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Article

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Discovery of Potent, Isoform-Selective Inhibitors of Histone Deacetylase containing Chiral Heterocyclic Capping Groups and a *N*-(2-Aminophenyl)-benzamide Binding Unit

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Abstract: The synthesis of a novel series of potent chiral inhibitors of histone deacetylase (HDAC) is described that contain a heterocyclic capping group and a *N*-(2-aminophenyl)-benzamide unit that binds in the active site. *In vitro* assays for the inhibition of HDAC1, HDAC2, HDAC3-NCoR1 and HDAC8 by the *N*-(2-aminophenyl)-benzamide **24a** gave respective IC_{50} values of 930, 85, 12 and 4100 nM, exhibiting Class I selectivity and potent inhibition of HDAC3-NCoR1. Both imidazolinone and thiazoline rings are shown to be effective replacements for the pyrimidine ring present in many other 2-(aminophenyl)-benzamides previously reported, an example of each ring system at 1 μM causing an increase

in histone H3K9 acetylation in the human cell lines Jurkat and HeLa, and an increase in cell death consistent with induction of apoptosis. Inhibition of the growth of MCF-7, A549, DU145 and HCT116 cell lines by **24a** was observed, with respective IC_{50} values of 5.4, 5.8, 6.4 and 2.2 mM.

Introduction

Epigenetic therapy represents a paradigm shift in terms of the selection of the biology to drug targets.[1,2] Of the post-translational modifications relevant to disease, histone protein acetylation status has received much attention in regard to potential for epigenetic cancer therapy. Deacetylation of histone protein at the ε-amino group of lysine residues in the *N*-terminal tails of core histones in the nucleosome through the action of histone deacetylases (HDACs) increases the number of protonated lysine termini; those bind closely to the negatively charged DNA phosphate groups, leading to chromatin compaction that reduces accessibility of transcription factors to DNA,[3] and hence to transcriptional repression.[4-6] Such hypoacetylation has been associated with precancerous or malignant states, and relief of transcriptional repression present in various leukemias[7,8] has been achieved using HDAC inhibitors.[1,2] In addition, the inappropriate recruitment of HDAC enzymes by oncogenic proteins may alter gene expression in favor of arrested differentiation and/or unregulated proliferation.[9] HDAC inhibitors reduce cancer cell proliferation by induction of cell cycle arrest, differentiation and/or apoptosis.[10-13] HDAC inhibitors have been shown to affect several pathways involving proliferation and differentiation, including the TGF-β and JAK-STAT pathways, and myc and Bcl-6 expression in lymphoid cells.[14]

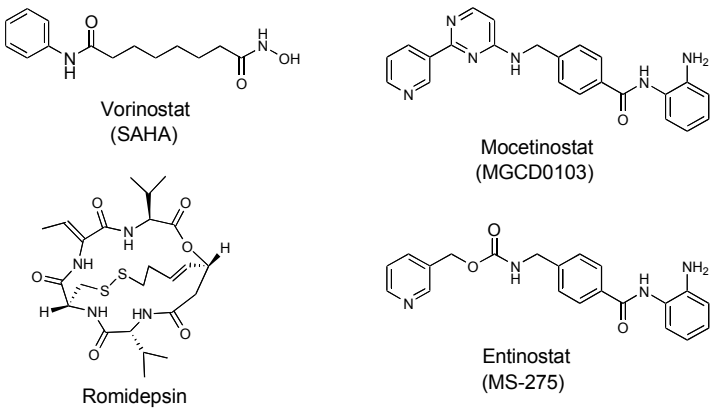


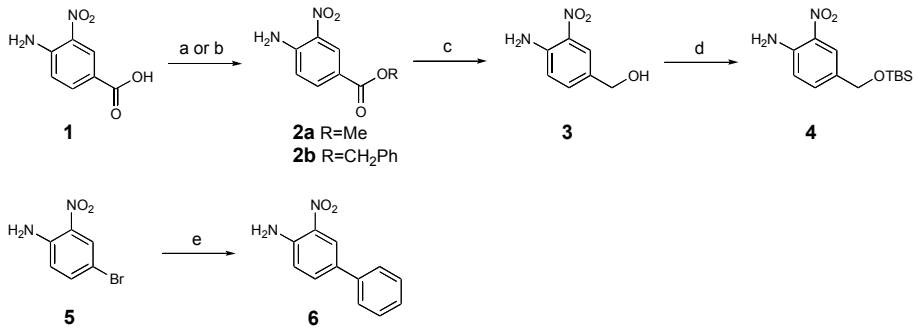
Figure 1. Examples of clinical HDAC inhibitors.

