C9–H9B	109.1	H9A–C9–C10	111.2
H9B–C9–C10	111.2	C9–C10–H10A	111.1
C9–C10–H10B	111.1	C9–C10–C11	103.52(12)
H10A–C10–H10B	109.0	H10A–C10–C11	111.1
H10B–C10–C11	111.1	N3–C11–C10	103.93(11)
N3–C11–H11A	111.0	N3–C11–H11B	111.0
C10–C11–H11A	111.0	C10–C11–H11B	111.0
H11A–C11–H11B	109.0		

Table S11. Torsion angles [°] for irh33.

O1–S2–N3–C8	43.50(12)	O1–S2–N3–C11	−176.03(10)
O2–S2–N3–C8	172.89(10)	O2–S2–N3–C11	−46.64(11)
C4–S2–N3–C8	−72.16(12)	C4–S2–N3–C11	68.31(11)
C7–N1–C1–S1	−1.58(14)	C7–N1–C1–N2	179.50(12)
C2–S1–C1–N1	1.62(10)	C2–S1–C1–N2	−179.40(11)
C1–S1–C2–C3	178.50(12)	C1–S1–C2–C7	−1.12(9)
S1–C2–C3–C4	−179.06(10)	C7–C2–C3–C4	0.51(18)
C2–C3–C4–S2	176.17(9)	C2–C3–C4–C5	−0.44(18)
O1–S2–C4–C3	153.40(10)	O1–S2–C4–C5	−29.95(12)
O2–S2–C4–C3	22.45(12)	O2–S2–C4–C5	−160.89(10)
N3–S2–C4–C3	−91.87(11)	N3–S2–C4–C5	84.79(11)
S2–C4–C5–C6	−176.52(10)	C3–C4–C5–C6	0.06(19)
C4–C5–C6–C7	0.28(19)	C1–N1–C7–C2	0.66(15)
C1–N1–C7–C6	−178.19(12)	C5–C6–C7–N1	178.60(12)
C5–C6–C7–C2	−0.21(18)	S1–C2–C7–N1	0.53(14)
S1–C2–C7–C6	179.46(9)	C3–C2–C7–N1	−179.11(11)
C3–C2–C7–C6	−0.19(19)	S2–N3–C8–C9	126.45(12)
C11–N3–C8–C9	−16.92(15)	N3–C8–C9–C10	34.68(16)
C8–C9–C10–C11	−39.86(16)	S2–N3–C11–C10	−151.76(10)
C8–N3–C11–C10	−7.81(15)	C9–C10–C11–N3	29.39(16)

Table S12. Anisotropic displacement parameters (Å^2) for irh33. The anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2}U^{11} + ... + 2hka^*b^*U^{12}]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
S1	0.01638(16)	0.01554(16)	0.02077(17)	−0.00111(11)	0.00896(12)	0.00200(11)
S2	0.01746(16)	0.01347(16)	0.01568(16)	0.00091(10)	0.00729(11)	0.00341(11)
O1	0.0283(5)	0.0164(4)	0.0218(5)	0.0040(3)	0.0136(4)	0.0031(4)
O2	0.0204(5)	0.0203(5)	0.0249(5)	0.0010(4)	0.0091(4)	0.0082(4)
N1	0.0183(5)	0.0143(5)	0.0183(5)	0.0011(4)	0.0082(4)	0.0040(4)
N2	0.0196(5)	0.0147(5)	0.0224(5)	0.0014(4)	0.0102(4)	0.0037(4)
N3	0.0167(5)	0.0213(5)	0.0156(5)	−0.0001(4)	0.0058(4)	0.0021(4)
C1	0.0161(5)	0.0167(5)	0.0140(5)	0.0006(4)	0.0048(4)	0.0055(4)
C2	0.0150(5)	0.0153(5)	0.0153(5)	0.0010(4)	0.0056(4)	0.0022(4)
C3	0.0162(6)	0.0170(6)	0.0165(6)	−0.0004(4)	0.0059(4)	0.0045(5)
C4	0.0175(5)	0.0143(5)	0.0143(5)	0.0008(4)	0.0058(4)	0.0037(4)
C5	0.0190(6)	0.0157(5)	0.0176(5)	0.0017(4)	0.0086(4)	0.0030(4)
C6	0.0195(6)	0.0172(6)	0.0181(6)	0.0015(4)	0.0098(4)	0.0050(5)
C7	0.0166(5)	0.0153(5)	0.0134(5)	0.0015(4)	0.0051(4)	0.0046(4)
C8	0.0161(6)	0.0354(8)	0.0269(7)	0.0014(6)	0.0063(5)	0.0036(5)
C9	0.0300(8)	0.0432(9)	0.0343(8)	0.0062(7)	0.0086(6)	0.0179(7)
C10	0.0329(8)	0.0332(8)	0.0212(7)	0.0047(5)	0.0049(6)	0.0103(6)
C11	0.0245(6)	0.0277(7)	0.0172(6)	0.0034(5)	0.0093(5)	0.0048(5)

