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Article

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Development of (4-Cyanophenyl)glycine Derivatives as Reversible Inhibitors of Lysine Specific Demethylase 1

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Abstract

Inhibition of lysine specific demethylase 1 (LSD1) has been shown to induce the differentiation of leukemia stem cells in acute myeloid leukaemia (AML). Irreversible inhibitors developed from the non-specific inhibitor tranylcypromine have entered clinical trials; however, the development of effective reversible inhibitors has proved more challenging. Herein, we describe our efforts to identify reversible inhibitors of LSD1 from a high throughput screen, and subsequent *in silico* modelling approaches. From a single hit (**12**) validated by biochemical and biophysical assays, we describe our efforts to develop acyclic scaffold-hops from GSK-690 (**1**). A further scaffold modification to a (4-cyanophenyl)glycinamide (e.g. **29a**) led to the development of compound **32**, with a K_d value of 32 nM and an EC_{50} value of 0.67 μM in a surrogate cellular biomarker assay. Moreover, this derivative does not display the same level of hERG liability as observed with **1** and represents a promising lead for further development.

Introduction

Lysine specific demethylase 1 (LSD1) was the first histone demethylase to be discovered,[1] and has been found to play an important role in normal and malignant cells as a transcriptional repressor.[2] LSD1 belongs to the FAD-dependent amine oxidase family of demethylases and catalyzes the demethylation of lysine residues of histones,[3] specifically H3K4me1/2 and H3K9me1/2.[4] Aside from this role, it has become clear that LSD1 plays important scaffolding roles as part of the CoREST complex,[5] especially in controlling the interaction with transcription factors such as growth factor independence (GFI) 1 and 1b.[6-7] LSD1 and GFI1 in combination appear to balance the regulation of hematopoietic cell proliferation and differentiation in hematopoietic stem and progenitor cells.[8] GFI1 interacts with LSD1 through binding of its C-terminal SNAG domain in the active site of LSD1, suggesting that LSD1 inhibitors may induce their phenotype through abrogation of this protein-protein interaction, and the downstream changes in gene expression this effects, rather than by inhibition of histone demethylation.[9] In addition, it has been established that LSD1 plays a key role in acute myeloid leukaemia (AML) driven by mixed lineage leukaemia (MLL) fusions,[10] whereby overexpression of LSD1 maintains malignant stem and progenitor cells in a self-renewing state. This has helped to establish a novel treatment hypothesis in a hematological malignancy where the standard of care has changed little in the past three decades.[11]

There is also evidence that LSD1 inhibition could have a beneficial effect in a number of other disease areas. It has been shown that small cell lung cancer cell lines have high levels of LSD1 overexpression in almost all cases, and some show sensitivity to LSD1 inhibition *in vitro* and in tumour xenografts.[12] The authors suggest a correlation between response and DNA hypomethylation. The consequence of inhibition is similar to AML, in that cells show a differentiation phenotype towards their original neuroendocrine state. LSD1 inhibition has also shown promise for the treatment of herpes simplex infection,[13, 14] memory deficit,[15] and patents have been filed describing the potential use of LSD1 inhibitors in diseases associated with alterations in protein conformation, such as Alzheimer's, Parkinson's and Huntingdon's diseases.[16, 17] Irreversible inhibitors of LSD1 developed from the clinical monoamine oxidase (MAO) inhibitor tranylcypromine entered clinical trials in 2014.

These inhibitors will provide valuable insights into the validation of targeting malignant stem and progenitor cells in AML patients. Preliminary clinical trials data of *rel*-N^1-[(1R,2S)-2-phenylcyclopropyl]-1,4-cyclohexanediamine (ORY-1001, Oryzon Genomics) has demonstrated promotion of blast cell differentiation in roughly two-thirds of patients in an extension cohort with specific subtypes of relapsed or refractory AML, although adverse effects limited the maximum tolerated dose.[18] Additional clinical trials are underway with tranylcypromine derivatives from GlaxoSmithKline (Clinicaltrials.gov identifier NCT02177812),[19] and Incyte Corporation (Clinicaltrials.gov identifier NCT02712905),[20] although no results have yet been disclosed. The clinical efficacy of LSD1 inhibitors may ultimately be enhanced by way of combination therapies. For example, all-trans-retinoic acid (ATRA) therapy is already successfully used as a differentiation therapy in acute promyelocytic leukaemia (APL), and research has suggested addition of an LSD1 inhibitor could enable this therapy to be more widely applicable to AML.[21] Emerging data suggests the wider applicability of this strategy to other epigenetic targets such as bromodomains,[22] and histone deacetylases.[23]

The development of reversible LSD1 inhibitors is not trivial, due to the large size and polarity of the LSD1 substrate binding pocket. As such, progress has not matched that of the covalent inactivators of LSD1.[24] Reversible inhibitors such as GSK-690 (**1**, Figure 1) have achieved good potency in biochemical and cellular assays,[25] however this compound strongly inhibits the human ether-a-go-go-related gene (hERG) cardiac ion channel, which prevented the progression of this series to the clinic.[26] Many other published reversible inhibitor series appear to have significant off-target and non-specific effects.[24] In the past year, it has become apparent that there is commercial interest in developing reversible inhibitors, as evidenced by a number of patent applications from Quanticel Pharmaceuticals (**2**–**6**, Figure 1) and Celgene.[27–32] Recently, the discovery and optimization of a series of thieno[3,2-*b*]pyrrole-5-carboxamides, and the crystal structures of quinazoline based reversible inhibitors has also been described.[33–35]

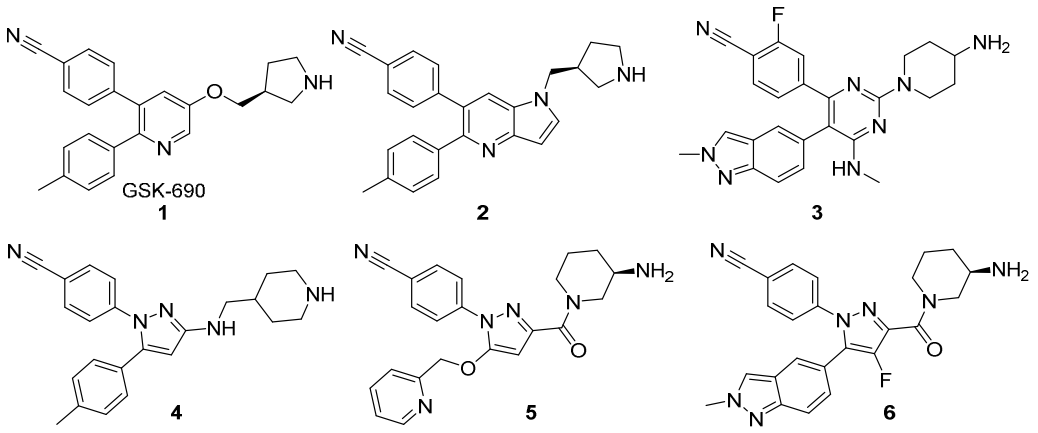


Figure 1. Compound **1** and examples from several series disclosed by Quanticel Pharmaceuticals(**2–6**).[28]

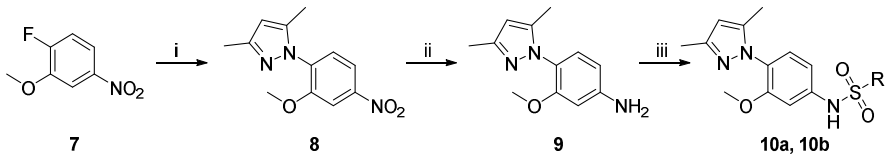
Herein, we describe our efforts towards the development of reversible inhibitors of LSD1 identified by high-throughput screening, and using *in silico* design to develop sub-micromolar inhibitors of LSD1 that show activity in cellular assays.

Chemistry

Aniline **9** (Scheme 1) was prepared from nitrobenzene **7** via aromatic nucleophilic substitution with 3,5-dimethylpyrazole to afford **8**, which was reduced with zinc metal and ammonium formate. Aniline **9** was reacted with the requisite sulfonyl chloride in pyridine to afford compounds **10a**–**b**. As described in Scheme 2, compound **12** was formed by the reaction of aniline **11** with 4-acetatamidobenzenesulfonyl chloride. Compound **11** was also reacted with 4-methylphenylsulfonyl chloride in acetonitrile in the presence of pyridine to afford sulfonamide **13**. Compound **13** was alkylated with tert-butyl bromoacetate to afford the ester **14**. Deprotection with trifluoroacetic acid afforded acid **15** as a key intermediate.[36] This was reacted with a variety of di-amines under standard amide coupling conditions, with a Boc-deprotection step using 4M HCl/dioxane if required, to give amides **16a**–**m**. Sulfonamides **18**–**20** were formed under standard conditions, then alkylated with a Boc-protected chloroacetylpiperidine derivative (see Supporting Information for synthesis) to afford derivatives **21**–**23** (Scheme 3). To synthesize the methylene replacement analogues (Scheme 4),

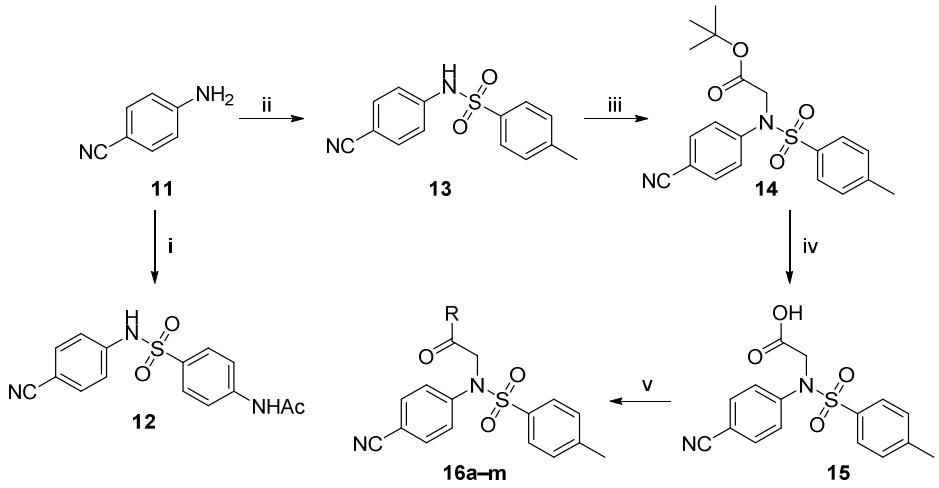
commercially available (4-cyanophenyl)glycine was reacted with a variety of Boc-protected substituted piperidines under standard amide coupling conditions to afford intermediates **25–28**. Alkylation with a variety of substituted benzyl bromides in DMF in the presence of potassium carbonate, and subsequent Boc-deprotection, afforded compounds **29a–e**, **30a–f**, **31** and **32**.

Scheme 1: Synthesis of compounds 10a–b[a]



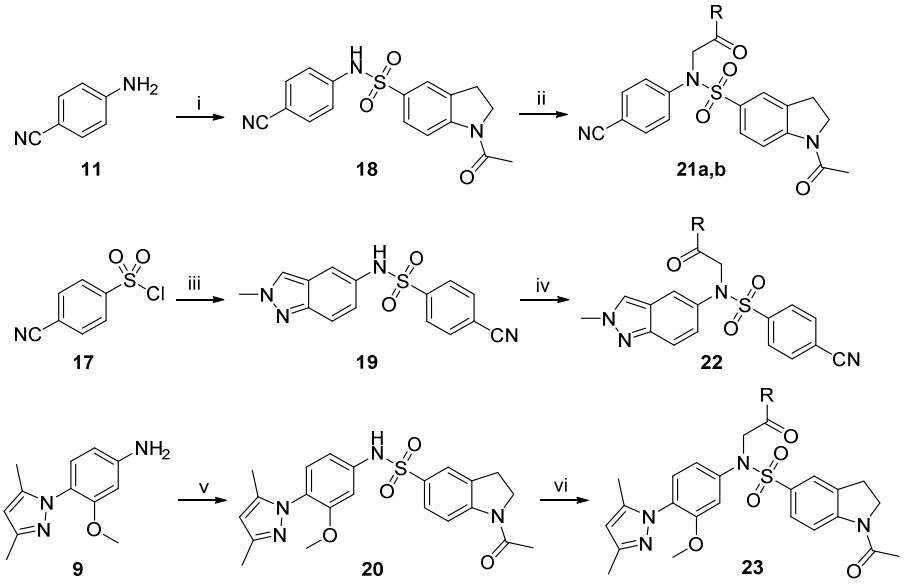
[a]Reagents and conditions: (i) 3,5-dimethylpyrazole, K_2CO_3, DMF, 80 °C, 16 h, 99%; (ii) Zn, ammonium formate, MeOH, 40 °C, 16 h, 78%; (iii) appropriate sulfonyl chloride, pyridine, 100 °C, 1 h, 41–70%.

Scheme 2: Synthesis of compounds 12 and 16a–m[a]



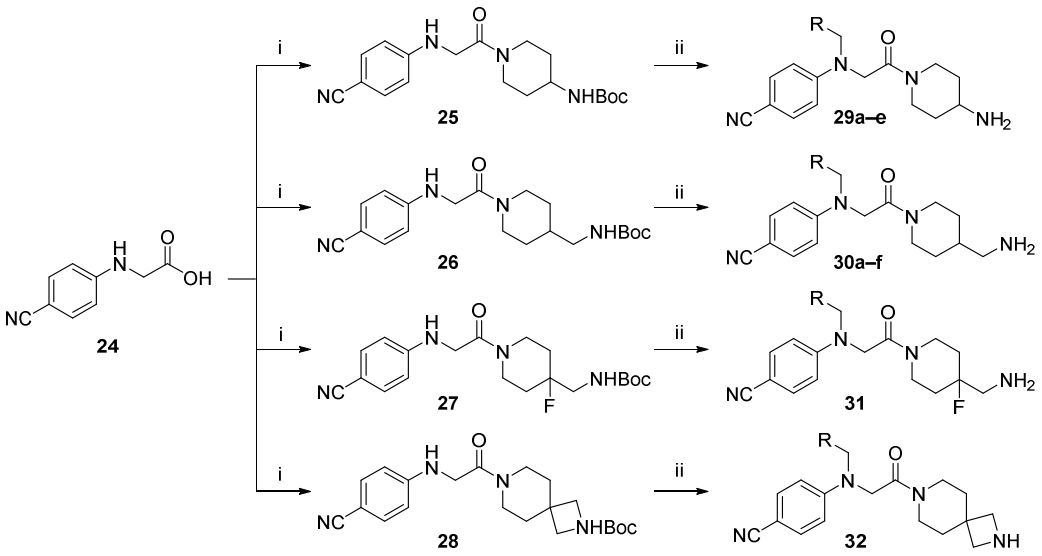
[a]Reagents and conditions: (i) 4-acetamidobenzenesulfonyl chloride, DMAP, MeCN, μW, 100 °C, 1 h, 37%; (ii) 4-methylphenylsulfonyl, pyridine, MeCN, RT, 12 h, 89%; (iii) tert-butyl bromoacetate, K_2CO_3, DMF, 1 h, 96%; (iv) TFA, DCM, 1 h, 98%; (v) amine, DIPEA, COMU, DMF, then 4M HCl/dioxane if Boc deprotection required, RT, 12–68%.

Scheme 3: Synthesis of compounds 21a–b, 22 and 23[a]



[a]Reagents and conditions: (i) 1-acetylindoline-5-sulfonyl chloride, pyridine, MeCN, RT, 12 h, 84%; (ii) (**21a)** *tert*-butyl *N*-[(3*R*)-1-(2-chloroacetyl)-3-piperidyl]carbamate, K_2CO_3, cat. KI, DMF, 80 °C, 3 h; then TFA, 1 h, rt, 42%; (**21b**) *tert*-butyl N-[1-(2-chloroacetyl)-4-piperidyl]carbamate, K_2CO_3, 80 °C, 6 h, then 4M HCl/dioxane, 70%; (iii) 2-methyl-2*H*-indazol-5-amine, pyridine, MeCN, rt, 12 h, 54%; (iv) *tert*-butyl *N*-[(3*R*)-1-(2-chloroacetyl)-3-piperidyl]carbamate, K_2CO_3, cat. KI, DMF, 80 °C, 3 h; then TFA, 1 h, rt, 25%; (v) 1-acetylindoline-5-sulfonyl chloride, pyridine, MeCN, RT, 12 h, 54%; (vi) tert-butyl N-[1-(2-chloroacetyl)-4-piperidyl]carbamate, K_2CO_3, DMF, 80 °C, 6 h, then 4M HCl/dioxane, 1 h RT, 52%.