HDAC inhibitors are a promising class of anti-cancer agents[1,2] of which those currently approved for clinical use (Fig. 1) comprise three structural classes: hydroxamic acids, including Vorinostat (suberoylanilide hydroxamic acid), Merck & Co., for the treatment of cutaneous T-cell lymphoma),[15] the aminoanilides Mocetinosat (MethylGene Inc., for the treatment of myelodysplastic syndromes and leukemia, especially acute myelogenous leukemia)[16] and MS-275 (Syndax Pharmaceuticals/Schering AG, for the treatment of metastatic melanoma),[17] and the cyclic disulfide Romidepsin (cyclodepsipeptide or FK-228, Gloucester Pharmaceuticals, for the treatment of cutaneous T-cell lymphoma).[18] With the wide range of known hydroxamic acids available, mainly with limitations of efficacy and toxicity, and the limited number of potential disulfide inhibitors, we sought alternative structures to Mocetinosat that were also aminoanilides, and could be expected to show similar slow, tight-binding[19] to HDACs, the slow decomplexation also leading to prolonged inhibitory effects and longer intervals between dosing.[19-21] In addition, Mocetinostat did not show acquired resistance, at least to HCT116 colon tumor cells, unlike hydroxamic acid HDAC inhibitors.[22] We describe here the synthesis and preliminary evaluation of new classes of aminoanilides containing chirality as part of a heterocyclic ring.

Chemistry

A series of analogs having in common the linker and cap region of Mocetinostat was prepared by coupling carboxylic acid **7** with a set of arylamines (Schemes 1 and 2). In order to ensure regiocontrolled acylation, routes *via* the corresponding *o*-nitroanilines were developed (Scheme 1).

Scheme 1.[a]

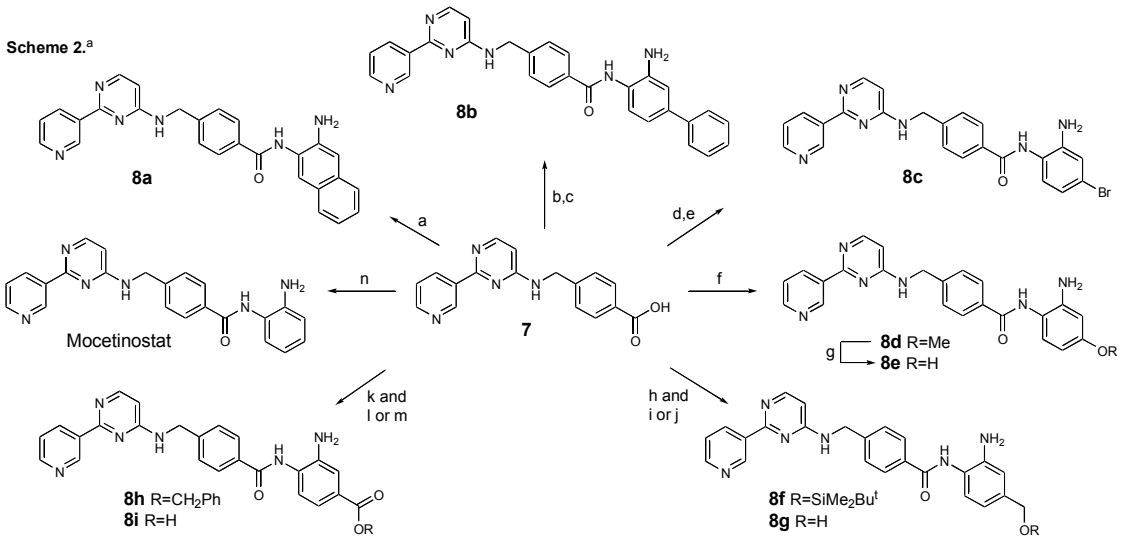


[a] Reagents and conditions: (a) $SOCl_2$, MeOH, reflux,16 h ; (b) $PhCH_2Br$, Na_2CO_3, DMF, 20 °C, 18 h. (c) **2a**, DIBAL, THF-CH_2Cl_2, 0 °C, 1 h, then 20 °C, 3 h; (d) TBSCl, imidazole, DMF, 20 °C, 16 h; (e) $PhB(OH)_2$, $(Ph_3P)_4Pd$, Na_2CO_3, aq. 1,2-dimethoxyethane, 80 °C, 48 h.