Table S13. Hydrogen coordinates and isotropic displacement parameters ($Å^2$) for irh33.

	x	y	z	U
H2A	1.361(4)	1.055(2)	0.908(2)	0.029(5)
H2B	1.185(4)	1.073(3)	0.964(2)	0.037(6)
H3	1.0851	0.4311	0.7400	0.020
H5	0.5110	0.3308	0.8577	0.021
H6	0.5965	0.5791	0.9406	0.021
H8A	0.2769	0.1849	0.6445	0.033
H8B	0.1893	0.0636	0.5212	0.033
H9A	0.3336	0.3809	0.5301	0.042
H9B	0.1118	0.2649	0.4293	0.042
H10A	0.4688	0.3588	0.3586	0.036
H10B	0.3392	0.1831	0.3267	0.036
H11A	0.6957	0.1716	0.4406	0.028
H11B	0.7559	0.3290	0.5244	0.028

Table S14. Hydrogen bonds for irh33 [Å and °].

D–H...A	d(D–H)	d(H...A)	d(D...A)	<(DHA)
N2–H2A...O1a	0.82(2)	2.16(2)	2.9448(15)	159(2)
N2–H2B...N1b	0.85(2)	2.11(2)	2.9608(16)	172(2)

Symmetry operations for equivalent atoms
a x+1,y+1,z b –x+2,–y+2,–z+2

Table S15. Crystal data and structure refinement for irh32 (**3i**).

Identification code	irh32	
CCDC reference	1410004	
Chemical formula (moiety)	$C_{10}H_{13}N_3O_2S_2$	
Chemical formula (total)	$C_{10}H_{13}N_3O_2S_2$	
Formula weight	271.35	
Temperature	150(2) K	
Radiation, wavelength	MoKα, 0.71073 Å	
Crystal system, space group	orthorhombic, $Pca2_1$	
Unit cell parameters	a = 14.3612(3) Å	α = 90°
	b = 9.07325(14) Å	β = 90°
	c = 18.9809(4) Å	γ = 90°
Cell volume	2473.26(8) $Å^3$	
Z	8	
Calculated density	1.457 g/cm^3	
Absorption coefficient μ	0.424 mm^{-1}	
F(000)	1136	
Crystal colour and size	colourless, 0.200 × 0.100 × 0.100 mm^3	
Reflections for cell refinement	6930 (θ range 3.0 to 29.6°)	
Data collection method	Oxford Diffraction Gemini A Ultra diffractometer thin-slice ω scans	
θ range for data collection	3.0 to 29.6°	
Index ranges	h −14 to 19, k −12 to 11, l −20 to 25	
Completeness to θ = 25.2°	99.8 %	
Reflections collected	11183	
Independent reflections	5361 (R_{int} = 0.0237)	
Reflections with $F^2>2\sigma$	4330	
Absorption correction	multi-scan	
Min. and max. transmission	0.920 and 0.960	
Structure solution	direct methods	
Refinement method	Full-matrix least-squares on F^2	
Weighting parameters a, b	0.0501,	
Data / restraints / parameters	5361 / 1 / 327	
Final R indices [$F^2>2\sigma$]	R1 = 0.0319, wR2 = 0.0764	
R indices (all data)	R1 = 0.0432, wR2 = 0.0790	
Goodness-of-fit on F^2	0.964	
Absolute structure parameter	0.40(4)	
Largest and mean shift/su	0.001 and 0.000	
Largest diff. peak and hole	0.38 and –0.39 e $Å^{-3}$	