Scheme 4: Synthesis of compounds 29a–e, 30a–f, 31 and 32[a]



[a]Reagents and conditions: i) Boc-protected diamine, DIPEA, COMU, DMF, rt, 1 h, 67 – 99%; ii) substituted benzyl-bromide, K_2CO_3, DMF, 80°C, 1 h, then 4 M HCl/dioxane, rt, 1 h, 6–58%

Results and Discussion

A library of ~150,000 compounds was screened against the LSD1 enzyme in a time-resolved fluorescence resonance energy transfer (TR-FRET) assay at 30 μM, with an initial hit rate of 0.3% (as defined by >70% inhibition). The resulting 472 compounds were tested for interference of the TR-FRET assay and for purity by LC-MS, then taken into 7- and 12- point IC_{50} testing, yielding 72 hits with IC_{50} values in the range of 4–102 μM.

After resynthesis of the original hit matter for validation, we chose to develop a series of sulfonamides that appeared to offer an attractive start-point with multiple vectors for diversification (Figure 2). This series was optimized from compound **10a** to give **10b** with sub-micromolar activity in the biochemical assay. Both sides of the sulfonamide could be extensively modified while retaining potency; however the aniline portion originating from the HTS could not be improved upon. Unfortunately, when tested in our previously described cellular assay,[37] **10b** and related compounds

were found to cause no induction of CD86 at 50 μM in human THP-1 AML cells (Figure 2B). This disconnect was reinforced when the compounds were tested by surface plasmon resonance (SPR), where the binding response increased linearly with compound concentration (Figure 2C, inset), suggesting that no specific binding between compound and LSD1 protein was occurring. In addition, a competition assay with tranylcypromine demonstrated no abrogation of the interactions of these compounds with LSD1 (data not shown). Several other chemotypes emerging from the HTS were shown to display a similar lack of activity by SPR, suggesting the biochemical assay may be particularly susceptible to false positives, and demonstrating the value of an orthogonal biophysical assay in the screening cascade.

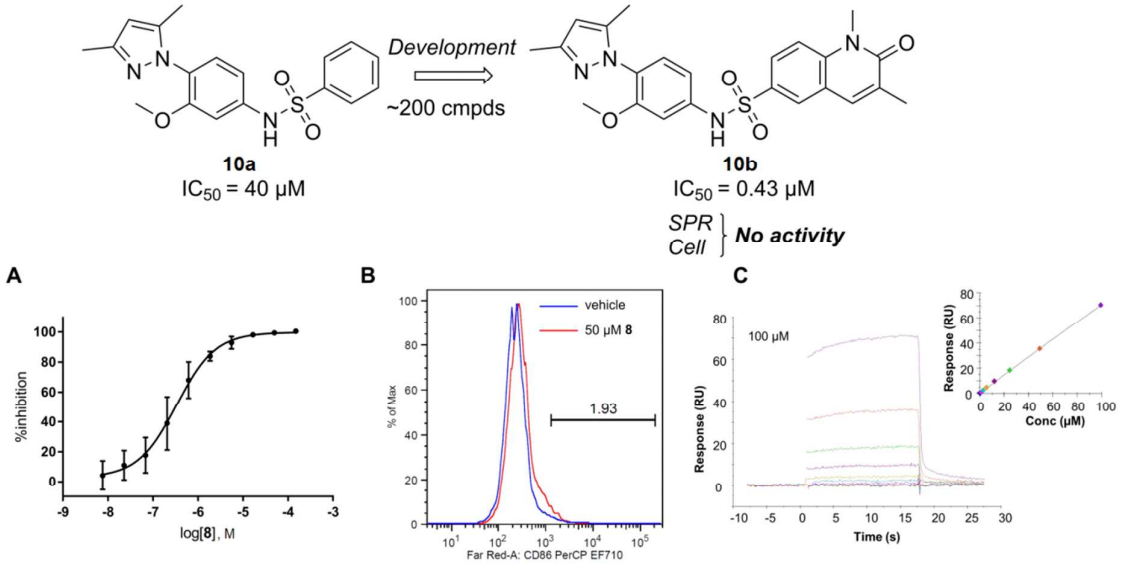


Figure 2. Development and characterization of compound **10b**. A: Biochemical assay; B: Surrogate cellular biomarker assay; C: Surface plasmon resonance sensorgram of the interaction between LSD1 and **10b** in two-fold dilution series (highest concentration indicated in the graph).

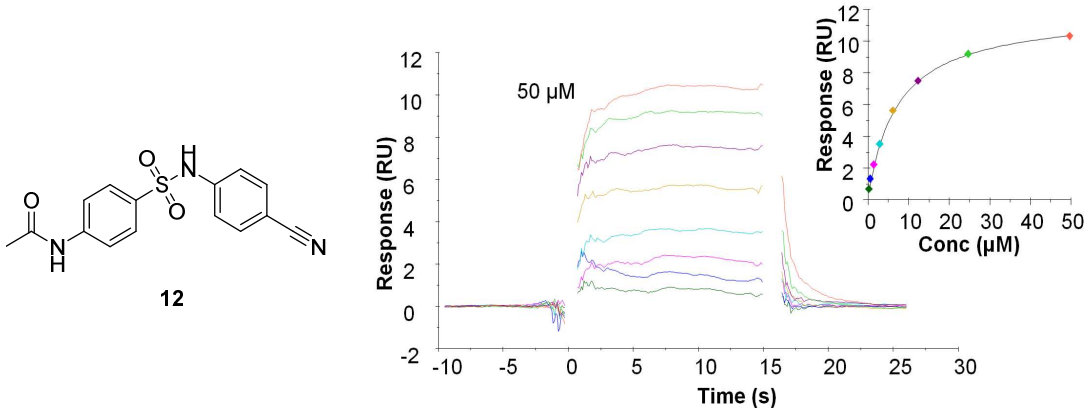


Figure 3. Structure of compound **12** with biochemical and SPR sensorgram indicating the highest tested concentration. K_d = 9.5 µM. K_d value was determined by SPR at 15 °C as the average of two experimental series (typically 10 concentrations).

SPR analysis did identify a single compound (**12**) that displayed reversible binding activity (Figure 3), a sulfonamide that we hypothesized might be acting as a ring-opened isostere of the pyridine scaffold of compound **1**. While **1** was disclosed by GSK at the AACR Annual Meeting 2013 (Washington DC, USA), very little work to develop this ligand had been published before 2015.[38] Certainly, the scope for potential core-hops had been seldom explored at the point at which we began to pursue this approach. To attempt to validate sulfonamides as a potential new scaffold, we used Cresset Spark and Torch software (Welwyn Garden City, U.K.) to align and identify potential new cores by searching fragment libraries for replacements with similar spatial and electronic features.[39]

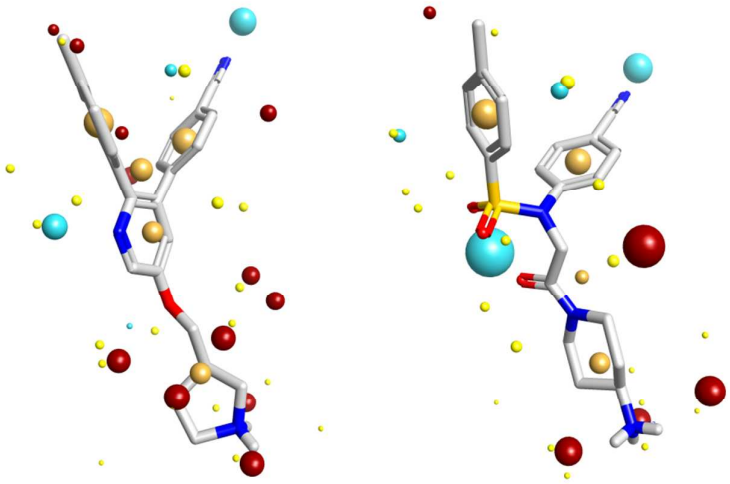
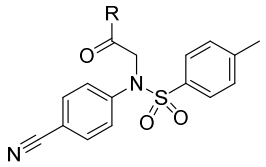
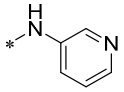
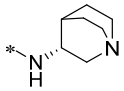
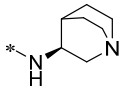
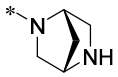
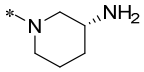
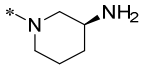
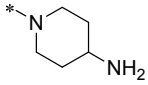
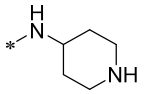
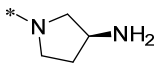
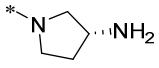
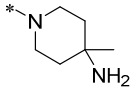


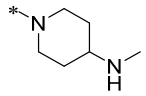
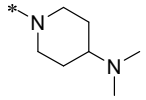
Figure 4. Cresset Torch alignment and structures of compound **1** (left) and **16g** (right). Similarity score 0.712. Blue field points (spheres) highlight energy minima for a positively charged probe, red for a negative probe. Yellow spheres represent an attractive van der Waals minimum for a neutral probe and brown spheres represent hydrophobic regions. Oxygen atoms are shown in red, nitrogen in blue. The similarity score is based on the likeness of the field points in terms of their magnitude and position. A score of greater than 0.7 is considered a 'good' score.

From this output we designed initial targets, a series of sulfonamidoacetamides (Figure 4) that looked to offer ease of synthesis while offering low two-dimensional (2D) similarity in comparison to more recognizable pyridine replacements such as pyridazines or pyrazines. We incorporated the *para*-cyano and *para*-tolyl aryl groups from **1** for the initial development, focused on optimizing the basic center, by coupling a series of amines to a key sulfonamidoacetic acid intermediate to give compounds **16a**–**m**. (Table 1). In contrast to the sulfonamides **10a**–**b**, these compounds showed specific and reversible binding to LSD1 by SPR, validating them as start points for further assay. Both primary and secondary amides decorated with basic amines were tolerated and displayed significantly improved levels of potency over **16a**. Initial optimization gave the 4-aminopiperidine derivative **16g**. Methylation α- to the basic center (**16k**) was also tolerated, alongside methylation on the nitrogen itself (**16l**, **16m**), however the activity appeared have reached a plateau, with no compounds achieving a K_d value below 2 μM.

Table 1. Optimization of basic center on the acetamidosulfonamide scaffold[a]



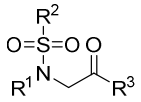
Compound	R	IC_{50} (μM)	SPR K_d (μM)
1	-	0.037 (0.012)	0.009
16a		>150	90
16b		11.9 (0.2)	3.6
16c		14.2 (0.3)	3.5
16d		13.1 (0.2)	2.4
16e		13.9 (0.1)	4.5
16f		24.3 (0.4)	10.3
16g		7.6 (1.2)	2.6
16h		15.6 (1.3)	6.5
16i		22.7 (1.6)	4.7
16j		16.4 (0.6)	3.2
16k		5.2 (0.7)	2.5

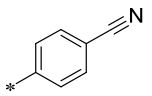
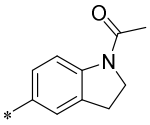
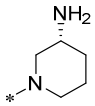
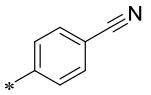
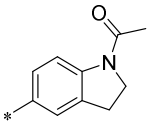
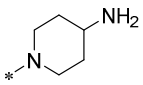
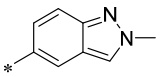
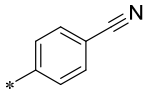
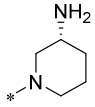
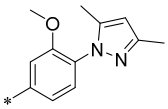
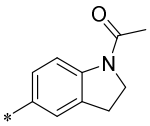
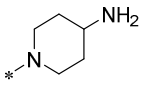
16l		7.5 (1.2)	2.9
16m		12.6 (1.8)	2.9

[a]IC_{50} and K_d values for selected compounds against the LSD1 enzyme in biochemical and biophysical assays. Standard deviation is given in parentheses. IC_{50} determined from 10 point concentration/effect experiments. Geometric mean of at least two independent experimental determinations given. Kd values were determined by SPR at 15 °C as the average of two experimental series (typically 10 concentrations).

Shortly after synthesizing these initial compounds, a patent from Quanticel disclosed several series of reversible LSD1 inhibitors featuring 6- and 5-membered monocyclic and 6,5-bicyclic core-hops (Figure 1).[28] Compounds from each series were claimed to display IC_{50} values against LSD1 of less than 100 nM in biochemical and cellular assays. While the patent reduced the diversity of chemical space available to explore, it also provided useful learning, both in terms of the essential features of the compounds, and which positions are more tolerant to change. For instance, the *para*-cyanophenyl and the presence of a basic nitrogen center are conserved throughout the examples in the patent, whereas the tolyl moiety was more amenable to optimization, and was successfully replaced with a number of mono- and bicyclic systems (e.g. **3**, Figure 1).

Table 2. Aryl modifications on the acetamidosulfonamide scaffold[a]



Compound	R^1	R^2	R^3	IC_{50} (μM)	SPR K_d (μM)
1	-	-	-	0.037 (0.012)	0.009
21a				5.2 (0.4)	2.4
21b				4.4 (1.0)	1.8
22				30.0 (2.4)	9.6
23				>30	>100

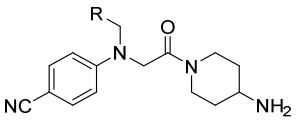
[a]IC_{50} and K_d values for selected compounds against the LSD1 enzyme in biochemical and biophysical assays. Standard deviation is given in parentheses. IC_{50} determined from 10-point concentration/effect experiments. Geometric mean of at least two independent experimental determinations given. K_d values were determined by SPR at 15 °C as the average of two experimental series (typically 10 concentrations).

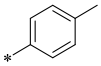
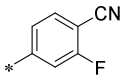
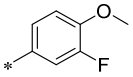
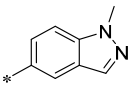
With this in mind, we investigated how the modification of the two aryl groups affected the activity (Table 2). Derivatives with an acetylindoline group were shown to give slightly better potency than the parent tolyl (**21a–b**). On the other hand, compound **22**, where the sulfonamide was reversed and

the tolyl replaced with the favored 2-methylindazole from the Quanticel patent, showed a disappointing drop-off in activity. Adopting the substituted aniline group from the prior HTS series (**23**) showed no activity by biochemical assay or SPR, while the parent **20** (biochemical IC_{50} = 1.6 μM; K_d > 100 μM) displayed the same non-specific binding effects by SPR. Despite our optimization attempts, we were disappointed to find that potency could not be significantly improved further, and thus we looked to modify the core itself to establish whether potency could be improved..

Toward this aim, we replaced the SO_2 moiety with a methylene linker to give a 4-cyanophenylglycinamide core. This alteration has the benefit of a significant reduction in molecular weight and total polar surface area, which could afford increased membrane permeability and bioavailability. However, this modification also introduces an aniline functionality that could potentially be a metabolic liability.[40] Initially, we chose to adopt the 4-aminopiperidine from compound **16g** as the basic center, and focused on modifications of the tolyl- group. Compound **29a** demonstrated a 5-fold improvement in potency compared to the matched-pair sulfonamide **16h** by both biochemical assay and SPR. In addition, a range of phenyl groups were tolerated and, in one case, improved potency further (**29c**). These beneficial modifications could be extended to bicyclic systems (**15e**). We then returned to investigate the basic center and found that homologating the 4-aminopiperidine unit to a piperidin-4-ylmethanamine gave a useful ~4-fold increase in potency (e.g. **29a** vs. **30a**). Modifying the pKa of the basic center by introducing of a fluorine atom at the *β*-carbon to the amine was deleterious to activity, however decreasing the flexibility of the amine by incorporation into a spirocycle (**32**) afforded a compound with <100 nM activity in the biochemical assay.