Methyl 4-(guanidinomethyl)benzoate[23] and *(E)*-3-(dimethylamino)-1-(pyridin-3-yl)prop-2-en-1-one[24] were heated in propan-2-ol for 20 h at reflux to give methyl 4-((4-(pyridin-3-yl)pyrimidin-2-ylamino)methyl)benzoate as previously described[24] (67%); hydrolysis of this ester to the carboxylic acid **7** was achieved using aqueous lithium hydroxide (24 h, 20 °C, 97%).[24]

Condensation of acid **7** with a range of arylamines (Scheme 2) afforded the corresponding aminoanilides. The nitroaniline derivatives (*e.g.* Scheme 1) could not be directly acylated; however, deprotonation of the aromatic amino group using NaH permitted coupling with carboxylic acids activated by (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) or, for the preparation of **8h** and **8i**, with the acid chloride of **7**. The aminoanilides were further modified by standard functional group manipulations, to afford **8a**-**8i**.

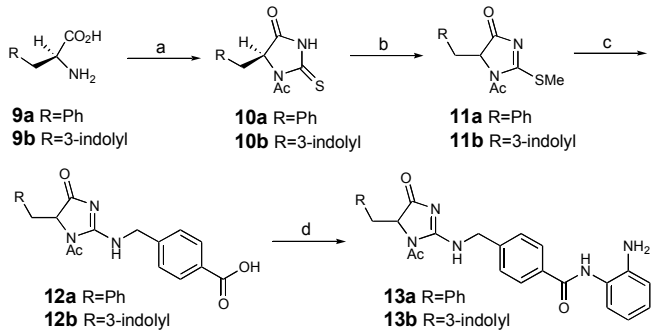
Scheme 2.[a]



[a] Reagents and conditions: (a) (i) $SOCl_2$, reflux, 3 h; (ii) naphthalene-2,3-diamine, Et_3N, CH_2Cl_2, DMF, 0 °C, 30 min then 20 °C, 1 h; (b) BOP, **6**, pyridine, then NaH, 20 °C, 7 h; (c) H_2, 10% Pd/C, MeOH, CH_2Cl_2, 20 °C, 6 h; (d) **5**, NaH, BOP, pyridine, Et_3N,20 °C, 2.5 h; (e) $Fe_2SO_4.7H_2O$, aq. NH_3, EtOH, 60 °C, 4 h; (f) 3-methoxyphenylene-1,2-diamine, BOP, Et_3N, DMF, 20 °C, 27 h; (g) BBr_3, CH_2Cl_2, 20 °C, 24 h; (h) BOP, **4**, pyridine, then NaH, 20 °C, 2.5 h; (i) H_2, 10% Pd/C, MeOH, $CHCl_3$, 20 °C, 7 h; (j) $SnCl_2.2H_2O$, NH_4OAc, 1:1:1 THF:MeOH:H_2O, 20 °C, 21 h then 50 °C, 1 h; (k) NaH and **2b**, anhyd. pyridine, -10 °C, 5 min; then add acid chloride (**7**, $SOCl_2$, toluene, reflux, 3 h), 20 °C, 2 h; (l) H_2, 10% Pd/C, MeOH, CH_2Cl_2, 20 °C, 5.5 h; (m) H_2, 10% Pd/C, MeOH, CH_2Cl_2, 20 °C, 45 h; (n) phenylene-1,2-diamine, BOP, DMF, 20 °C, 24 h.

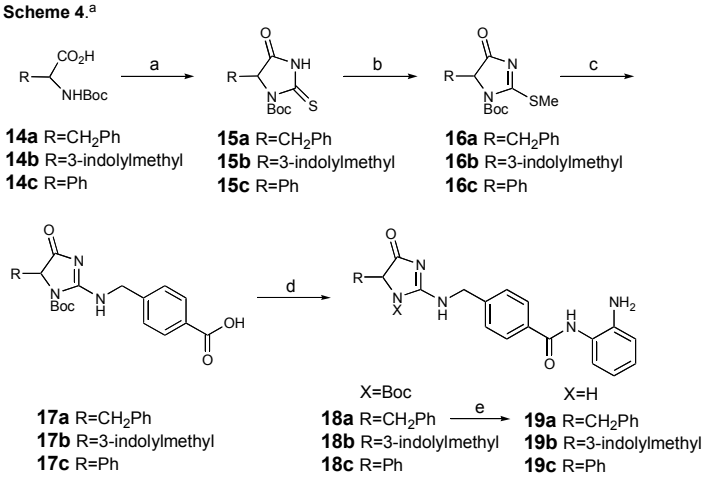
Substituted imidazolin-4-ones (Schemes 3 and 4) were prepared from the corresponding thiohydantoins **10**[25] and **15**.[26] During *S*-methylation to give the corresponding methylthio derivatives **11** and **16**, racemisation occurred, presumably because of enolate formation in the presence of K_2CO_3. Those 2-(methylthio)imidazolin-4-ones were reacted with 4-(methylamino)benzoic acid in ethanol at reflux to give the corresponding carboxylic acids **12** and **17** which could then be converted into the aminoanilides **13** and **18** by BOP-mediated amide formation, and subsequent deprotection of **18** to give **19**.