Table S16. Atomic coordinates and equivalent isotropic displacement parameters (Å^2) for irh32. U_{eq} is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U_{eq}
S1	0.27921(6)	0.01637(9)	0.19391(5)	0.0214(2)
S2	0.06800(6)	0.01041(8)	0.43813(5)	0.01748(19)
O1	0.03330(17)	–0.1245(2)	0.40849(14)	0.0243(6)
O2	0.09346(19)	0.0121(2)	0.51156(13)	0.0245(6)
N1	0.3895(2)	0.1910(3)	0.26541(15)	0.0196(6)
N2	0.4384(2)	0.1442(3)	0.15061(17)	0.0252(7)
N3	–0.0120(2)	0.1342(3)	0.42809(17)	0.0195(6)
C1	0.3793(2)	0.1281(3)	0.20342(19)	0.0185(7)
C2	0.2480(2)	0.0606(3)	0.28081(19)	0.0179(7)
C3	0.1724(2)	0.0127(3)	0.3184(2)	0.0171(8)
C4	0.1650(2)	0.0621(3)	0.38776(19)	0.0180(7)
C5	0.2310(2)	0.1556(3)	0.41747(19)	0.0216(7)
C6	0.3069(2)	0.2022(3)	0.37883(18)	0.0210(7)
C7	0.3167(2)	0.1543(3)	0.30941(18)	0.0166(7)
C8	–0.0438(2)	0.1655(3)	0.35604(17)	0.0212(7)
C9	–0.1386(2)	0.2409(3)	0.3574(2)	0.0252(7)
C10	–0.1353(2)	0.3953(3)	0.3869(2)	0.0260(7)
S3	0.70877(6)	0.51415(9)	0.24353(5)	0.0221(2)
S4	0.92127(6)	0.49211(8)	–0.00048(5)	0.0185(2)
O3	0.88497(17)	0.4912(2)	–0.07063(14)	0.0261(6)
O4	0.95704(18)	0.6280(2)	0.02740(14)	0.0259(6)
N4	0.5566(2)	0.3766(4)	0.29375(18)	0.0285(7)
N5	0.61468(19)	0.2999(3)	0.18474(16)	0.0201(6)
N6	1.0033(2)	0.3732(3)	0.00170(17)	0.0198(6)
C11	0.6179(2)	0.3836(3)	0.2410(2)	0.0191(7)
C12	0.7450(2)	0.4505(3)	0.16152(18)	0.0163(7)
C13	0.8175(2)	0.5012(3)	0.1199(2)	0.0194(8)
C14	0.8307(2)	0.4335(3)	0.05510(19)	0.0175(7)
C15	0.7743(2)	0.3169(3)	0.0326(2)	0.0205(7)
C16	0.7022(2)	0.2683(3)	0.07430(19)	0.0206(7)
C17	0.6858(2)	0.3360(3)	0.13921(18)	0.0179(7)
C18	1.0790(3)	0.3723(4)	0.0535(2)	0.0370(10)
C19	1.0834(3)	0.2378(4)	0.0980(2)	0.0459(12)
C20	1.0053(4)	0.2283(5)	0.1518(3)	0.0586(13)

Table S17. Bond lengths [Å] and angles [°] for irh32.