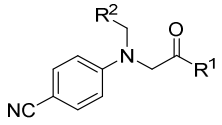
Table 3. Aryl optimization on the (4-cyanophenyl)glycine scaffold[a]

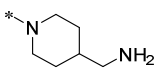
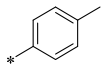
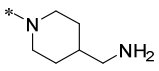
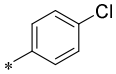
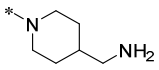
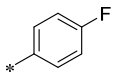
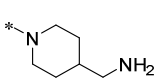
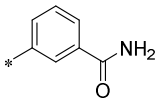
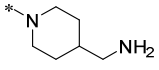
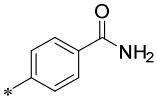
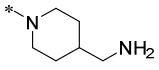
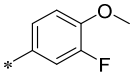
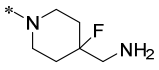
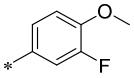
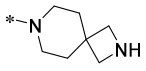
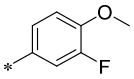


Compound	R	IC_{50} (μM)	SPR K_d (μM)
1	-	0.037 (0.012)	0.009
29a		1.3 (0.02)	0.43
29b		5.5 (0.07)	1.9
29c		0.81 (0.02)	0.19
29d		4.1 (0.1)	1.1
29e		0.62 (0)	0.14

[a]IC_{50} and K_d values for selected compounds against the LSD1 enzyme in biochemical and biophysical assays. Standard deviation is given in parentheses. IC_{50} determined from 10 point concentration/effect experiments. Geometric mean of at least two independent experimental determinations given. Kd values were determined by SPR at 15 °C as the average of two experimental series (typically 10 concentrations).

Table 4. Basic center optimization and aryl variation on the (4-cyanophenyl)glycine scaffold[a]



Compound	R^1	R^2	IC_{50} (μM)	SPR K_d (μM)
1	-	-	0.037 (0.012)	0.009
30a			0.29 (0.03)	0.11
30b			0.40 (0.11)	0.29
30c			1.3 (0.15)	0.46
30d			1.0 (0.14)	0.42
30e			0.83 (0.08)	0.49
30f			0.21 (0.06)	0.058
31			0.99 (0.23)	0.23
32			0.083 (0.003)	0.032

[a]IC_{50} and K_d values for selected compounds against the LSD1 enzyme in biochemical and biophysical assays. Standard deviation is given in parentheses. IC_{50} determined from 10 point concentration/effect experiments. Geometric mean of at least two independent experimental determinations given. K_d values were determined by SPR at 15 °C as the average of two experimental series (typically 10 concentrations).

In an attempt to rationalize the observed SAR, we performed docking studies, using the previously reported structure of LSD1 bound to tetrahydrofolate (PDB accession code 4KUM).[41] Protein preparation and docking were performed in Glide (Schrödinger, New York, USA).[42] The crystal structure of **1** bound to LSD1 has been disclosed,[26] and from these data, the binding mode of compound **30f** was predicted, as displayed in Figure 5. In this predicted pose, the nitrile forms a key hydrogen bonding interaction with K661. The basic center forms a network of ionic interactions with Asp555 and Asp556. This ionic interaction has been exploited previously by Vianello and co-workers.[33–34] The 3-fluoro-4-methoxyphenyl group sits in a channel formed by Trp695, Ile356, Leu677 and Leu693. The ether oxygen is predicted to make a hydrogen bonding interaction with Gln358, however, if the phenyl group were to flip then it could also potentially form a similar interaction with Asn535. It is worthy of note that a wide variety of scaffold-hops can be tolerated, and this may be explained by a lack of contact between the protein and the scaffold itself, with the key interactions being located at distal ends of the ligands.

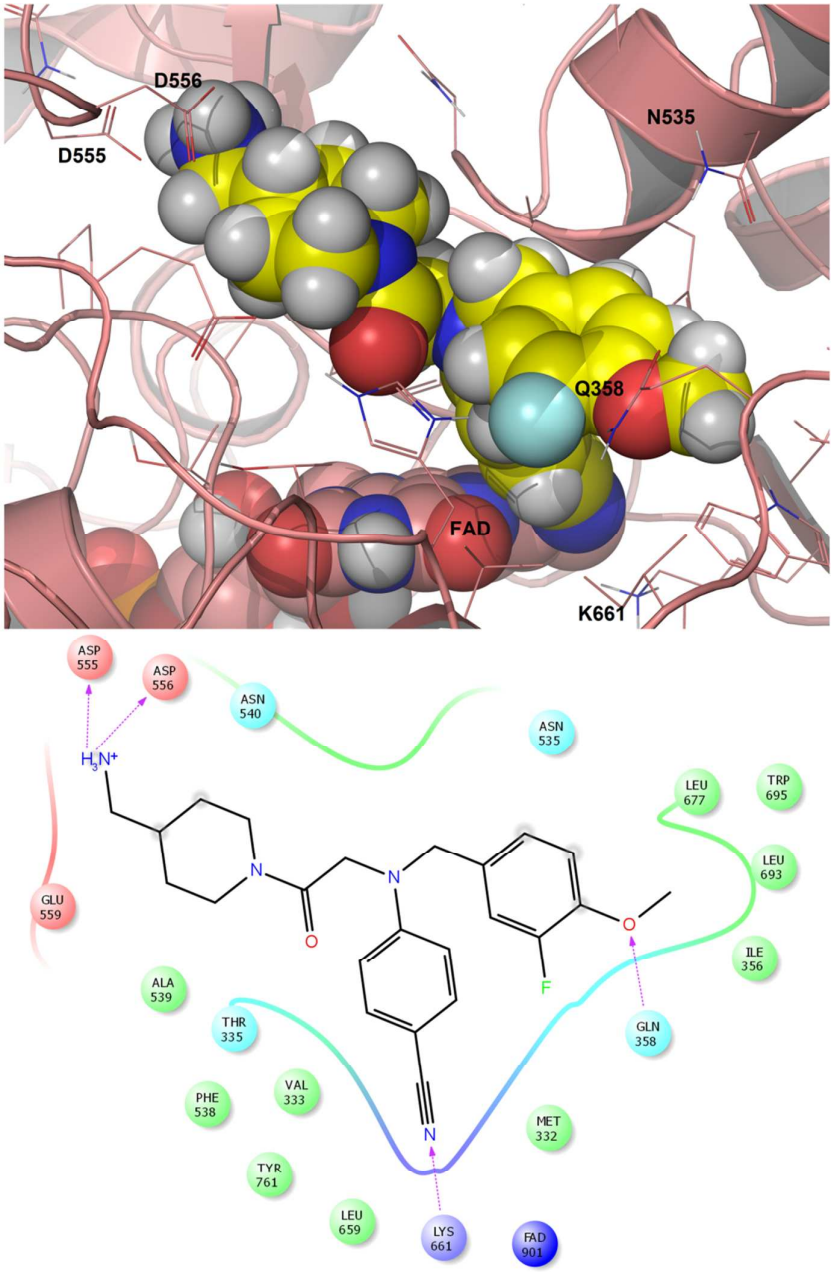


Figure 5. Left: Predicted binding mode of compound **30f** in the LSD1 active site (4KUM). Visualized using Maestro. Right: Protein-ligand interaction diagram with key hydrogen bond interactions highlighted (pink arrows).

To assess the effects of these derivatives on cells, compounds **1**, **30f** and **32** were tested at 7 concentrations in the THP-1 AML cell line to measure their effect on expression levels of CD86, a surrogate cellular biomarker of LSD1 inhibition (Figure 6).[37] Although compound **1** displayed the highest levels of potency in this assay (EC_{50} = 0.30 μM), we were satisfied to find only a 2-fold drop off in activity to our most active derivative, **32** (EC_{50} = 0.67 μM). The less active analogue **30f** displayed more modest activity (EC_{50} = 4.1 μM), suggesting good correlation between the biochemical and cellular assay.

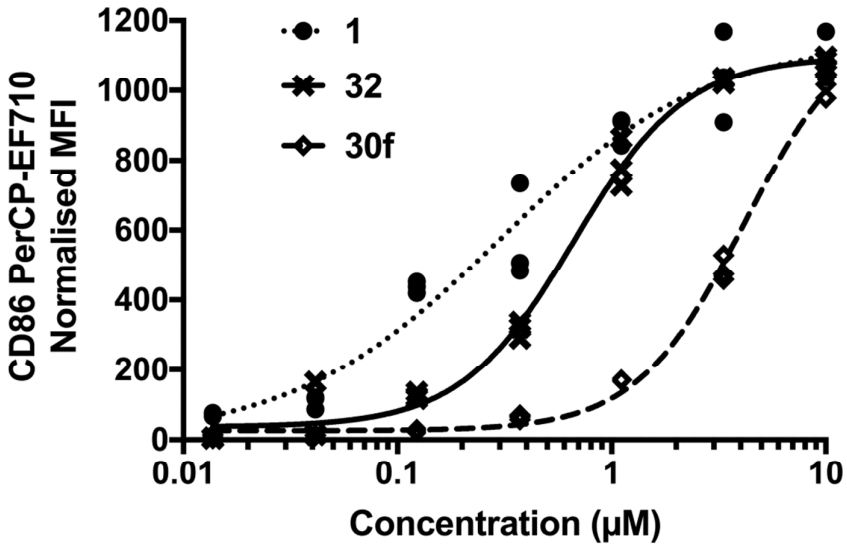


Figure 6. CD86 cell assay results for compounds **1**, **30f** and **32**.

To further assess cellular effects, we performed colony forming assays using two AML cell lines. This assay format indicates both proliferation of a cell population and the percentage of cells within that population with colony initiating activity.[10] THP1, and MV4-11 cells were treated with 10 μM of **1** or **32** for 7 days in semi-solid methylcellulose-based culture. Both compounds substantially reduced both colony forming frequency and the size of the colonies that did develop (Figure 7) indicating significant anti-proliferative activity, in addition to inducing up-regulation of the cellular differentiation marker CD86. To explore whether these compounds induced apoptosis, we performed

Annexin V-perCP EF710/7AAD staining on THP-1 cells treated for four days with 10 μM of each of the compounds. A modest but significant increase in apoptosis was observed (Figure 8). These results, combined with the CD86 data, suggest that compounds such as **32** act predominantly to promote differentiation of AML cells, with concomitant reduction in clonogenic potential.

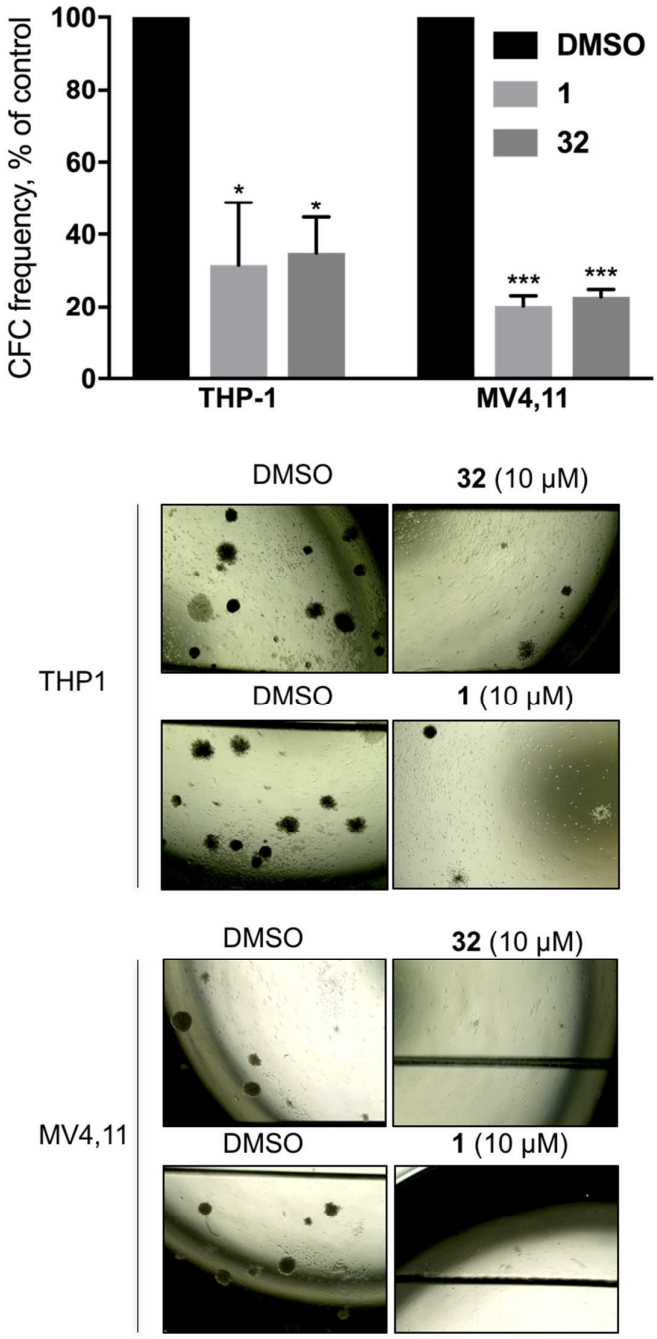


Figure 7. Top: Colony formation assay for THP-1 and MV4-11 cells dosed with 10 μM of compounds **1** and **32** for 7 days. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, statistical significance calculated using a one-way ANOVA with Dunnett's tests between vehicle treated and compound dosed cells. Bottom: representative images of colonies.

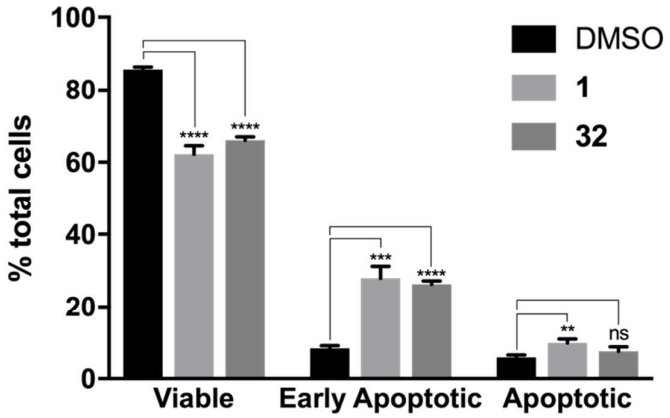


Figure 8. Annexin V-perCP EF710/7AAD double staining experiments results with THP-1 cell dosed with vehicle, or 10 μM **1** or **32** for 4 days in liquid culture. Early apoptotic cells = Annexin $V^{pos}7\text{-}AAD^{neg}$; Apoptotic cells = Annexin $V^{pos}7\text{-}AAD^{pos}$. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, statistical significance calculated using one-way ANOVA analysis with Dunnett's tests.

in vitro ADME properties

As inhibition of the hERG ion channel proved to be an insurmountable issue in furthering the development of **1** towards the clinic,[26] we were keen to assess our compounds against this target. Compounds **29c**, **30f**, and **32** were tested in a patch clamp assay, alongside being profiled for their metabolic stability (Table 5). Gratifyingly these compounds displayed improved selectivity against hERG in comparison to **1** (IC_{50} = 3.2 μM), with compound **32** displaying a ~30-fold window of selectivity between LSD1 inhibition in cells and hERG inhibition. In addition, we observed low levels of clearance in mouse microsomes with all three compounds. Disappointingly, these compounds displayed poor membrane permeability and high levels of efflux in comparison to compound **1**, which

could make achieving oral bioavailability challenging, although this liability does not appear to preclude the ability of the compounds to inhibit LSD1 in a cellular context.

Table 5. In vitro parameters for 1, 29c, 30f and 32

	1	**29c**	**30f**	**32**
Caco2 A–B mean P_{app} (10^{-6} cm/s); (efflux ratio)	5.6 (3.2)	0.44 (48)	0.26 (58)	0.09 (58)
Mo Mic CL_{int} (µL/min/10^6 cells)	3.43	3.6	4.5	<1
Mo Mic $T_{½}$ (min)	404	385	305	>1000
hERG EC_{50} (µM); (standard error)	3.2	>25	12.4 (3.4)	20.1 (4.6)

CONCLUSION

In conclusion, we have developed a series of (4-cyanophenyl)glycine derivatives that show potent activity against LSD1 by both biochemical assay and SPR. Starting from a weakly active HTS hit **12**, we identified acyclic lead-like analogues of compound **1**. We successfully developed these initial start points, leading to the development of compound **32**, which potently inhibits LSD1 in biochemical, biophysical and cellular assays. These compounds display much greater levels of selectivity over the hERG cardiac ion channel than compound **1**, a liability which precluded further development of that series. Compound **32** is an attractive tool compound for use in in vitro systems and start-point for future development. More generally, this work suggests the scaffold of compound **1** is broadly modifiable, and while the *p*-cyanophenyl group appears to form a key interaction with K661, modification of the tolyl- functionality and the position and orientation of basic center is much more tolerant to modification.

Experimental Section

Chemistry

Flash column chromatography was performed using pre-packed silica gel cartridges (KP-Sil SNAP, Biotage, Hengoed UK or RediSep Rf, Isco). Thin layer chromatography was conducted with 5 × 10 cm plates coated with Merck Type 60 F_{254} silica gel to a thickness of 0.25 mm. All reagents obtained from commercial sources were used without further purification. Anhydrous solvents were obtained from the Sigma-Aldrich Company Ltd. (Dorset, UK) or Fisher Scientific UK Ltd. (Loughborough, UK), and used without further drying. HPLC grade solvents were obtained from Fisher Chemicals Ltd.