Scheme 3.[a]



[a] Reagents and conditions: (a) NH_4SCN, Ac_2O, 100 °C (1 h, **9a**; 10 min, **9b**); (b) MeI, K_2CO_3, MeCN, 20 °C, 2.5 h; (c) 4-(methylamino)benzoic acid, ethanol, reflux, 16 h; (d) BOP, Et_3N, phenylene-1,2-diamine, DMF, 20 °C, 3.5 h.

Scheme 4.[a]



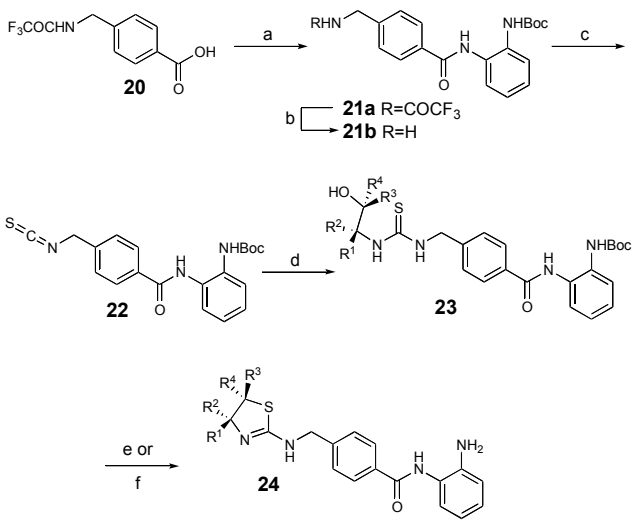
[a] Reagents and conditions: (a) ethoxycarbonyl isothiocyanate, pyridine, MeCN, 20 °C, 18 h; (b) MeI, K_2CO_3, MeCN, 20 °C, 2.5 h; (c) 4-(methylamino)benzoic acid, ethanol, reflux, 4 h (18 h for **17a**); (d) BOP, Et_3N, phenylene-1,2-diamine, DMF, 20 °C, 3 h; (e)TFA, CH_2Cl_2, 20 °C, 3 h.

In view of the lack of stereointegrity in the imidazolin-4-one series, an unsaturated five-membered ring system that lacked a carbonyl group was sought; a set of 2-aminothiazoline derivatives **24** was prepared (Scheme 5). Several approaches were attempted, the first by analogy with Scheme 4. Addition of 4-(methylamino)benzoic acid to 2-(methylthio)-4-substituted thiazolines failed, although addition of the corresponding methyl ester was successful. However, this could not be progressed because phenylene-1,2-diamine could not be satisfactorily reacted with the carboxylic acids derived from latter adducts. In the second approach, reaction of *N*-(2-aminophenyl)-4'-(aminomethyl)benzamide, or its Boc derivative **21b**, with 2-(methylthio)-4-substituted thiazolines was also unsuccessful.

Since displacement of the 2-thiomethyl group by substituted benzylamines could not be achieved, advantage was taken of the *S*-nucleophilic nature of thioureas (Scheme 5). By analogy with previous work[27] the isothiocyanate **22** was reacted with enantiopure 1,2-aminoalcohols to give the corresponding thioureas **23** which underwent acid-catalyzed ring-closure to the desired 2-aminothiazolines **24** (Scheme 5). However, ring-closure to the thiazoline was unsuccessful for **24** where R=(3-indolyl)methyl and for **24c**. Consequently,

Mitsunobu conditions[28] were applied to **23c** and afforded the desired thiazoline which was then deprotected to **24c**.