S1–C1	1.768(3)	S1–C2	1.756(4)
S2–O1	1.437(2)	S2–O2	1.441(3)
S2–N3	1.618(3)	S2–C4	1.753(3)
N1–C1	1.316(4)	N1–C7	1.379(4)
N2–H2A	0.93(4)	N2–H2B	0.75(4)
N2–C1	1.322(5)	N3–H3	0.79(4)
N3–C8	1.469(4)	C2–C3	1.369(5)
C2–C7	1.412(4)	C3–H3A	0.950
C3–C4	1.396(5)	C4–C5	1.391(5)
C5–H5	0.950	C5–C6	1.381(5)
C6–H6A	0.950	C6–C7	1.395(5)
C8–H8A	0.990	C8–H8B	0.990
C8–C9	1.524(4)	C9–H9A	0.990
C9–H9B	0.990	C9–C10	1.509(4)
C10–H10A	0.980	C10–H10B	0.980
C10–H10C	0.980	S3–C11	1.763(3)
S3–C12	1.740(4)	S4–O3	1.430(3)
S4–O4	1.436(2)	S4–N6	1.598(3)
S4–C14	1.757(3)	N4–H4A	0.94(4)
N4–H4B	0.87(4)	N4–C11	1.335(5)
N5–C11	1.311(4)	N5–C17	1.378(4)
N6–H6	0.86(3)	N6–C18	1.466(5)
C12–C13	1.385(5)	C12–C17	1.408(4)
C13–H13	0.950	C13–C14	1.389(5)
C14–C15	1.400(4)	C15–H15	0.950
C15–C16	1.376(5)	C16–H16	0.950
C16–C17	1.397(5)	C18–H18A	0.990
C18–H18B	0.990	C18–C19	1.485(6)
C19–H19A	0.990	C19–H19B	0.990
C19–C20	1.519(7)	C20–H20A	0.980
C20–H20B	0.980	C20–H20C	0.980

C1–S1–C2	88.91(16)	O1–S2–O2	118.43(15)
O1–S2–N3	107.38(16)	O1–S2–C4	106.85(16)
O2–S2–N3	106.66(16)	O2–S2–C4	108.84(17)
N3–S2–C4	108.34(15)	C1–N1–C7	110.7(3)
H2A–N2–H2B	122(4)	H2A–N2–C1	119(3)
H2B–N2–C1	119(3)	S2–N3–H3	108(3)
S2–N3–C8	117.7(2)	H3–N3–C8	116(3)
S1–C1–N1	115.5(3)	S1–C1–N2	120.5(3)
N1–C1–N2	124.0(3)	S1–C2–C3	128.2(3)
S1–C2–C7	108.7(2)	C3–C2–C7	123.1(3)
C2–C3–H3A	121.6	C2–C3–C4	116.7(3)
H3A–C3–C4	121.6	S2–C4–C3	119.3(2)
S2–C4–C5	118.9(3)	C3–C4–C5	121.8(3)
C4–C5–H5	119.7	C4–C5–C6	120.6(3)
H5–C5–C6	119.7	C5–C6–H6A	120.5
C5–C6–C7	119.1(3)	H6A–C6–C7	120.5
N1–C7–C2	116.3(3)	N1–C7–C6	125.0(3)
C2–C7–C6	118.7(3)	N3–C8–H8A	109.6
N3–C8–H8B	109.6	N3–C8–C9	110.4(3)
H8A–C8–H8B	108.1	H8A–C8–C9	109.6
H8B–C8–C9	109.6	C8–C9–H9A	108.9
C8–C9–H9B	108.9	C8–C9–C10	113.3(3)
H9A–C9–H9B	107.7	H9A–C9–C10	108.9
H9B–C9–C10	108.9	C9–C10–H10A	109.5