All compounds prepared were >95% purity as determined by examination of both the LC-MS and ^{1}H NMR spectra unless otherwise indicated. Where Cl or Br were present, expected isotopic distribution patterns were observed.

Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on a 300 MHz Bruker Avance spectrometer. Solutions were typically prepared in either deuterochloroform ($CDCl_3$), deuterated dimethylsulfoxide (DMSO-d_6) or deuterated methanol (methanol-d_4) with chemical shifts referenced to tetramethylsilane (TMS) or deuterated solvent as an internal standard. ^{1}H NMR data are reported indicating the chemical shift (δ), the integration (e.g. 1H), the multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad; dd, doublet of doublets etc.), and the coupling constant (J) in Hz. Deuterated solvents were obtained from Goss Scientific (Crewe, UK). In general, basic N-H protons were not observed.

LC-MS analyses were performed on a Waters (Elstree, UK) Acquity UPLC system fitted with BEH C18 1.7 μM columns (2.1 × 50 mm) and with UV diode array detection (210–400 nm) with a Waters SQD detector. Analyses were performed with either buffered acidic or basic solvents, and using gradients detailed in the Supporting Information.

Some compounds were purified by preparative (prep.) HPLC on a Waters FractionLynx MS autopurification system, with a Waters XBridge 5 μm C18, 100 mm × 19 mm i.d. column, running at a flow rate of 20 mL/min with UV diode array detection (210–400 nm) and mass-directed collection using both positive and negative mass ion detection. Purifications were performed using buffered

acidic or basic solvent systems as appropriate. Compound retention times on the system were routinely assessed using a 30–50 μL test injection and a standard gradient, then purified using an appropriately chosen focused gradient as detailed in the Supporting Information, based upon observed retention time.

Preparation of compounds 10a–b

1-(2-Methoxy-4-nitro-phenyl)-3,5-dimethylpyrazole (8).

A suspension of 3-methoxy-4-fluoronitrobenzene (25 g, 146 mmol), 3,5-dimethylpyrazole (15.5 g, 161 mmol) and potassium carbonate (60.5 g, 438 mmol) in DMF (200 mL) was stirred overnight at 80 °C. The reaction mixture was added to water (500 mL) and the resulting yellow precipitate collected by filtration and dried to the title compound (35.8 g, 99%) as a yellow powder. ^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.95 (dd, *J* = 8.5, 2.4 Hz, 1H), 7.90 (d, *J* = 2.4 Hz, 1H), 7.54 (d, *J* = 8.5 Hz, 1H), 6.03 (s, 1H), 3.94 (s, 3H), 2.31 (s, 3H), 2.14 (s, 3H). LC-MS: (High pH) t_R 1.09 min, *m/z* 248.1 $[M+H]^+$, 100% purity.

4-(3,5-Dimethylpyrazol-1-yl)-3-methoxyaniline (9).

A stirred suspension of 1-(2-methoxy-4-nitro-phenyl)-3,5-dimethylpyrazole (36 g, 0.145 mol), zinc powder (28.6 g, 0.44 mol) and ammonium formate (91.2 g, 1.46 mol) in methanol (250 mL) was heated overnight to 40 °C. The reaction mixture was passed through a hydrophobic frit and concentrated to dryness. The resulting residue was suspended between brine (250 mL) and DCM (500 mL), the organic layer passed through a hydrophobic frit and concentrated to dryness to afford the crude product. Recrystallization from hexanes afforded the title compound (24.7 g, 78%) as off-white needles. ^{1}H NMR (300 MHz, Chloroform-$_d$) δ 7.13–7.03 (m, 1H), 6.36 (m, 2H), 5.94 (s, 1H), 3.74 (s, 3H), 2.30 (s, 3H), 2.08 (s, 3H). LC-MS: (High pH) t_R 0.88 min, *m/z* 218.2 $[M+H]^+$, 98% purity.

***N*-[4-(3,5-Dimethylpyrazol-1-yl)-3-methoxyphenyl]benzenesulfonamide (10a).**

A solution of benzenesulfonyl chloride (18 μL, 0.14 mmol) and 4-(3,5-dimethylpyrazol-1-yl)-3-methoxy-aniline (30 mg, 0.14 mmol) in pyridine (0.45 mL) was stirred at 100 °C for 1 hour. The

reaction was concentrated to dryness then purified by preparative HPLC (low pH gradient 3) to afford the title compound (20 mg, 41%) as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.39 (s, 1H), 7.90–7.75 (m, 2H), 7.65–7.42 (m, 3H), 7.02 (d, J = 8.4 Hz, 1H), 6.83 (d, J = 2.2 Hz, 1H), 6.67 (dd, J = 8.4, 2.2 Hz, 1H), 5.94–5.81 (m, 1H), 3.62 (s, 3H), 2.09 (s, 3H), 1.91 (s, 3H).

***N*-[4-(3,5-Dimethylpyrazol-1-yl)-3-methoxyphenyl]-1,3-dimethyl-2-oxo-quinoline-6-sulfonamide (10b).**

A solution of 4-(3,5-dimethylpyrazol-1-yl)-3-methoxyaniline (28 mg, 0.13 mmol) and 1,3-dimethyl-2-oxo-quinoline-6-sulfonyl chloride (34 mg, 0.12 mmol) in pyridine (300 μL) was stirred at room temperature overnight. The reaction mixture was diluted with 2:1:1 (v:v:v) DMSO:water:MeCN (700 μL), then purified by preparative HPLC (low pH) to afford the title compound (39 mg, 70%) as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.62 (s, 1H), 8.15 (d, J = 2.2 Hz, 1H), 7.97–7.86 (m, 2H), 7.67 (d, J = 9.0 Hz, 1H), 7.08 (d, J = 8.4 Hz, 1H), 6.93 (d, J = 2.2 Hz, 1H), 6.75 (dd, J = 8.4, 2.2 Hz, 1H), 5.90 (s, 1H), 3.66 (s, 3H), 3.64 (s, 3H), 2.12 (s, 3H), 2.07 (s, 3H), 1.91 (s, 3H). ^{13}C NMR (75 MHz, Methanol-d_4) δ 144.1, 140.5, 137.6, 135.1, 133.2, 130.5, 128.7, 124.8, 122.1, 114.7, 114.0, 110.8, 106.8, 57.4, 30.9, 22.4, 15.7, 15.2. HRMS (ESI) *m/z* $[M-H]^-$ calcd for $C_{23}H_{23}N_4O_4S$: 451.1445. Found: 451.1443.

Preparation of compound 12

***N*-[4-[(4-Cyanophenyl)sulfamoyl]phenyl]acetamide (12).**

A solution of DMAP (50 mg, 0.42mmol), *p*-cyanoaniline (50 mg, 0.42 mmol) and 4-acetamidobenzenesulfonyl chloride (99 mg, 0.42 mmol) in acetonitrile (5 mL) was heated by microwave irradiation to 100 °C for 1 h. The reaction was concentrated to dryness then purified by preparative HPLC (low pH gradient 2) to afford the title compound (49 mg, 37%) as colourless crystals. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.95 (s, 1H), 10.34 (s, 1H), 7.83–7.62 (m, 6H), 7.28–7.15 (m, 2H), 2.06 (s, 3H).

Preparation of compounds 16a–16m

_N_-(4-Cyanophenyl)-4-methylbenzenesulfonamide (13).

To a stirred solution of 4-cyanoaniline (5 g, 42.3 mmol) in pyridine (3.6 mL) and acetonitrile (9 mL) was added portionwise *p*-toluenesulfonyl chloride (8.88 g, 46.6 mmol). The reaction mixture was stirred at room temperature overnight, then water (50 mL) was added and the resulting light orange precipitate collected by filtration, washed with water (3 × 20 mL) and dried under vacuum at 50 °C to afford the title compound (8.92 g, 77%) as a light orange powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.99 (s, 1H), 7.78–7.61 (m, 4H), 7.43–7.33 (m, 2H), 7.32–7.15 (m, 2H), 2.34 (s, 3H). LC-MS: (High pH) t_R 0.82 min, *m/z* 271.1 $[M-H]^-$, 100% purity.

***tert*-Butyl 2-[4-cyano-*N*-(p-tolylsulfonyl)anilino]acetate (14).**

To a stirred suspension of N-(4-cyanophenyl)-4-methylbenzenesulfonamide (2 g, 7.34 mmol) and potassium carbonate (2.02 g, 14.7 mmol) in DMF (20 mL) was added tert-butyl bromoacetate (1.30 mL, 8.81 mmol) and the reaction mixture heated to 80°C for 1 h. The reaction mixture was diluted with water (50 mL) then extracted with DCM (2 × 20 mL). The combined organic layers were passed through a hydrophobic frit and concentrated to dryness to afford the title compound (2.71g, 97%) as an orange oil. ^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.66–7.51 (m, 4H), 7.41–7.23 (m, 4H), 4.35 (s, 2H), 2.43 (s, 3H), 1.40 (s, 9H). LC-MS: (High pH) t_R 1.32 min, *m/z* 387.1 $[M+H]^+$, 94% purity.

2-[4-Cyano-N-(*p*-tolylsulfonyl)anilino]acetic acid (15).

A stirred solution of *tert*-butyl 2-[4-cyano-N-(*p*-tolylsulfonyl)anilino]acetate (2.9 g, 7.5 mmol) in trifluoroacetic acid (2.79 mL, 37.5 mmol) and DCM (30 mL) was heated to 30 °C for 1 h. The reaction mixture was concentrated to dryness to afford an orange oil. Purification by column chromatography (0 to 60% EtOAc in isohexane, then 10% MeOH in DCM) afforded the title compound as a colourless oil that solidified upon standing to give colourless crystals. ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.06 (s, 1H), 7.87–7.75 (m, 2H), 7.65–7.53 (m, 2H), 7.39 (m, 4H), 4.55 (s, 2H), 2.38 (s, 3H). LC-MS: (High pH) t_R 0.63 min, *m/z* 319.1 $[M-H]^-$, 96% purity.

General method A: To a stirred solution of 2-[4-cyano-*N*-(p-tolylsulfonyl)anilino]acetic acid (30. mg, 0.09 mmol), the appropriate bis-amine (1.2 eq.) and N,N-Diisopropylethylamine (45 µL, 0.27 mmol) in DMF (300 µL) was added COMU (58 mg, 0.14 mmol) and the reaction mixture stirred at room temperature for 6 h. The reaction mixture was concentrated to dryness and purified by mass directed preparative HPLC (high pH), to afford the desired product.

General method B: To a stirred solution of 2-[4-cyano-*N*-(p-tolylsulfonyl)anilino]acetic acid (30 mg, 0.09 mmol), the appropriate Boc-protected bis-amine (1.2 eq.) and *N*,*N*-diisopropylethylamine (45 µL, 0.27 mmol) in DMF (300 µL) was added COMU (58 mg, 0.14mmol) and the reaction mixture stirred at room temperature for 6 h. The reaction mixture was concentrated to dryness and 4 M HCl in dioxane (1 mL) was added. This was stirred for 1 h, then concentrated to dryness and purified by mass directed preparative HPLC (high pH), to afford the desired product.

2-[4-Cyano-*N*-(p-tolylsulfonyl)anilino]-*N*-(3-pyridyl)acetamide (16a).

Prepared by general method A using 3-aminopyridine to afford the title compound (24 mg, 65%) as a light brown powder. ^{1}H NMR (300 MHz, Methanol-d_4) δ 8.68 (dd, J = 2.6, 0.8 Hz, 1H), 8.27 (dd, J = 4.8, 1.5 Hz, 1H), 8.07 (ddd, J = 8.4, 2.6, 1.5 Hz, 1H), 7.72–7.65 (m, 2H), 7.63–7.57 (m, 2H), 7.54–7.43 (m, 2H), 7.44–7.28 (m, 3H), 4.61 (s, 2H), 2.42 (s, 3H).

2-[4-Cyano-*N*-(p-tolylsulfonyl)anilino]-*N*-[(3*R*)-quinuclidin-3-yl]acetamide (16b).

Prepared by general method A using (*R*)-3-aminoquinuclidine dihydrochloride to afford the title product as an orange oil (14 mg, 35%). ^{1}H NMR (300 MHz, Methanol-d_4) δ 8.49 (s, 1H), 7.75–7.63 (m, 2H), 7.60–7.50 (m, 2H), 7.42 (m, 2H), 7.40–7.34 (m, 2H), 4.42 (s, 2H), 4.17 (m, 1H), 3.74–3.60 (m, 1H), 3.34–3.19 (m, 4H), 3.02 (dd, J = 13.8, 4.7 Hz, 1H), 2.44 (s, 3H), 2.12 (q, J = 3.1 Hz, 1H), 1.99 (s, 3H), 1.86 (s, 1H).

2-[4-Cyano-*N*-(p-tolylsulfonyl)anilino]-*N*-[(3*S*)-quinuclidin-3-yl]acetamide (16c).

Prepared by general method A using (*S*)-3-aminoquinuclidine dihydrochloride to afford the title product as a yellow powder (11 mg, 28%). ^{1}H NMR (300 MHz, Methanol-d_4) δ 8.46 (s, 1H), 7.70 (dd,

J = 1.9, 8.7 Hz, 2H), 7.60–7.50 (m, 2H), 7.47–7.40 (m, 2H), 7.37 (d, *J* = 7.9 Hz, 2H), 4.43 (s, 2H), 4.19 (m, 1H), 3.72–3.62 (m, 1H), 3.29–3.22 (m, 3H), 3.04 (dd, *J* = 13.8, 4.7 Hz, 1H), 2.44 (s, 3H), 2.13 (m, 1H), 2.06–1.97 (m, 4H), 1.87 (s, 1H).

_N_-(4-Cyanophenyl)-_N_-[2-[(1_S_,4_S_)-2,5-diazabicyclo[2.2.1]heptan-2-yl]-2-oxo-ethyl]-4-methyl-benzenesulfonamide (16d).

Prepared by general method B using (1*S*,4*S*)-2-Boc-2,5-diazabicyclo[2.2.1]heptane to afford the title compound (28 mg, 75%) as a white powder. ^{1}H NMR (300 MHz, Methanol-d_4) δ 8.50 (s, 1H), 7.78–7.60 (m, 2H), 7.64–7.47 (m, 2H), 7.50–7.25 (m, 4H), 4.43 (s, 2H), 4.18 (ddd, *J* = 8.3, 5.3, 3.3 Hz, 1H), 3.74–3.59 (m, 1H), 3.34–3.15 (m, 4H), 3.02 (ddd, *J* = 13.5, 5.3, 2.2 Hz, 1H), 2.43 (s, 3H), 2.18–1.90 (m, 2H), 1.88– 1.76 (m, 1H)

_N_-[2-[(3_R_)-3-Amino-1-piperidyl]-2-oxo-ethyl]-N-(4-cyanophenyl)-4-methyl-benzenesulfonamide (16e).

Prepared by general method B using (*R*)-3-Boc-aminopiperidine to afford the title compound (14 mg, 37%) as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.85–6.78 (m, 2H), 6.78–6.68 (m, 2H), 6.60–6.53 (m, 2H), 6.51 (d, *J* = 8.1 Hz, 2H), 3.89 (d, *J* = 16.5 Hz, 1H), 3.81 (d, *J* = 16.5 Hz, 1H), 3.38 (d, *J* = 12.5 Hz, 1H), 3.29–3.08 (m, 1H), 2.96 (d, *J* = 13.7 Hz, 1H), 2.61–2.06 (m, 3H), 1.58 (s, 3H), 1.08–1.00 (m, 1H), 0.92–0.78 (m, 2H).

_N_-[2-[(3_S_)-3-Amino-1-piperidyl]-2-oxo-ethyl]-_N_-(4-cyanophenyl)-4-methyl-benzenesulfonamide (16f).

Prepared by general method B using (*S*)-3-Boc-aminopiperidine to afford the title compound (18 mg, 48%) as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.81–7.74 (m, 2H), 7.72–7.60 (m, 2H), 7.43–7.27 (m, 4H), 4.85 (d, *J* = 17.3 Hz, 1H), 4.70 (d, *J* = 17.3 Hz, 1H), 4.16–4.04 (m, 1H), 3.71 (d, *J* = 12.5 Hz, 2H), 3.10–2.86 (m, 1H), 2.86 (s, 1H), 2.73–2.51 (m, 1H), 2.38 (s, 3H), 1.95–1.12 (m, 4H).