Scheme 5.[a]



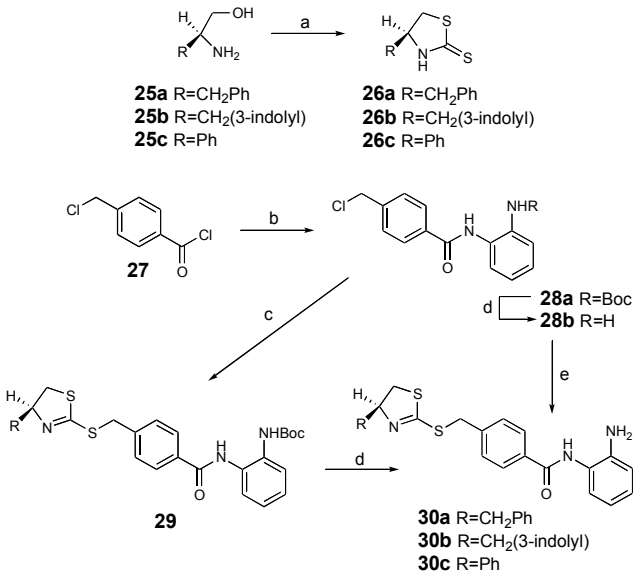
		R^1	R^2	R^3	R^4
23/24	**a**	Ph	H	H	H
	b	H	Ph	H	H
	c	*p*-HOC_6H_4	H	H	H
	d	$PhCH_2$	H	H	H
	e	H	$PhCH_2$	H	H
	f	H	H	Ph	H
	g	H	H	H	Ph
	h	H	Ph	Ph	H
	i	Ph	H	H	Ph

[a] Reagents and conditions: (a) Et_3N, $(COCl)_2$, DMF, CH_2Cl_2; then *N*-Boc-phenylene-1,2-diamine, 20 °C, 20 h; (b) K_2CO_3, aq. MeOH, 70 °C, 4 h; (c) CS_2, Et_3N,CH_2Cl_2, 20 °C, 10 min, then 0 °C and TsCl, 10 min; (d) 1,2-amino alcohol, MeCN, 20 °C, 16 h; (e) conc. HCl, 90 °C (for all **23** except **23c**); (f) diethyl azodicarboxylate, Ph_3P, THF, 20 °C, 75 min, then HCl in Et_2O, 20 °C, 5 h (for **23c**).

The sulfur-linked thiazolines **30** (Scheme 6) were prepared by *S*-alkylation of the corresponding thiazoline-2-thiones.[26] The method was established by reaction of the chloromethyl compound **28a** with **26a**, affording, after Boc deprotection (conditions d, Scheme 6) the thiazoline **30a**. The unprotected alkylating agent **28b** was then tested, and proved effective, affording the thiazolines **30b** and **30c**. To our knowledge, the use of pre-assembled aminoanilide linkers of types **22** and **28** that contain electrophilic centers appropriate for subsequent reaction is a novel approach to aminoanilide HDAC inhibitors;

this route has greater convergence and also avoids a late-stage amination with the feebly nucleophilic 1,2-phenylenediamine derivative.

Scheme 6.[a]



[a] Reagents and conditions: (a) CS_2, aq. KOH, reflux, 20 h; (b) *N*-Boc-phenylene-1,2-diamine, Et_3N, CH_2Cl_2, -10 °C to 20 °C, 2 h; (c) **28a**, NaI, acetone, reflux, 2 h; then **26a**, 20 °C, 28 h;(d) TFA, CH_2Cl_2, 20 °C, 2.5 h; (e) **26b/26c**, K_2CO_3, acetone, reflux, 17 h;

Results and Discussion

To our knowledge, few SAR data or details of Mocetinosat analogues have been reported, and no chiral heterocyclic analogs have been reported; indeed some planar analogs showed less potency to the extent they were evaluated, namely against HDAC1 and a few cell lines).[29,30] A 5-(2-thienyl) substituent on the *o*-phenylenediamine ring enhanced potency in some cases;[31] regarding the zinc-binding group, replacement of the 2-amino group by hydroxy retained activity in some aminoanilides,[32] but that specific analogue of Mocetinostat has not been described. Since effects of 4-substitution on the terminal aryl ring of Mocetinostat were largely unknown,[31] a range of 4-substituted derivatives of Mocetinostat were prepared and evaluated (Table 1). Of that series, 4-methoxy and 4-hydroxy substituents (entries 4 and 5) were most potent against HDAC2 and HDAC3-NCoR1. For both isoforms,