C9–C10–H10B	109.5	C9–C10–H10C	109.5
H10A–C10–H10B	109.5	H10A–C10–H10C	109.5
H10B–C10–H10C	109.5	C11–S3–C12	88.52(16)
O3–S4–O4	118.60(15)	O3–S4–N6	106.78(16)
O3–S4–C14	106.71(16)	O4–S4–N6	107.85(16)
O4–S4–C14	107.65(16)	N6–S4–C14	109.00(15)
H4A–N4–H4B	124(4)	H4A–N4–C11	119(3)
H4B–N4–C11	117(3)	C11–N5–C17	110.3(3)
S4–N6–H6	108(2)	S4–N6–C18	124.6(2)
H6–N6–C18	124(2)	S3–C11–N4	120.0(3)
S3–C11–N5	116.0(3)	N4–C11–N5	124.0(3)
S3–C12–C13	128.6(3)	S3–C12–C17	109.5(2)
C13–C12–C17	121.9(3)	C12–C13–H13	121.3
C12–C13–C14	117.4(3)	H13–C13–C14	121.3
S4–C14–C13	120.0(2)	S4–C14–C15	118.3(3)
C13–C14–C15	121.8(3)	C14–C15–H15	119.9
C14–C15–C16	120.1(3)	H15–C15–C16	119.9
C15–C16–H16	120.2	C15–C16–C17	119.6(3)
H16–C16–C17	120.2	N5–C17–C12	115.8(3)
N5–C17–C16	125.0(3)	C12–C17–C16	119.2(3)
N6–C18–H18A	108.6	N6–C18–H18B	108.6
N6–C18–C19	114.7(3)	H18A–C18–H18B	107.6
H18A–C18–C19	108.6	H18B–C18–C19	108.6
C18–C19–H19A	108.9	C18–C19–H19B	108.9
C18–C19–C20	113.4(4)	H19A–C19–H19B	107.7
H19A–C19–C20	108.9	H19B–C19–C20	108.9
C19–C20–H20A	109.5	C19–C20–H20B	109.5
C19–C20–H20C	109.5	H20A–C20–H20B	109.5
H20A–C20–H20C	109.5	H20B–C20–H20C	109.5

Table S18. Torsion angles [°] for irh32.

O1–S2–N3–C8	−58.1(3)	O2–S2–N3–C8	174.0(2)
C4–S2–N3–C8	56.9(3)	C7–N1–C1–S1	−0.5(3)
C7–N1–C1–N2	179.7(3)	C2–S1–C1–N1	0.8(2)
C2–S1–C1–N2	−179.4(3)	C1–S1–C2–C3	−178.8(3)
C1–S1–C2–C7	−0.7(2)	S1–C2–C3–C4	178.4(2)
C7–C2–C3–C4	0.6(5)	C2–C3–C4–S2	177.8(2)
C2–C3–C4–C5	0.1(5)	O1–S2–C4–C3	24.6(3)
O1–S2–C4–C5	−157.6(3)	O2–S2–C4–C3	153.6(2)
O2–S2–C4–C5	−28.7(3)	N3–S2–C4–C3	−90.8(3)
N3–S2–C4–C5	87.0(3)	S2–C4–C5–C6	−178.1(2)
C3–C4–C5–C6	−0.4(5)	C4–C5–C6–C7	0.1(5)
C1–N1–C7–C2	−0.1(4)	C1–N1–C7–C6	179.6(3)
C5–C6–C7–N1	−179.2(3)	C5–C6–C7–C2	0.5(4)
S1–C2–C7–N1	0.7(3)	S1–C2–C7–C6	−179.1(2)
C3–C2–C7–N1	178.9(3)	C3–C2–C7–C6	−0.9(5)
S2–N3–C8–C9	160.5(2)	N3–C8–C9–C10	68.7(4)
O3–S4–N6–C18	158.0(3)	O4–S4–N6–C18	29.6(3)
C14–S4–N6–C18	−87.0(3)	C17–N5–C11–S3	−0.2(3)
C17–N5–C11–N4	178.4(3)	C12–S3–C11–N4	−178.4(3)
C12–S3–C11–N5	0.2(3)	C11–S3–C12–C13	177.7(3)
C11–S3–C12–C17	−0.2(2)	S3–C12–C13–C14	−178.3(2)
C17–C12–C13–C14	−0.7(5)	C12–C13–C14–S4	180.0(2)
C12–C13–C14–C15	−1.0(5)	O3–S4–C14–C13	−139.9(3)
O3–S4–C14–C15	41.0(3)	O4–S4–C14–C13	−11.6(3)
O4–S4–C14–C15	169.3(2)	N6–S4–C14–C13	105.1(3)
N6–S4–C14–C15	−74.0(3)	S4–C14–C15–C16	−179.6(2)
C13–C14–C15–C16	1.4(5)	C14–C15–C16–C17	0.0(5)
C11–N5–C17–C12	0.0(4)	C11–N5–C17–C16	−179.8(3)
C15–C16–C17–N5	178.3(3)	C15–C16–C17–C12	−1.6(5)
S3–C12–C17–N5	0.1(3)	S3–C12–C17–C16	180.0(2)
C13–C12–C17–N5	−177.9(3)	C13–C12–C17–C16	2.0(5)
S4–N6–C18–C19	119.1(3)	N6–C18–C19–C20	−71.7(5)