_N_-[2-(4-Amino-1-piperidyl)-2-oxo-ethyl]-_N_-(4-cyanophenyl)-4-methyl-benzenesulfonamide (16g).

Prepared by general method B using 4-[(tert-Butoxycarbonyl)amino]piperidine to afford the title compound (21 mg, 56%) as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.87–7.69 (m, 2H), 7.74–7.59 (m, 2H), 7.43–7.30 (m, 4H), 4.85 (d, J = 17.3 Hz, 1H), 4.70 (d, J = 17.3 Hz, 1H), 4.18 (d, J = 13.7 Hz, 1H), 3.88 (d, J = 13.7 Hz, 1H), 3.09 (m, 2H), 2.73–2.53 (m, 1H), 2.38 (s, 3H), 1.85 (m, 2H), 1.50–1.30 (m, 1H), 1.31–1.07 (m, 1H).

2-[4-Cyano-*N*-(p-tolylsulfonyl)anilino]-*N*-(4-piperidyl)acetamide (16h).

Prepared by general method B using 4-amino-1-Boc-piperidine to afford the title compound (19 mg, 51%) as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.15 (d, J = 7.6 Hz, 1H), 7.90–7.71 (m, 2H), 7.62–7.50 (m, 2H), 7.48–7.30 (m, 4H), 4.33 (s, 2H), 3.70–3.53 (m, 2H), 3.11–2.97 (m, 2H), 2.75–2.61 (m, 2H), 2.39 (s, 3H), 1.72–1.59 (m, 2H), 1.45–1.27 (m, 2H).

***N*-[2-[(3*S*)-3-Aminopyrrolidin-1-yl]-2-oxo-ethyl]-*N*-(4-cyanophenyl)-4-methyl-benzenesulfonamide (16i).**

Prepared by general method B using (*S*)-3-(Boc-amino)pyrrolidine to afford the title compound (18 mg, 50%) as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.84–7.72 (m, 2H), 7.66 (dq, J = 8.6, 2.2 Hz, 2H), 7.50–7.27 (m, 5H), 4.65 (s, 1H), 4.61 (s, 1H), 3.69–3.48 (m, 1H), 3.47–3.07 (m, 3H), 2.38 (s, 3H), 2.13–1.85 (m, 1H), 1.85–1.52 (m, 1H).

***N*-[2-[(3*R*)-3-Aminopyrrolidin-1-yl]-2-oxo-ethyl]-*N*-(4-cyanophenyl)-4-methyl-benzenesulfonamide (16j).**

Prepared by general method B using (*R*)-3-(Boc-amino)pyrrolidine to afford the title compound (23 mg, 64%) as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.84–7.72 (m, 2H), 7.72–7.58 (m, 2H), 7.42–7.34 (m, 4H), 4.75–4.55 (m, 2H), 3.69–3.56 (m, 1H), 3.62–3.21 (m, 3H), 3.16 (m, 1H), 2.38 (s, 3H), 2.13–1.85 (m, 1H), 1.85–1.51 (m, 1H).

***N*-[2-(4-Amino-4-methyl-1-piperidyl)-2-oxo-ethyl]-*N*-(4-cyanophenyl)-4-methyl-benzenesulfonamide (16k).**

Prepared by general method B using *tert*-butyl N-(4-methyl-4-piperidyl)carbamate to afford the title compound (26 mg, 67%) as a white powder. ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.57 (d, 2H), 7.49 (d, J = 8.2 Hz, 2H), 7.32 (d, J = 8.5 Hz, 2H), 7.26 (d, J = 8.0 Hz, 2H), 4.68 (d, J = 16.7 Hz, 1H), 4.57 (d, J = 16.7 Hz, 1H), 3.91 (m, 2H), 3.43–3.28 (m, 1H), 3.17–2.97 (m, 1H), 2.33 (s, 3H), 1.81 (s, 2H), 1.75–1.53 (m, 2H), 1.38 (s, 3H).

_N_-(4-Cyanophenyl)-4-methyl-_N_-[2-[4-(methylamino)-1-piperidyl]-2-oxo-ethyl]benzenesulfonamide (16l).

Prepared by general method B using *tert*-butyl *N*-methyl-*N*-(piperidin-4-yl)carbamate to afford the title compound (21 mg, 39%) as a white powder. ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.73–7.64 (m, 2H), 7.65–7.53 (m, 2H), 7.45–7.39 (m, 2H), 7.39–7.32 (m, 2H), 4.77 (d, J = 16.5 Hz, 1H), 4.63 (d, J = 16.5 Hz, 1H), 4.51 (d, J = 14.0 Hz, 1H), 4.18 (d, J = 14.0 Hz, 1H), 3.31–3.13 (m, 2H), 2.72 (s, 3H), 2.43 (s, 3H), 2.17 (m, 2H), 1.66 (qd, J = 12.3, 4.3 Hz, 1H), 1.43 (qd, J = 12.3, 4.3 Hz, 1H).

_N_-(4-Cyanophenyl)-_N_-[2-[4-(dimethylamino)-1-piperidyl]-2-oxo-ethyl]-4-methyl-benzenesulfonamide (16m).

Prepared by general method A using 4-dimethylaminopiperidine to afford the title compound (26 mg, 65%) as a white powder. ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.71–7.63 (m, 2H), 7.63–7.55 (m, 2H), 7.45–7.39 (m, 2H), 7.39–7.32 (m, 2H), 4.76 (d, J = 16.5 Hz, 1H), 4.64 (d, J = 16.5 Hz, 1H), 4.56 (d, J = 14.0 Hz, 1H), 4.20 (d, J = 14.0 Hz, 1H), 3.19 (t, J = 12.6 Hz, 1H), 2.78 (s, 6H), 2.74–2.59 (m, 1H), 2.43 (s, 3H), 2.12 (m, 2H), 1.75 (qd, J = 12.2, 4.2 Hz, 1H), 1.51 (dd, J = 12.2, 4.2 Hz, 1H).

Preparation of compounds 21–27

1-Acetyl-_N_-(4-cyanophenyl)indoline-5-sulfonamide (18).

Prepared in a manner analogous to compound **11** to afford the title compound as a light orange powder (486 mg, 84%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.95 (s, 1H), 8.11 (d, J = 8.4 Hz, 1H), 7.76–7.61 (m, 4H), 7.29–7.17 (m, 2H), 4.11 (t, J = 8.6 Hz, 2H), 3.15 (t, J = 8.6 Hz, 2H), 2.15 (s, 3H). LC-MS: (High pH) t_R 0.76 min, *m/z* 340.1 $[M-H]^-$, 96% purity.

4-Cyano-*N*-(2-methylindazol-5-yl)benzenesulfonamide (19).

To a stirred solution of 2-methyl-2*H*-indazol-5-amine (1 g, 6.8 mmol) in pyridine (0.9 mL) and acetonitrile (4 mL) was added 4-cyanobenzenesulfonyl chloride (1.51 g, 7.5 mmol). The reaction mixture was stirred at room temperature overnight, then water (50 mL) was added and the resulting light orange precipitate collected by filtration, washed with water (3 × 20 mL) and dried under vacuum at 50 °C to the title compound (1.15 g, 54%) as a light orange powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.36 (s, 1H), 8.25 (s, 1H), 8.00 (d, *J* = 8.4 Hz, 2H), 7.88–7.82 (m, 2H), 7.82–7.74 (m, 1H), 7.49 (dd, *J* = 9.1, 0.9 Hz, 1H), 7.36 (d, *J* = 2.0 Hz, 1H), 6.98 (dd, *J* = 9.1, 2.0 Hz, 1H), 4.11 (s, 3H). LC-MS: (High pH) t_R 0.74 min, *m/z* 311.1 $[M-H]^-$, 100% purity.

1-Acetyl-*N*-[2-[(3*R*)-3-amino-1-piperidyl]-2-oxo-ethyl]-*N*-(4-cyanophenyl)indoline-5-sulfonamide (21a)

A stirred suspension of **18** (30 mg, 0.09 mmol), *tert*-butyl *N*-[(3*R*)-1-(2-chloroacetyl)-3-piperidyl]carbamate (**SI1**, 27 mg, 0.10 mmol), potassium carbonate (18 mg, 0.13 mmol) and potassium iodide (2 mg) in DMF (800 µL) was stirred at 80 °C for 3 hours. The reaction mixture was adsorbed onto silica and concentrated to dryness. Purification by CombiFlash (0 to 30% EtOAc in isohexane) afforded the Boc protected product. This was stirred in TFA (0.5 mL) and DCM (3 mL) then concentrated to dryness to afford the title compound (32 mg, 76%) as a white powder, TFA salt. ^{1}H NMR (300 MHz, Methanol-d_4) δ 8.19 (d, *J* = 8.8 Hz, 1H), 7.76–7.61 (m, 2H), 7.55 (d, *J* = 8.4 Hz, 2H), 7.44 (d, *J* = 8.4 Hz, 2H), 4.79 (d, *J* = 16.5 Hz, 1H), 4.67 (d, *J* = 16.5 Hz, 1H), 4.21 (t, *J* = 8.6 Hz, 2H), 3.93–3.77 (m, 1H), 3.67 (s, 3H), 3.24 (t, *J* = 8.9 Hz, 2H), 2.27 (s, 3H), 2.20–2.09 (m, 1H), 1.99–1.86 (m, 1H), 1.83–1.64 (m, 2H), 1.45 (d, *J* = 10.9 Hz, 1H).

1-Acetyl-*N*-[2-(4-amino-1-piperidyl)-2-oxo-ethyl]-*N*-(4-cyanophenyl)indoline-5-sulfonamide (21b).

A stirred suspension of **18** (50 mg, 0.15 mmol), *tert*-butyl *N*-[1-(2-chloroacetyl)-4-piperidyl]carbamate (**SI2**, 49 mg, 0.18 mmol) and potassium carbonate (40 mg, 0.29 mmol) in DMF (1 mL) was heated to 80 °C for 6 h. EtOAc (5 mL) was added and the reaction mixture filtered

through an Isolute filter cartridge, then concentrated to dryness. 4 M HCl/dioxane (1 mL) was then added. After 1 h the reaction mixture was concentrated to dryness under vacuum, and the resulting residue was purified by preparative HPLC (high pH) to afford the title compound (29 mg, 41%) as a light yellow powder. ^{1}H NMR (300 MHz, Methanol-d_4) δ 8.16 (d, J = 8.6 Hz, 1H), 7.71–7.59 (m, 2H), 7.53 (d, J = 7.8 Hz, 2H), 7.48–7.34 (m, 2H), 4.79 (d, J = 16.5 Hz, 1H), 4.65 (d, J = 16.5 Hz, 1H), 4.47 (d, J = 13.8 Hz, 1H), 4.29–4.09 (m, 3H), 3.40 (ddt, J = 11.8, 7.4, 4.1 Hz, 1H), 3.26–3.15 (m, 3H), 2.73 (td, J = 13.3, 12.6, 2.8 Hz, 1H), 2.26 (s, 3H), 2.14 (d, J = 12.5 Hz, 1H), 2.04 (d, J = 12.5 Hz, 1H), 1.68 (qd, J = 11.8, 4.1 Hz, 1H), 1.46 (qd, J = 11.8, 4.1 Hz, 1H).

_N_-[2-[(3_R_)-3-Amino-1-piperidyl]-2-oxo-ethyl]-4-cyano-_N_-(2-methylindazol-5-yl)benzenesulfonamide (22).

Prepared in a manner analogous to compound **21a** using sulfonamide **19** and **SI1**, to afford the title compound as a white powder, TFA salt (18 mg, 25%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.32 (m, 2H), 8.17 (s, 2H), 8.13–7.99 (m, 3H), 7.83 (t, J = 8.6 Hz, 2H), 7.59–7.45 (m, 2H), 7.01 (dd, J = 9.0, 2.1 Hz, 1H), 4.81–4.68 (m, 2H), 4.63 (d, J = 16.9 Hz, 1H), 4.14 (s, 3H), 3.67 (m, 1H), 3.15–3.05 (m, 1H), 3.02–2.83 (m, 1H), 1.93 (s, 1H), 1.80–1.28 (m, 2H).

1-Acetyl-_N_-[2-(4-amino-1-piperidyl)-2-oxo-ethyl]-_N_-[4-(3,5-dimethylpyrazol-1-yl)-3-methoxy-phenyl]indoline-5-sulfonamide (23).

Prepared in a manner analogous to compound **21b** using sulfonamide **20** and **SI2**, to afford the title compound as a white powder (34 mg, 52%). ^{1}H NMR (300 MHz, Methanol-d_4) δ 8.55 (s, 1H), 8.20 (d, J = 9.0 Hz, 1H), 7.60–7.49 (m, 2H), 7.21 (dd, J = 8.4, 1.6 Hz, 1H), 7.07 (d, J = 2.2 Hz, 1H), 6.90 (dd, J = 8.4, 2.2 Hz, 1H), 6.05–5.98 (m, 1H), 4.73 (d, J = 16.2 Hz, 1H), 4.62 (d, J = 16.2 Hz, 1H), 4.51 (d, J = 13.9 Hz, 1H), 4.20 (t, J = 8.7 Hz, 3H), 3.71 (s, 3H), 3.71 (s, 1H), 3.39 (td, J = 7.3, 3.6 Hz, 1H), 3.24 (t, J = 8.7 Hz, 3H), 2.82–2.64 (m, 1H), 2.27 (s, 3H), 2.22 (d, J = 1.6 Hz, 3H), 2.12 (d, J = 11.9 Hz, 1H), 2.06 (s, 3H), 1.73–1.55 (m, 1H), 1.54–1.37 (m, 1H).

Preparation of compounds 29–32

Route to glycine core

***tert*-Butyl *N*-[1-[2-(4-cyanoanilino)acetyl]-4-piperidyl]carbamate (25).**

To a solution of *N*-(4-cyanophenyl)glycine (1 g, 5.7 mmol), COMU (2.95 g, 6.8 mmol) and 4-[(tert-butoxycarbonyl)amino]piperidine (1.36 g, 6.8 mmol) in DMF (10 mL) was added N,N-diisopropylethylamine (1.93 mL, 11.35 mmol). After 10 min a white precipitate formed. Stirring was continued for 30 min, then water (50 mL) was added. The precipitate was filtered, washed with water (3 × 10 mL) and dried to afford the title compound (2.02 g, 99%) as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.65–7.50 (m, 2H), 6.86–6.73 (m, 2H), 6.62 (t, J = 5.1 Hz, 1H), 6.21 (d, J = 9.1 Hz, 1H), 4.10 (d, J = 5.1 Hz, 2H), 3.66–3.48 (m, 1H), 3.56–3.35 (m, 3H), 3.42–3.29 (m, 1H), 1.82–1.67 (m, 4H), 1.40 (s, 9H). f: (High pH) t_R 1.07 min, *m/z* 357.2 $[M-H]^-$, 100% purity.

***tert*-Butyl *N*-[[1-[2-(4-cyanoanilino)acetyl]-4-piperidyl]methyl]carbamate (26).**

To a solution of *N*-(4-cyanophenyl)glycine (0.5 g, 2.8 mmol), COMU (730 mg, 3.4 mmol) in DMF (10 mL) was added *N*,*N*-diisopropylethylamine (0.97 mL, 5.7 mmol). After 10 min a white precipitate formed. Stirring was continued for 30 min, and then water (50 mL) was added. The resulting white precipitate was filtered, washed with water (3 × 10 mL) and dried to afford the title compound (1.04 g, 98%) as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.51–7.40 (m, 2H), 6.90 (t, J = 5.9 Hz, 1H), 6.82–6.68 (m, 2H), 6.63 (t, J = 5.1 Hz, 1H), 4.34 (d, J = 13.3 Hz, 1H), 3.98 (t, J = 5.1 Hz, 2H), 3.88 (d, J = 13.3 Hz, 1H), 2.97 (t, J = 12.8 Hz, 1H), 2.87–2.78 (m, 2H), 2.58 (d, J = 12.8 Hz, 1H), 1.64 (t, J = 10.8 Hz, 4H), 1.38 (s, 9H), 1.20–0.79 (m, 2H). LC-MS: (High pH) t_R 1.07 min, *m/z* 372.3 $[M-H]^-$, 100% purity.

***tert*-Butyl *N*-[[1-[2-(4-cyanoanilino)acetyl]-4-fluoro-4-piperidyl]methyl]carbamate (27).**

Prepared in a manner analogous to compound **25** using **24** and *tert*-butyl N-[(4-fluoropiperidin-4-yl)methyl]carbamate to afford the title compound (356 mg, 87%) as a pink powder. This product was used in the next step without further purification.