the 4-methoxy substituent conferred some six-fold greater potency than the 4-hydroxy compound. An aminonaphthyl terminus (entry 1) conferred good selectivity for HDAC3-NCoR1 over all other isoforms tested, including HDAC1 (>20 mM). In contrast, a 4-phenyl derivative (entry 2) showed no inhibition, suggesting that there is a strict limit on the length of the 4-substituent to retain HDAC3 inhibition. The greater inhibition of HDAC2 and HDAC3-NCoR1 for entries 4 and 5 in this series may reflect a stronger binding to zinc arising from increased electron density provided by the mesomeric effect of the methoxy and hydroxy groups, respectively. Since investigation of the series of compounds **8a-8i** did not disclose a substituted *N*-(2-aminophenyl)-benzamide unit of improved potency, the binding moiety present in Mocetinostat was used in further studies.

Table 1

There have been few reports of alternative ring systems to replace the pyrimidine ring in Mocetinostat; only planar, fused rings have been substituted, leading to high molecular weight compounds with non-optimal aqueous solubility, albeit with medicinal potential.[29] Our view was that five-membered semi-saturated ring systems could prove effective, also offering the possibility of introducing a chiral centre, as well as variable substituents that would be alternatives to the terminal 3-pyridyl ring present in Mocetinostat. The imidazolin-4-one ring indeed proved effective (Table 2, entries 1-6); these compounds showing appreciable isoform selectivity for HDAC3-NCoR1. Of particular note is entry 6; this suggests that the imidazolin-4-one ring is a good mimic of pyrimidine, and that the 5-phenyl substituent occupies a closely similar location in HDAC to that of the 3-pyridyl group in Mocetinostat. Entry 6 showed at least as great potency as did Mocetinosat against HDAC3-NCoR1.

Table 2

A thiazoline ring was next examined, in order to establish whether a single nitrogen atom in the ring was compatible with HDAC inhibitory activity; that is the case, as shown by entries 7-9 (Table 2). Once again, a phenyl substituent was the most potent in inhibiting HDAC3-NCoR1, with the 2-arylmethylthio linkage in entry 9 being notable as the first *N*-(2-aminophenyl)-benzamide HDAC inhibitor lacking an aminomethyl linker that exhibits an appreciably low nanomolar IC_{50} value. Its aminomethyl analog (Table 3, entry 1) was an even more potent inhibitor, and likely more potent than Mocetinostat against HDAC3-NCoR1. For inhibition of HDAC1 and HDAC2, a (4*R*)-configuration (entries 2, 5 and 7) shows somewhat greater inhibition than the corresponding (4*S*)-enantiomer (entries 1, 4 and 6). However for **24a**/**24b**, the (4*S*)-enantiomer (entry 1) is the more potent inhibitor of HDAC3-NCoR1. Although phenyl, 4-hydroxyphenyl and benzyl monosubstitution was consistent with good potency, bulkier 4,5-diphenyl substitution (entries 8 and 9) was found to be deleterious to all HDAC inhibition investigated.

Table 3

As expected, most thiazolines prepared (Tables 2 and 3) inhibited HDAC8 only weakly, binding to the cap region being ineffective owing to the short tunnel that leads to the catalytic site in HDAC8. As for compounds studied previously,[33] inhibition of HDAC6 by any of the novel benzamides was not detected. Molecular modeling of thiazoline **24a** bound to HDAC3 (modification of PDB code: 4A69) showed the co-ordination to zinc to both the carbonyl group (Fig. 2) of the benzamide linkage and to the aniline nitrogen atom. The largely hydrophobic tunnel (including Leu266, Phe144, Phe200) accommodates the benzamide ring, whereas contacts to the protein periphery (including Phe199) are made by the terminal phenyl and thiazoline rings.

Figure 2

Two benzamide inhibitors, including MS-275, were previously shown to exhibit slow, tight-binding kinetics for HDAC1, HDAC2 and HDAC3, although the mechanism and rate of binding for HDAC3 differed from HDAC1 and HDAC2.[19] This assay, in our hands, identified a two-step binding mechanism for the binding of Mocetinostat to HDAC3-NCoR1 and additionally revealed compound **24a** as a slow tight-binding inhibitor of HDAC3-NCoR1 (see supporting information for graphs). These results contrasted with the hydroxamic acid **31** which showed the expected fast, competitive inhibition of HDAC3-NCoR1. The above slow tight-binding behavior is therefore attributable to the *N*-(2-aminophenyl)-benzamide moiety.