Table S19. Anisotropic displacement parameters ($Å^2$) for irh32. The anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2}U^{11} + ...+ 2hka^*b^*U^{12}]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
S1	0.0226(4)	0.0284(4)	0.0130(5)	–0.0035(4)	0.0012(3)	–0.0059(3)
S2	0.0220(4)	0.0165(4)	0.0139(5)	0.0003(4)	0.0037(3)	–0.0005(3)
O1	0.0332(14)	0.0187(10)	0.0211(16)	0.0005(10)	0.0076(11)	–0.0040(9)
O2	0.0332(13)	0.0263(12)	0.0141(14)	0.0024(10)	0.0024(11)	0.0032(10)
N1	0.0214(14)	0.0232(13)	0.0141(15)	–0.0012(12)	0.0003(12)	–0.0013(12)
N2	0.0288(16)	0.0348(17)	0.0121(17)	–0.0028(14)	0.0018(15)	–0.0072(14)
N3	0.0219(14)	0.0188(12)	0.0177(16)	–0.0055(12)	–0.0010(13)	0.0008(11)
C1	0.0214(16)	0.0193(14)	0.0150(19)	0.0033(14)	0.0004(15)	–0.0016(12)
C2	0.0214(16)	0.0182(14)	0.0140(18)	–0.0013(14)	0.0001(14)	0.0028(13)
C3	0.0184(17)	0.0167(15)	0.016(2)	–0.0013(13)	0.0007(12)	–0.0012(12)
C4	0.0178(15)	0.0180(14)	0.0182(18)	0.0017(14)	0.0026(14)	0.0014(12)
C5	0.0252(17)	0.0255(14)	0.0141(18)	–0.0040(13)	0.0019(15)	–0.0004(13)
C6	0.0243(17)	0.0228(14)	0.0160(18)	–0.0058(14)	–0.0007(15)	–0.0039(13)
C7	0.0183(17)	0.0153(13)	0.0161(18)	0.0012(13)	–0.0007(14)	0.0003(12)
C8	0.0279(17)	0.0236(15)	0.0120(17)	–0.0008(14)	–0.0010(14)	–0.0002(13)
C9	0.0241(16)	0.0285(16)	0.0231(19)	–0.0016(15)	–0.0056(16)	–0.0022(14)
C10	0.0252(17)	0.0255(15)	0.027(2)	–0.0009(15)	–0.0017(16)	0.0034(14)
S3	0.0242(4)	0.0277(4)	0.0144(5)	–0.0048(4)	0.0025(4)	–0.0061(3)
S4	0.0227(4)	0.0174(4)	0.0156(5)	0.0009(3)	0.0039(4)	–0.0003(3)
O3	0.0275(13)	0.0371(13)	0.0138(14)	0.0020(10)	0.0047(11)	0.0050(10)
O4	0.0366(15)	0.0174(10)	0.0236(16)	–0.0032(10)	0.0122(12)	–0.0058(10)
N4	0.0298(17)	0.0398(17)	0.0158(18)	–0.0053(14)	0.0083(15)	–0.0144(15)
N5	0.0215(14)	0.0232(12)	0.0157(16)	0.0002(12)	0.0021(12)	–0.0036(11)
N6	0.0191(14)	0.0184(12)	0.0219(17)	–0.0079(12)	–0.0015(13)	–0.0033(11)
C11	0.0202(17)	0.0229(14)	0.0142(18)	0.0040(15)	–0.0010(16)	0.0001(12)
C12	0.0196(15)	0.0187(14)	0.0106(17)	0.0001(13)	–0.0031(14)	0.0033(12)
C13	0.0231(19)	0.0160(16)	0.019(2)	0.0003(13)	–0.0022(14)	–0.0026(12)
C14	0.0205(16)	0.0169(14)	0.0153(19)	0.0022(13)	0.0045(14)	0.0017(12)
C15	0.0251(17)	0.0196(14)	0.0168(18)	–0.0032(13)	0.0009(15)	0.0025(13)
C16	0.0214(15)	0.0205(14)	0.0200(18)	–0.0026(13)	–0.0025(15)	–0.0040(13)
C17	0.0196(16)	0.0179(14)	0.0161(18)	0.0013(13)	0.0005(15)	0.0001(12)
C18	0.035(2)	0.0339(19)	0.042(3)	–0.0041(18)	–0.0134(19)	–0.0023(16)
C19	0.055(3)	0.036(2)	0.046(3)	–0.0012(19)	–0.023(2)	0.004(2)
C20	0.072(3)	0.051(2)	0.053(3)	0.015(2)	–0.012(3)	–0.015(2)