***tert*-Butyl 7-[2-(4-cyanoanilino)acetyl]-2,7-diazaspiro[3.5]nonane-2-carboxylate (28).**

To a solution of *N*-(4-cyanophenyl)glycine (370 mg, 2.1 mmol), COMU (1.08 g, 2.5 mmol) and *tert*-butyl-2,7-diazaspiro[3.5]nonane-2-carboxylate (500 mg, 2.2 mmol) in DMF (5 mL) at 0 °C was added N,N-diisopropylethylamine (0.71 mL, 4.2 mmol). After 10 min a white precipitate formed. Stirring was continued for 30 min, then water (50 mL) was added. The precipitate was filtered, washed with water (3 × 10 mL) and recrystallized from ethyl acetate (30 mL) to afford the title compound (598 mg, 74%) as an off-white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.50–7.41 (m, 2H), 6.82–6.70 (m, 2H), 6.65 (t, *J* = 5.0 Hz, 1H), 3.99 (d, *J* = 5.0 Hz, 2H), 3.59 (br s, 4H), 3.40 (br s, 4H), 1.71 (t, *J* = 5.5 Hz, 2H), 1.61 (t, *J* = 5.5 Hz, 2H), 1.38 (s, 9H). LC-MS: (High pH) t_R 1.11 min, *m/z* 383.3 $[M-H]^-$, 98% purity.

4-[[2-(4-Amino-1-piperidyl)-2-oxo-ethyl]-(*p*-tolylmethyl)amino]benzonitrile (29a).

A stirred suspension of *tert*-butyl *N*-[1-[2-(4-cyanoanilino)acetyl]-4-piperidyl]carbamate (50 mg, 0.14 mmol), 4-methylbenzyl bromide (31 mg, 0.17 mmol) and potassium carbonate (48 mg, 0.35 mmol) in DMF (500 µL) was heated to 80°C for 1 h, then concentrated to dryness. 4 M HCl in dioxane (1 mL) was added and the reaction mixture stirred for 1 h, then concentrated to dryness and purified by preparative HPLC (high pH) to afford the title compound (17 mg, 34%) as a white powder. ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.53–7.32 (m, 2H), 7.16 (s, 4H), 6.76–6.62 (m, 2H), 4.63 (s, 2H), 4.56 (d, *J* = 8.4 Hz, 1H), 4.48 (d, *J* = 4.5 Hz, 2H), 4.03 (d, *J* = 14.1 Hz, 1H), 3.47–3.33 (m, 1H), 3.30–3.14 (m, 1H), 2.79 (t, *J* = 12.3 Hz, 1H), 2.32 (s, 3H), 2.12–2.05 (m, 2H), 1.65 (qd, *J* = 12.3, 4.2 Hz, 1H), 1.51 (qd, *J* = 12.3, 4.2 Hz, 1H).

4-[(*N*-[2-(4-Amino-1-piperidyl)-2-oxo-ethyl]-4-cyano-anilino)methyl]-2-fluoro-benzonitrile (29b).

Prepared in a manner analogous to compound **29a** using 4-cyano-3-fluorobenzyl bromide to afford the title compound (17 mg, 15%) as a white powder. ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.74 (dd, *J* = 8.2, 6.7 Hz, 1H), 7.51–7.44 (m, 2H), 7.42–7.31 (m, 2H), 6.72–6.60 (m, 2H), 4.78 (s, 2H), 4.58 (d, *J* = 9.1 Hz, 2H), 4.06 (d, *J* = 14.3 Hz, 1H), 3.51–3.34 (m, 2H), 3.30–3.17 (m, 1H), 2.81 (t, *J* = 13.2 Hz, 1H), 2.10 (t, *J* = 13.2 Hz, 2H), 1.65 (qd, *J* = 12.3, 4.2 Hz, 1H), 1.51 (qd, *J* = 12.3, 4.2 Hz, 1H).

4-[[2-(4-Amino-1-piperidyl)-2-oxo-ethyl]-[(3-fluoro-4-methoxy-phenyl)methyl]amino]benzonitrile (29c).

Prepared in a manner analogous to compound **29a** using 3-fluoro-4-methoxybenzyl bromide to afford the title compound (17 mg, 31%) as a white powder. ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.55–7.36 (m, 2H), 7.15–6.94 (m, 4H), 6.78–6.64 (m, 2H), 4.61 (s, 3H), 4.51 (d, J = 6.0 Hz, 2H), 4.04 (d, J = 14.2 Hz, 1H), 3.86 (s, 3H), 3.23 (t, J = 12.8 Hz, 1H), 2.80 (t, J = 12.8 Hz, 1H), 2.09 (t, J = 11.9 Hz, 2H), 1.65 (m, 1H), 1.50 (m, 1H).^{19}F NMR (300 MHz, Methanol-d_4) δ -135.7. ^{13}C NMR (75 MHz, Chloroform-*d*) δ 168.6, 154.0 (d, J = 245 Hz), 153.2, 148.9, 133.8, 131.0 (d, J = 5.4 Hz), 122.5 (d, J = 3.6 Hz), 120.7, 115.8 (d, J = 18.8 Hz), 114.5, 113.4, 99.1, 86.4, 58.2, 56.1, 54.7, 49.2, 43.4, 33.0. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{22}H_{26}O_2N_4F$: 397.2034. Found: 397.2022.

4-[[2-(4-Amino-1-piperidyl)-2-oxo-ethyl]-benzyl-amino]benzonitrile (29d).

Prepared in a manner analogous to **29a** using benzyl bromide to afford the title compound (12 mg, 25%) as a white powder. ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.51–7.39 (m, 2H), 7.41–7.21 (m, 5H), 6.77–6.65 (m, 2H), 4.68 (s, 2H), 4.64–4.41 (m, 3H), 4.03 (d, J = 14.0 Hz, 1H), 3.43–3.27 (m, 1H), 3.21 (t, J = 13.0 Hz, 1H), 2.79 (t, J = 13.0 Hz, 1H), 2.17–1.95 (m, 2H), 1.70–1.55 (m, 1H), 1.58–1.39 (m, 1H).

4-[[2-(4-Amino-1-piperidyl)-2-oxo-ethyl]-[(1-methylindazol-5-yl)methyl]amino]benzonitrile (29e).

Prepared in a manner analogous to **29a** using 5-(bromomethyl)-1-methyl-1H-indazole hydrobromide to afford the title compound (13 mg, 21%) as a white powder. ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.93 (d, J = 0.9 Hz, 1H), 7.65 (dd, J = 1.7, 0.9 Hz, 1H), 7.56 (dt, J = 8.6, 0.9 Hz, 1H), 7.50–7.35 (m, 3H), 6.80–6.69 (m, 2H), 4.79 (s, 2H), 4.61 (s, 1H), 4.54 (d, J = 6.0 Hz, 2H), 4.06 (s, 3H), 4.01 (s, 1H), 3.46–3.28 (m, 1H), 3.22 (t, J = 13.0 Hz, 1H), 2.80 (q, J = 12.1, 11.7 Hz, 1H), 2.13–2.01 (m, 2H), 1.71–1.45 (m, 2H).

4-[[2-[4-(Aminomethyl)-1-piperidyl]-2-oxo-ethyl]-(p-tolylmethyl)amino]benzonitrile (30a).

Prepared in a manner analogous to **30b** (see below), affording the title compound (25 mg, 47%) as a white powder, bis-hydrochloride salt. ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.50–7.38 (m, 2H), 7.16 (s, 4H), 6.78–6.66 (m, 2H), 4.62 (s, 2H), 4.55 (d, J = 13.7 Hz, 1H), 4.45 (s, 2H), 3.96 (d, J = 14.0 Hz, 1H), 3.15 (q, J = 11.1, 9.4 Hz, 1H), 2.95–2.84 (m, 2H), 2.72 (t, J = 13.1 Hz, 1H), 2.32 (s, 3H), 1.97 (tt, J = 7.3, 3.8 Hz, 1H), 1.86 (d, J = 13.4 Hz, 2H), 1.40–1.15 (m, 2H). ^{13}C NMR (75 MHz, Methanol-d_4) δ 168.9, 153.7, 138.1, 135.7, 134.3, 130.5, 127.8, 121.3, 113.7, 99.0, 56.4, 52.9, 45.4, 42.9, 35.6, 30.1, 21.1. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{23}H_{29}ON_4$: 377.2336. Found: 377.2320.

4-[[2-[4-(Aminomethyl)-1-piperidyl]-2-oxo-ethyl]-[(4-chlorophenyl)methyl]amino]benzonitrile (30b).

A stirred suspension of tert-butyl N-[[1-[2-(4-cyanoanilino)acetyl]-4-piperidyl]methyl]carbamate (50. mg, 0.14 mmol), 4-chlorobenzyl bromide (43 mg, 0.21 mmol) and potassium carbonate (48 mg, 0.35 mmol) in DMF (0.5 mL) was heated to 80 °C for 1 h, then purified by HPLC (high pH). 4 M HCl in dioxane (1 mL) was added and the reaction mixture concentrated to dryness to afford the title compound (27 mg, 37%) as a white powder, bis-hydrochloride salt. ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.42–7.29 (m, 2H), 7.35–7.13 (m, 6H), 6.58 (dd, J = 9.8, 2.8 Hz, 2H), 4.78 (d, J = 1.1 Hz, 2H), 4.55 (d, J = 7.0 Hz, 3H), 3.87 (d, J = 14.1 Hz, 1H), 3.14–2.98 (m, 1H), 2.79 (dd, J = 7.2, 2.9 Hz, 2H), 1.76 (s, 2H), 1.43–1.01 (m, 3H).

4-[[2-[4-(Aminomethyl)-1-piperidyl]-2-oxo-ethyl]-[(4-fluorophenyl)methyl]amino]benzonitrile (30c).

Prepared in a manner analogous to **30b** using 4-fluorobenzyl bromide to afford the title compound (13 mg, 21%) as a white powder, bis-hydrochloride salt. ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.40–7.28 (m, 2H), 7.28–7.14 (m, 2H), 7.04–6.89 (m, 2H), 6.66–6.54 (m, 2H), 4.55 (s, 2H), 4.44 (d, J = 13.2 Hz, 1H), 4.37 (s, 2H), 3.88 (d, J = 13.9 Hz, 1H), 3.70–3.54 (m, 1H), 3.06 (t, J = 13.0 Hz, 1H), 2.90–2.71 (m, 2H), 2.62 (td, J = 13.0, 2.7 Hz, 1H), 1.97–1.66 (m, 3H), 1.35 (d, J = 13.0 Hz, 1H), 1.30–1.00 (m, 2H).

3-[(*N*-[2-[4-(Aminomethyl)-1-piperidyl]-2-oxo-ethyl]-4-cyano-anilino)methyl]benzamide (30d).

Prepared in a manner analogous to **30b** using 3-(chloromethyl)benzamide to afford the title compound (14 mg, 22%) as a white powder, bis-hydrochloride salt. ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.76–7.62 (m, 2H), 7.49–7.29 (m, 4H), 6.67–6.55 (m, 2H), 4.63 (d, J = 1.8 Hz, 2H), 4.42 (s, 3H), 3.89 (d, J = 13.8 Hz, 1H), 3.21 (t, J = 1.8 Hz, 1H), 3.06 (t, J = 12.9 Hz, 1H), 2.84–2.74 (m, 2H), 2.71–2.53 (m, 1H), 1.87 (tt, J = 7.3, 3.8 Hz, 2H), 1.76 (m, 3H), 1.30–1.00 (m, 2H).

4-[(*N*-[2-[4-(Aminomethyl)-1-piperidyl]-2-oxo-ethyl]-4-cyano-anilino)methyl]benzamide (30e).

Prepared in a manner analogous to **30b** using 4-(chloromethyl)benzamide to afford the title compound (12 mg, 18%) as a white powder, bis-hydrochloride salt. ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.81–7.70 (m, 2H), 7.39–7.33 (m, 2H), 7.33–7.25 (m, 2H), 6.59 (d, J = 9.0 Hz, 2H), 4.64 (s, 2H), 4.49–4.42 (m, 1H), 4.40 (s, 2H), 3.88 (d, J = 14.1 Hz, 1H), 3.68–3.53 (m, 4H), 3.06 (t, J = 12.7 Hz, 1H), 2.78 (dd, J = 6.9, 4.1 Hz, 2H), 2.62 (t, J = 12.6 Hz, 1H), 2.00–1.62 (m, 2H), 1.44–1.00 (m, 1H).

4-[[2-[4-(Aminomethyl)-1-piperidyl]-2-oxo-ethyl]-[(3-fluoro-4-methoxy-phenyl)methyl]amino]benzonitrile (30f).

Prepared in a manner analogous to **30b** using 3-fluoro-4-methoxybenzyl bromide to afford the title compound (23 mg, 40%) as a white powder, bis-hydrochloride salt. ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.42–7.29 (m, 2H), 7.03–6.89 (m, 3H), 6.67–6.55 (m, 2H), 4.50 (s, 2H), 4.43 (d, J = 14.7 Hz, 1H), 4.36 (s, 2H), 3.89 (d, J = 14.7 Hz, 1H), 3.76 (s, 3H), 3.06 (t, J = 12.8 Hz, 1H), 2.79 (dd, J = 6.8, 3.7 Hz, 2H), 2.62 (t, J = 12.8 Hz, 1H), 1.96–1.53 (m, 3H), 1.26–1.04 (m, 2H). ^{13}C NMR (75 MHz, MeOD) δ 169.0, 153.9 (d, J = 247 Hz), 153.5, 148.4, 134.1, 131.9 (d, J = 5.4 Hz), 124.2 (d, J = 3.6 Hz), 121.0, 115.9, 114.5 (d, J = 18.9 Hz), 114.2, 99.7, 56.8, 55.9, 53.1, 45.2, 42.9, 35.6, 30.1. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{23}H_{28}O_2N_4F$: 411.2191. Found: 411.2175.

4-[[2-[4-(Aminomethyl)-4-fluoro-1-piperidyl]-2-oxo-ethyl]-[(3-fluoro-4-methoxy-phenyl)methyl]amino]benzonitrile (31)

Prepared in a manner analogous to **30b** using acid **27** as a starting material to afford the title compound as a white powder (9 mg, 17%), bis-hydrochloride salt. ^{1}H NMR (300 MHz, Methanol-d_4)

δ 7.45 (dd, *J* = 8.9, 4.0 Hz, 2H), 7.22–6.93 (m, 2H), 6.83–6.62 (m, 2H), 4.68–4.34 (m, 4H), 3.88 (s, 3H), 3.86–3.54 (m, 2H), 3.67 (s, 1H), 3.45 (t, *J* = 12.5 Hz, 1H), 3.28 (d, *J* = 3.0 Hz, 1H), 3.22 (s, 1H), 3.05 (t, *J* = 12.8 Hz, 1H), 2.17–1.57 (m, 8H). ^{19}F NMR (282 MHz, Methanol-d_4) δ -136.50 (m), -169.24 (dddt, *J* = 38.0, 29.5, 20.2, 10.0 Hz).

4-[[2-(2,7-Diazaspiro[3.5]nonan-7-yl)-2-oxo-ethyl]-[(3-fluoro-4-methoxy-phenyl)methyl]amino]benzonitrile (32).

A stirred suspension of **28** (330 mg, 0.64 mmol), 4-(bromomethyl)-2-fluoro-1-methoxybenzene (282 mg, 1.3 mmol) and potassium carbonate (222 mg, 1.6 mmol) in DMF (3 mL) was heated to 120 °C by microwave irradiation for 0.5 h. The reaction mixture was adsorbed onto silica then purified by automated column chromatography (0 to 70% EtOAc in isohexane). 4M HCl/dioxane (1 mL) was added and the mixture stirred for 1 hour, then concentrated to dryness. The resulting residue was purified by preparative HPLC (high pH) to afford the title compound (33 mg, 12%) as a white powder. ^{1}H{^{19}F} NMR (300 MHz, Methanol-d_4) δ ^{1}H NMR (300 MHz, Methanol-d_4) δ 8.58 (s, 1H), 7.51–7.39 (m, 2H), 7.13–6.99 (m, 3H), 6.76–6.65 (m, 2H), 4.60 (s, 2H), 4.46 (s, 2H), 3.86 (s, 3H), 3.57 (br m, 2H), 3.50 (br m, 3H), 3.36 (s, 1H), 1.93 (br m, 2H), 1.86 (br m, 2H). ^{19}F NMR (282 MHz, Methanol-d_4) δ -136.57 (m). ^{13}C NMR (75 MHz, MeOD) δ 168.8, 153.8 (d, $J_{C,F}$ = 247 Hz), 153.3, 148.2, 134.2, 131.9 (d, $J_{C,F}$ = 5.4 Hz), 123.8 (d, $J_{C,F}$ = 3.6 Hz), 121.3, 115.5 (d, $J_{C,F}$ = 18.9 Hz), 115.0, 113.8, 99.2, 86.4, 56.8, 56.3, 52.9, 49.9, 42.4, 40.0, 38.2, 35.3. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{24}H_{28}O_2N_4F$: 423.2191. Found: 423.2181.