Thiazoline **24a** possesses good physicochemical properties as exemplified by its calculated lipophilicity value (Daylight clog P = 2.91), measured lipophilicity values (Chrom log $D_{pH7.4}$[35] = 4.24; Chrom log P[35] = 4.65), and its calculated polar surface area (tpsa[36] = 79.5 Å^2). Inhibitor **24a** has good measured solubility, with chemiluminescent nitrogen detection solubility[37] = 239 μM, and is also predicted to have good cell permeability (artificial membrane permeability[38] = 510 nm/s). The cytochrome P450 inhibition profile of **24a** showed only weak inhibition of all tested isoforms except CYP2D6 (isoform IC_{50} values: 2C19 = 32 μM; 2C9 = 16 μM; 2D6 = 80 nM; 3A4 (Vivid® Red substrate) = 25 μM; 3A4 (Vivid® Green substrate) = 16 μM). Owing to the inhibition of CYP2D6, the risk of drug-drug interactions would need to be evaluated further during any development of **24a**.

Cell growth inhibition data for four cell lines are given in Table 4. Despite good *in vitro* inhibitory potency against HDAC2 and HDAC3, the imidazolin-4-ones **19a** and **19c** showed poor inhibition of the cancer cell lines. However, thiazolines **24a**, **24d** and **30b** were more potent, having low micromolar IC_{50} values for the four cell lines tested, their pIC_{50} values approaching those of Mocetinostat and Vorinostat.

Table 4

Changes in histone acetylation status were assayed by analyzing histone H3 acetylated on lysine 9 (H3K9Ac) following addition of *N*-(2-aminophenyl)-benzamides. To determine whether Mocetinostat, **19c** or **24a** increase histone H3 acetylation levels, the human cell lines HeLa and Jurkat were cultured in the presence of each compound (10 μM or 1 μM), or an equivalent volume of the diluent (DMSO) added as the control. The cells were cultured for 24 h and H3K9Ac was analyzed by Western blotting. The data show that 10 μM or 1 μM Mocetinostat and **24a** both cause an increase in histone H3K9Ac with respect to histone H3 expression in Jurkat and in HeLa cells. Imidazolin-4-one **19c** (10 μM or 1 μM) also caused an increase in H3K9Ac in Jurkat cells but not in HeLa cells (Fig. 3). These data are consistent with an inhibition of endogenous cellular HDAC activity which would cause net acetylation of histone H3K9.

Previous studies showed that Mocetinostat causes an increase in H3K9Ac in HeLa cells and leads to cell death by apoptosis.[39] Consequently, in the present study flow cytometry analyses of DNA and protein content of cells cultured with the compounds were carried out to determine whether **19c** and **24a** also cause apoptosis. These data (Figs. 4 and 5) show that Mocetinostat (10 μM or 1 μM) and **24a** increase the percentage of Jurkat cells with sub-G_1 DNA content, consistent with an induction of apoptosis. However, HeLa cells cultured with either **19c** or **24a** at 10 μM appeared to accumulate in G_2/M, whereas there was an increase in the percentage of Jurkat cells in S-phase, indicating a difference in underlying mechanisms in these two cell types. In contrast, culturing Jurkat cells with **19c** did not result in the appearance of cells with sub-G_1 DNA content and did not significantly alter the percentage of cells in each cell cycle phase. The Poly-ADP Ribose Polymerase (PARP) protein is degraded during apoptosis by activation of Caspase 3 as was here observed in Jurkat cells (Fig. 3). However, no apparent cleavage of PARP occurred in HeLa cells, consistent with data previously reported[39] on apoptosis induced by Mocetinostat.

Summary

For the first time, chiral cyclic capping groups have been shown to be effective for *N*-(2-aminophenyl)-benzamide HDAC inhibitors. Inhibition of HDAC1, HDAC2 and HDAC3 (Class I) was generally potent, and in a few of the examples studied, this novel class of inhibitors exceeded (low nanomolar IC_{50}) potency against HDAC3-NCoR1, compared with Mocetinostat, one of the most potent benzamide HDAC inhibitors previously reported. The present study shows the importance of future work to address the central question of whether improved isoform selectivity and potency over existing *N*-(2-aminophenyl)-benzamide inhibitors can be achieved by new, enantiopure compounds of the appropriate absolute configuration and of the classes here disclosed. This study has shown the efficacy of chiral heterocyclic capping groups, in particular substituted imidazolin-4-ones and thiazolines, especially the latter, which conferred low-nanomolar potency for the inhibition of HDAC3-NCoR1 and may be of use as an alternative to the 2-[3-(3'-pyridyl)]pyrimidinyl group present in the clinical benzamide inhibitor MGCD0103. Both Mocetinostat and thiazoline **24a** (each at 1 μM) increased histone H3K9 acetylation in Jurkat and HeLa cell lines and caused cell death of Jurkat cells consistent with induction of apoptosis. Additionally, convergent routes were established to HDAC inhibitors containing a variety of chiral heterocyclic capping groups by use of a preassembled *N*-(2-aminophenyl)-benzamide unit, new methodology that should apply to heterocyclic systems other than those described here.