Table S20. Hydrogen coordinates and isotropic displacement parameters (Å^2) for irh32.

	x	y	z	U
H2A	0.493(3)	0.198(4)	0.158(2)	0.030
H2B	0.426(3)	0.114(4)	0.115(2)	0.030
H3	0.002(3)	0.203(4)	0.451(2)	0.023
H3A	0.1273	–0.0511	0.2982	0.020
H5	0.2237	0.1878	0.4648	0.026
H6A	0.3519	0.2660	0.3993	0.025
H8A	–0.0482	0.0723	0.3291	0.025
H8B	0.0019	0.2300	0.3321	0.025
H9A	–0.1637	0.2447	0.3089	0.030
H9B	–0.1819	0.1810	0.3862	0.030
H10A	–0.1976	0.4390	0.3851	0.039
H10B	–0.0922	0.4552	0.3589	0.039
H10C	–0.1139	0.3920	0.4359	0.039
H4A	0.506(3)	0.310(4)	0.290(2)	0.034
H4B	0.566(3)	0.436(4)	0.329(2)	0.034
H6	0.986(2)	0.297(4)	–0.0227(19)	0.024
H13	0.8567	0.5793	0.1352	0.023
H15	0.7857	0.2712	–0.0116	0.025
H16	0.6638	0.1892	0.0591	0.025
H18A	1.0718	0.4591	0.0846	0.044
H18B	1.1389	0.3830	0.0282	0.044
H19A	1.1439	0.2360	0.1230	0.055
H19B	1.0808	0.1499	0.0671	0.055
H20A	1.0165	0.1446	0.1833	0.088
H20B	0.9458	0.2146	0.1274	0.088
H20C	1.0033	0.3195	0.1794	0.088

Table S21. Hydrogen bonds for irh32 [Å and °].

D–H...A	d(D–H)	d(H...A)	d(D...A)	<(DHA)
N2–H2A...N5	0.93(4)	2.04(4)	2.970(4)	173(4)
N2–H2B...O2a	0.75(4)	2.19(4)	2.935(4)	173(4)
N3–H3...O4b	0.79(4)	2.19(4)	2.973(4)	172(4)
N4–H4A...N1	0.94(4)	2.05(5)	2.981(4)	168(4)
N4–H4B...O3c	0.87(4)	2.09(4)	2.900(4)	154(4)
N6–H6...O1d	0.86(3)	2.06(4)	2.915(4)	170(3)

Symmetry operations for equivalent atoms
a –x+1/2,y,z–1/2 b –x+1,–y+1,z+1/2 c –x+3/2,y,z+1/2
d –x+1,–y,z–1/2