LSD1 enzymatic assay

Assays were performed in Corning® 384 well low flange white flat bottom polystyrene (#3574) microplates in a 10 µL reaction volume consisting of 50 mM TrisHCl, 50 mM NaCl, 1mM DTT, 0.01% Tween-20, 1% DMSO with or without compound in a 10-point, 3-fold dilution series, 0.2 µM Histone H3(1–21)K4(Me1) biotin peptide substrate (AnaSpec Inc., Freemont, CA) and 1 nM LSD1 (Enzo Life Sciences, New York, NY). The reaction was allowed to proceed for 30 min at 25 °C before stopping the reaction with the addition of 0.3 mM tranylcypromine in LANCE detection buffer and

quantifying the level of demethylated peptide by the addition of 1 nM Europium-α-unmodified H3K4 antibody and 25 nM ULight Streptavidin (both from Perkin Elmer, Waltham, MA), also in LANCE detection buffer. Following a further 60 min incubation period the TR-FRET signal was read on a PHERAstar FS plate reader (BMG LabTech, Ortenberg, DE) with excitation at 340 nm and emission at 665 nm.

SPR binding assay

Direct binding between compounds and LSD1 was assessed by SPR using Biacore T200, S200 and S51 instruments (GE Healthcare, Uppsala, Sweden). LSD1 was immobilized to CM5 or CM7 chips using amine coupling. Interaction experiments were performed at 15 °C in 10 mM HEPES, pH 7.4, 150 mM NaCl, 1% DMSO, 0.05% Tween, at a flow rate of 30 mL/min. Compounds were diluted in the buffer and injected for 15 – 25 s at increasing concentrations over the prepared surfaces. Sensorgrams were double-referenced by subtracting signals from untreated reference channel and responses from blank injections. Affinities were derived by either dose-response analysis of steady state responses or regressions analysis of whole sensorgrams (1:1 interaction model including a term for mass transport limitation) using the T200 evaluation software 3.0 (GE Healthcare).

Cell lines

THP1 and MV4-11 cell lines were obtained from DSMZ (Braunschweig, Germany). All cell lines were cultured in RPMI supplemented with 10% FBS and 2mM L-glutamine (Sigma Aldrich, Poole, UK).

Clonogenic assays

Clonogenic assays of human leukaemic cell lines were performed in methylcellulose medium (H4320, Stem Cell Technologies, Vancouver, BC) with no supplemental growth factors. Cells were seeded at $2.5x10^3$ and colonies enumerated after 7 days in culture.

CD86 and apoptosis assays

For CD86 assays, leukaemic cell lines were incubated in culture medium containing compounds or DMSO (vehicle control) for 48 h at a density of $1x10^5$/ml. Cells were pelleted, resuspended in 100 ml SM buffer (Phenol red free RPMI, 5 mM EDTA and 2% BSA (Sigma Aldrich)) containing 0.25ul CD86-PerCP EF710 (Clone IT2.2, eBioscience, Thermo Fisher Scientific) and incubated at 4 °C for 10 mins. Cells were washed, resuspended in SM buffer and analyzed by flow cytometry. To determine the effects of compounds on apoptosis, leukaemic cell lines were incubated with 10 μM compound or vehicle for 4 days. Apoptosis was assessed using a BD Pharmingen PerCP-Cy5.5 Annexin V/7-AAD Kit (BD Biosciences), according to manufacturer's instructions. All FACS analyses were performed using either an LSR Model II (BD Biosciences, Oxford, UK) or NovoCyte (ACEA Biosciences) flow cytometer or FACSArray Bioanalyser (BD Biosciences).

Glide docking

Docking into the active site of LSD1 was carried out for each of the synthesized ligands. Molecules were docked in their predicted charged form at pH 7.4. Compounds were docked into the crystal structure of tetrahydrofolate bound to LSD1 (PDB code 4KUM) with all structural waters removed. Docking was performed in Glide 6.7, in standard precision mode and without further minimisation. Glidescore was used for conformation scoring of each ligand.

ASSOCIATED CONTENT

Supporting Information

The supporting information is available free of charge via the Internet at http://pubs.acs.org. SPR sensorgrams for selected compounds; LC-MS methods and solvent gradients; preparative HPLC instrument and solvent gradients; summary of purity data; molecular formula strings.

Abbreviations

AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; ATRA, all-trans retinoic acid; COMU, 1-Cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate; DIPEA, N,N-diisopropylethylamine; GFI, growth factor independence; K_d,

dissociation constant; LSD1**,** lysine specific demethylase 1; MeCN, acetonitrile; MLL, mixed lineage leukemia; SPR, surface plasmon resonance; TR-FRET, time resolved fluorescence resonance energy transfer.

AUTHOR INFORMATION

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Notes

Tim Somervaille has ongoing research collaborations with Oryzon Genomics and consults for Imago Biosciences. The other authors declare no competing financial interest.

Acknowledgments

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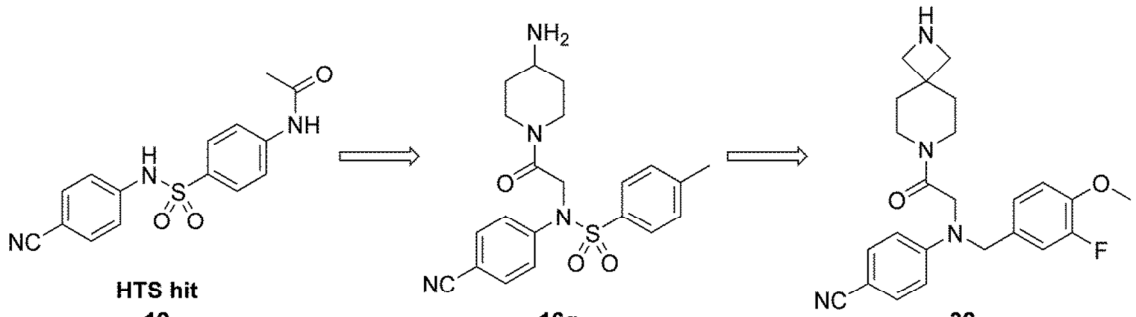
TABLE OF CONTENTS GRAPHIC



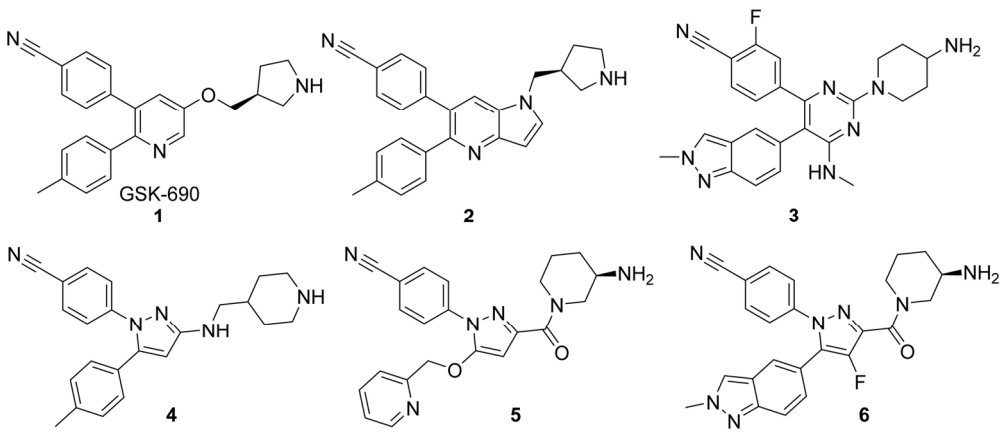


Figure 1. Compound **1** and examples from several series disclosed by Quanticel Pharmaceuticals in WO2015/089192 (**2–6**).[25]

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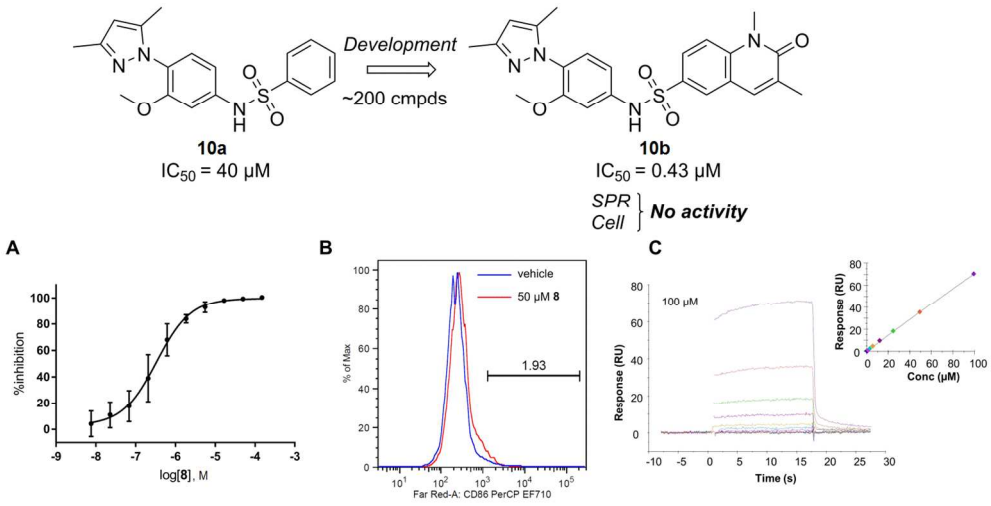

Figure 2. Development and characterization of compound **10b**. A: Biochemical assay; B: Surrogate cellular biomarker assay; C: Surface plasmon resonance sensorgram of the interaction between LSD1 and **10b** in two-fold dilution series (highest concentration indicated in the graph).

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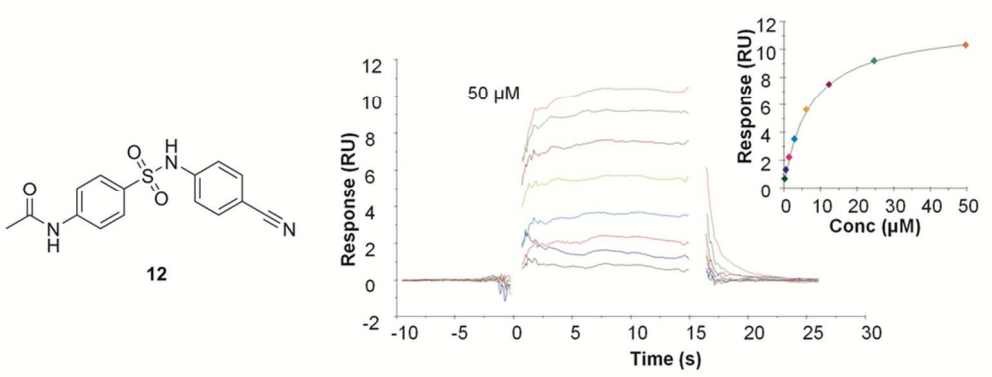

Figure 3. Structure of compound **12** with biochemical and SPR sensorgram indicating the highest tested concentration. K_d = 9.5 µM. K_d value was determined by SPR at 15 °C as the average of two experimental series (typically 10 concentrations).

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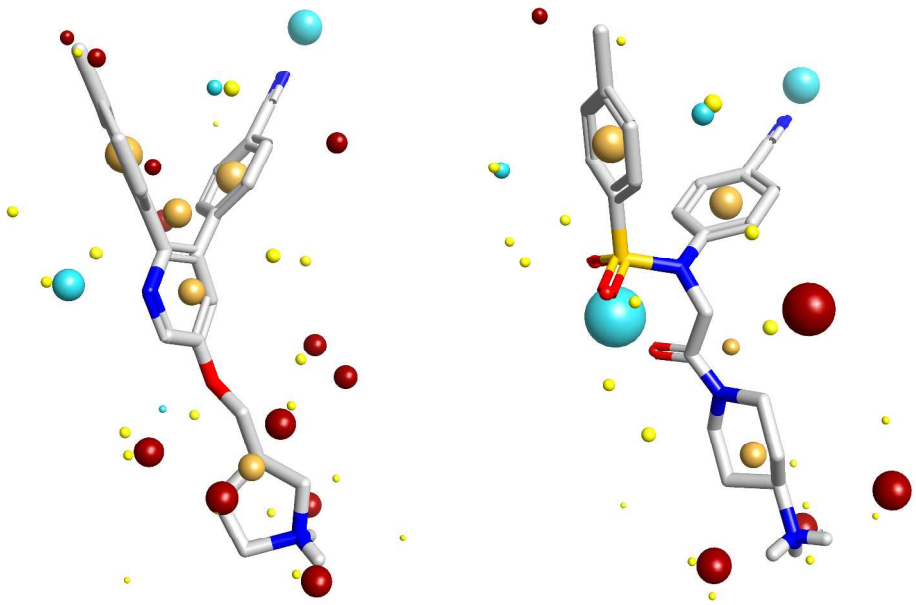

Figure 4. Cresset Torch alignment and structures of compound **1** (left) and **16g** (right). Similarity score 0.712. Blue field points (spheres) highlight energy minima for a positively charged probe, red for a negative probe. Yellow spheres represent an attractive van der Waals minimum for a neutral probe and brown spheres represent hydrophobic regions. Oxygen atoms are shown in red, nitrogen in blue. The similarity score is based on the likeness of the field points in terms of their magnitude and position. A score of greater than 0.7 is considered a 'good' score.

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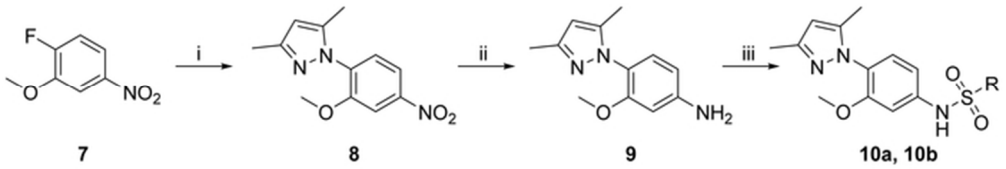

7 8 9 10a, 10b

Scheme 1: Synthesis of compounds 10a–b[a]

[a]Reagents and conditions: (i) 3,5-dimethylpyrazole, K_2CO_3, DMF, 80 °C, 16 h, 99%; (ii) Zn, ammonium formate, MeOH, 40 °C, 16 h, 78%; (iii) appropriate sulfonyl chloride, pyridine, 100 °C, 1 h, 41–70%.

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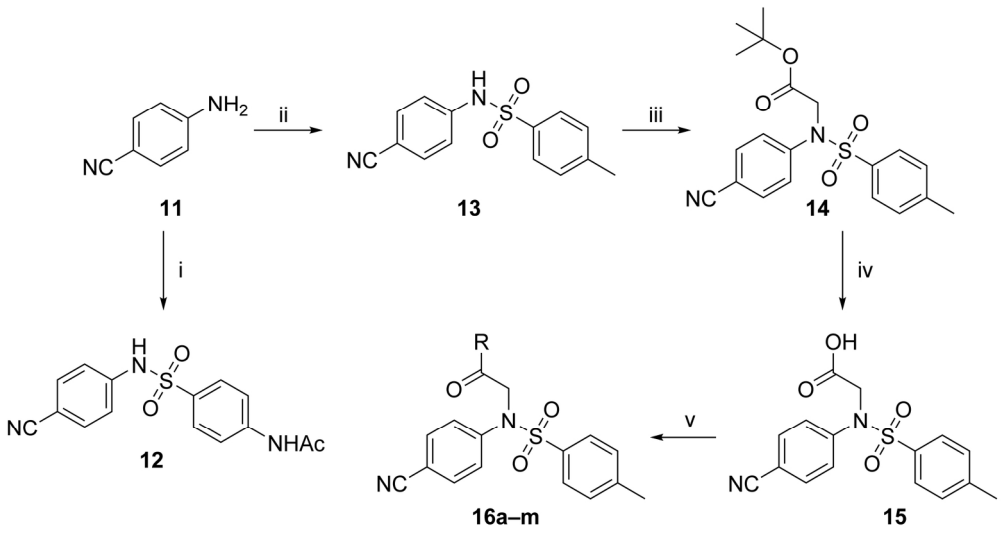

Scheme 2: Synthesis of compounds 12 and 16a–m[a]

[a]Reagents and conditions: (i) 4-acetamidobenzenesulfonyl chloride, DMAP, MeCN, μW, 100 °C, 1 h, 37% (ii) 4-methylphenylsulfonyl, pyridine, MeCN, RT, 12 h, 89%; (iii) tert-butyl bromoacetate, K_2CO_3, DMF, 1 h, 96%; (iv) TFA, DCM, 1 h, 98%; (v) amine, DIPEA, COMU, DMF, then 4M HCl/dioxane if Boc deprotection required, RT, 12–68%.

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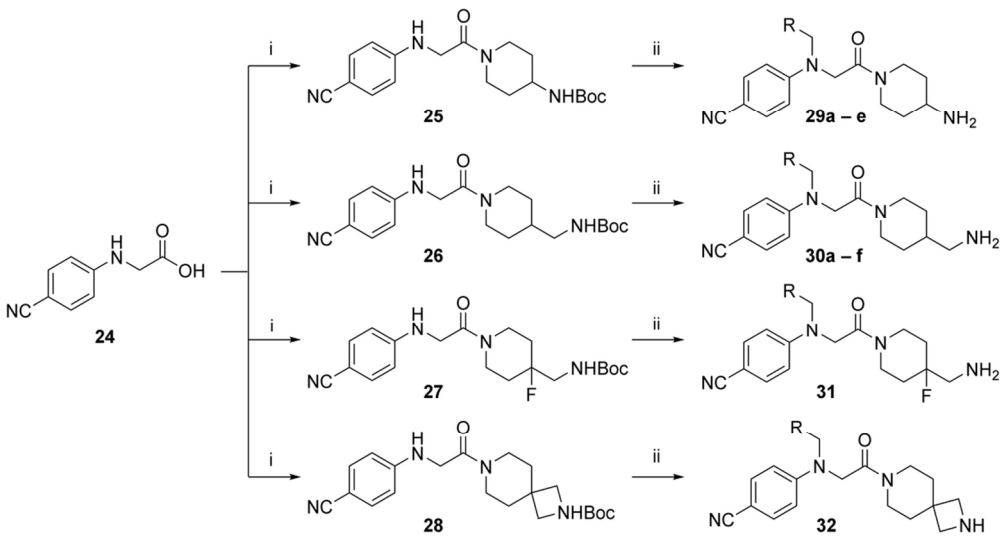

Scheme 4: Synthesis of compounds 29a–e, 30a–f, 31 and 32[a] [a]Reagents and conditions: i) Boc-protected diamine, DIPEA, COMU, DMF, rt, 1 h, 67 – 99%; ii) substituted benzyl-bromide, K_2CO_3, DMF, 80°C, 1 h, then 4 M HCl/dioxane, rt, 1 h, 6–58%

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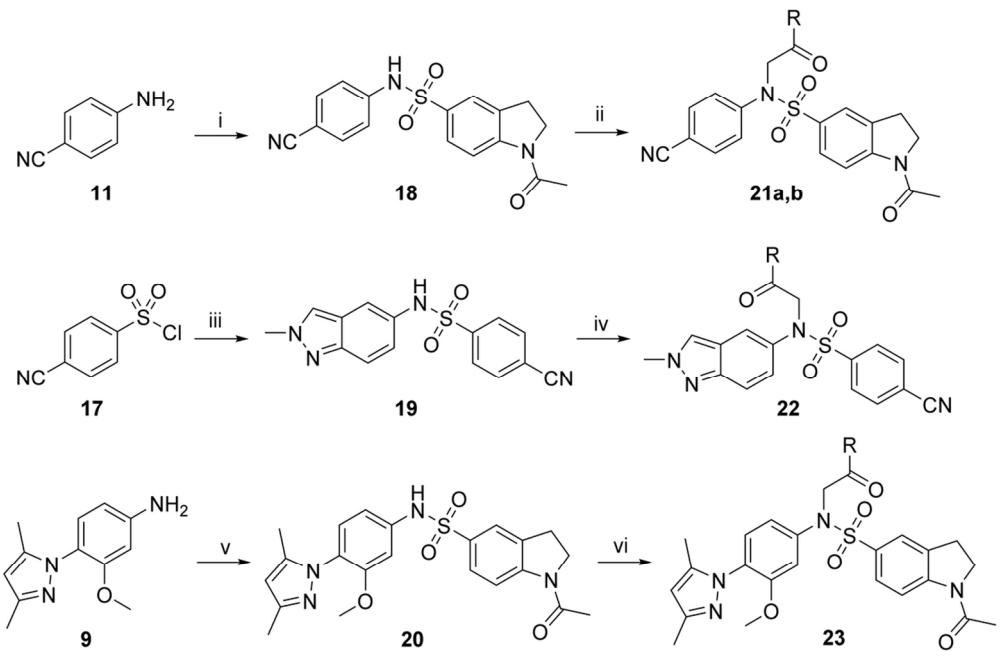

Scheme 3: Synthesis of compounds 21a–b, 22 and 23[a] [a]Reagents and conditions: (i) 1-acetylindoline-5-sulfonyl chloride, pyridine, MeCN, RT, 12 h, 84%; (ii) (**21a**) tert-butyl N-[(3R)-1-(2-chloroacetyl)-3-piperidyl]carbamate, K_2CO_3, cat. KI, DMF, 80 °C, 3 h; then TFA, 1 h, RT, 42%; (**21b**) tert-butyl N-[1-(2-chloroacetyl)-4-piperidyl]carbamate, K_2CO_3, 80 °C, 6 h, then 4M HCl/dioxane, 70%; (iii) 2-methyl-2H-indazol-5-amine, pyridine, MeCN, RT, 12 h, 54%; (iv) tert-butyl N-[(3R)-1-(2-chloroacetyl)-3-piperidyl]carbamate, K_2CO_3, cat. KI, DMF, 80 °C, 3 h; then TFA, 1 h, RT, 25%; (v) 1-acetylindoline-5-sulfonyl chloride, pyridine, MeCN, RT, 12 h, 54%; (vi) tert-butyl N-[1-(2-chloroacetyl)-4-piperidyl]carbamate, K_2CO_3, DMF, 80 °C, 6 h, then 4M HCl/dioxane, 1 h RT, 52%.

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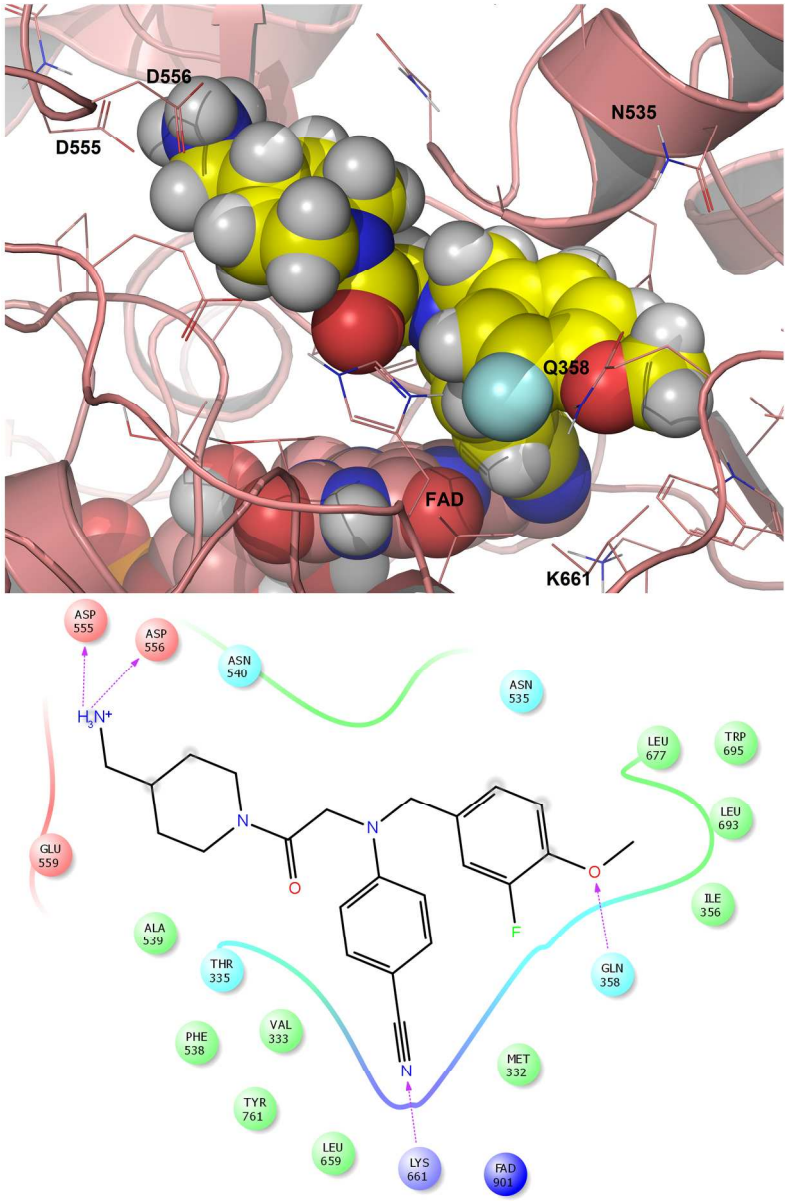

Figure 5. Top: Predicted binding mode of compound **30f** in the LSD1 active site (4KUM). Visualized using Maestro. Bottom: Protein-ligand interaction diagram with key hydrogen bond interactions highlighted (pink arrows).

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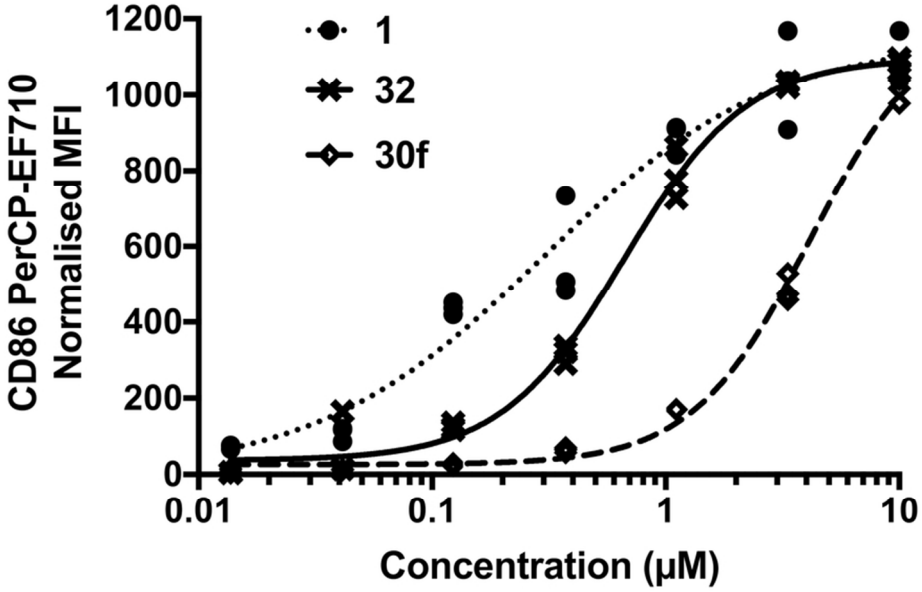

Figure 6. CD86 cell assay results for compounds **1**, **30f** and **32**.

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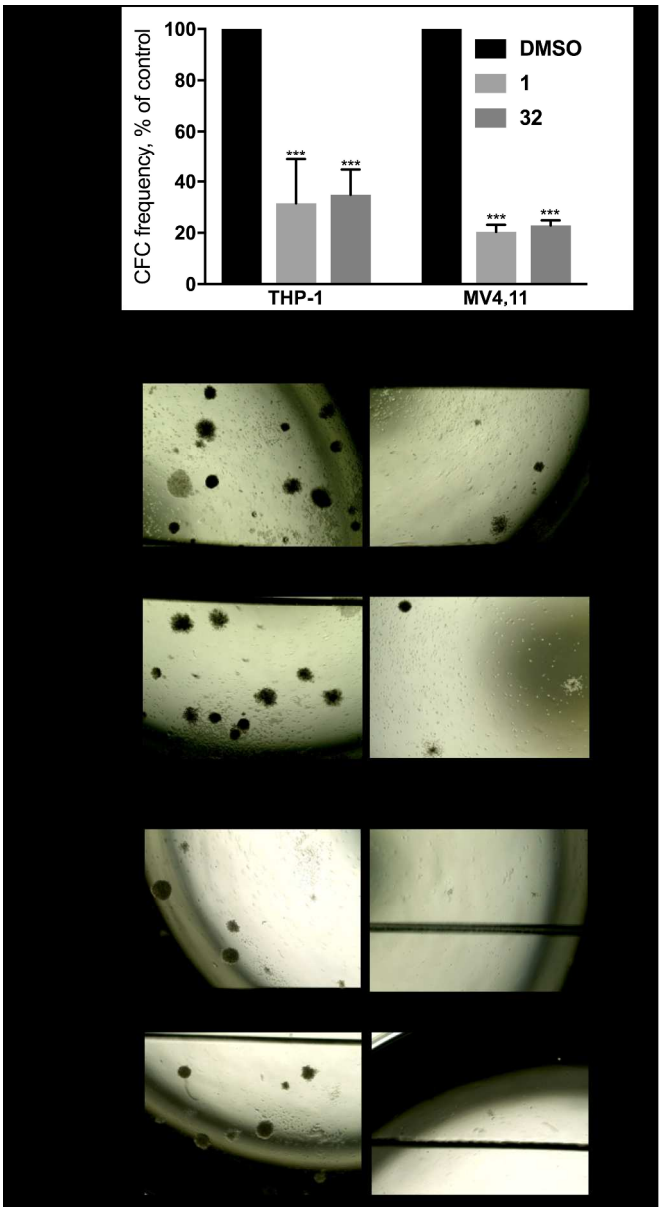

Figure 7. Top: Colony formation assay for THP-1 and MV4-11 cells dosed with 10 μM of compounds 1 and 32 for 7 days. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, statistical significance calculated using a one-way ANOVA with Dunnett's tests between vehicle treated and compound dosed cells. Bottom: representative images of colonies.

251x461mm (300 x 300 DPI)

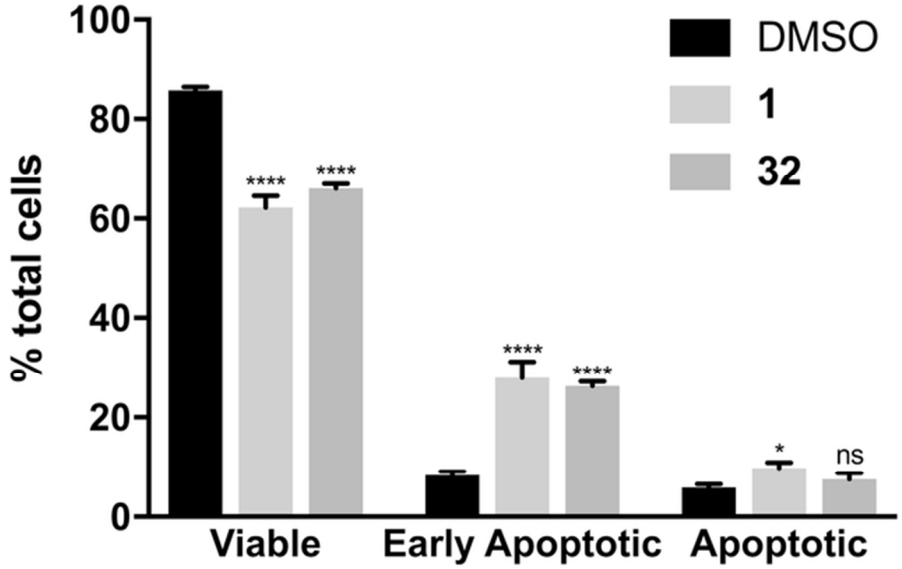

Figure 8. Annexin V-perCP EF710/7AAD double staining experiments results with THP-1 cell dosed with vehicle, or 10 µM **1** or **32** for 4 days in liquid culture. Early apoptotic cells = Annexin $V^{pos}7\text{-}AAD^{neg}$; Apoptotic cells = Annexin $V^{pos}7\text{-}AAD^{pos}$. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, statistical significance calculated using one-way ANOVA analysis with Dunnett's tests.

60x35mm (300 x 300 DPI)