Further work is needed to establish the detailed advantages of this novel class of HDAC inhibitors over existing *N*-(2-aminophenyl)-benzamides, but these may include benefits from the absence of the commonly required 3-pyridyl terminal capping group and its metabolism to the *N*-oxide, and of particular interest, a new means by which HDAC isoform selectivity could be increased. This study demonstrates that, in terms of HDAC inhibition, other ring systems can replace either of or both the pyridine and pyrimidine rings present in Mocetinostat, and hence supports design of new, chiral, Class I-selective HDAC inhibitors.

Experimental Section

Chemistry. All chemicals were used as supplied, except 1,2-phenylenediamine which was recrystallized from ethanol. Solvents used were reagent grade, and anhydrous solvents were obtained from Anhydrous Engineering (USA) solvent systems after passing through an alumina column. Compound homogeneity was monitored by ascending thin-layer chromatography, performed on Merck 0.2 mm aluminum-backed silica gel 60 F_{254} plates and visualized using an alkaline potassium permanganate dip or by ultraviolet light. Flash column chromatography was performed using Merck 0.040 to 0.063 mm, 230 to 400 mesh silica gel. Evaporation refers to the removal of solvent under reduced pressure. Melting points were determined using an Electrothermal digital melting point apparatus. Infrared (IR) spectra were recorded on a Perkin-Elmer spectrum 100 FT-IR spectrometer as neat powders or as thin films. ^{1}H NMR spectra were recorded at 300 MHz on a Bruker AMX300 spectrometer, at 400 MHz on a Bruker AMX400, at 500 MHz on a Bruker Avance 500 spectrometer, or at 600 MHz on a Bruker Avance 600 spectrometer in the stated solvent; chemical shifts are reported in δ (ppm) relative to the internal reference tetramethylsilane. Mass spectra were obtained on a Fisons VG70-SE mass spectrometer or Thermo Finnigan MAT900xp instrument. Purity of tested compounds was assessed to be at least 95% by HPLC analysis unless indicated otherwise. (Dionex) on an Supelco Discovery® BIO widepore C18 column (250 mm × 4.6 mm, 10 μm particle size) using gradient elution of acetonitrile in water, 2 to 98% for 20 min at a flow rate of 1 mL/min with detection at 254 nm wavelength. For all samples 0.1% TFA was added to both eluents.

The following compounds were prepared according to the literature: methyl 4-amino-3-nitrobenzoate (**2a**);[40] 2-nitro-4-phenylaniline (**6**);[41] methyl 4-(guanidinomethyl)benzoate;[23] (*E*)-3-(dimethylamino)-1-(pyridin-3-yl)prop-2-en-1-one;[24] methyl 4-((4-(pyridin-3-yl)pyrimidin-2-ylamino)methyl)benzoate;[24] 4-((4-(pyridin-3-yl)pyrimidin-2-

ylamino)methyl)benzoic acid (**7**);[24] *N*-(2-aminophenyl)-4-({[4-(pyridin-3-yl)pyrimidin-2-yl]amino}methyl)benzamide (Mocetinostat).[24] (5*S*)-1-acetyl-5-benzyl-2-thiohydantoin (**10a**);[25] (5*S*)-1-acetyl-5-(1*H*-indol-3-ylmethyl)-2-thiohydantoin (**10b**);[25] *N*-(*tert*-butoxycarbonyl)-L-phenylalanine (**14a**),[42] *N*-(*tert*-butoxycarbonyl)-L-tryptophan (**14b**);[43] *N*-*(tert*-butoxycarbonyl)-D-phenylglycine (**14c**);[44] (5*S*)-1-(*tert*-butoxycarbonyl)-5-benzyl-2-thiohydantoin (**15a**);[26] 4-[(trifluoroacetamido)methyl]benzoic acid (**20**);[45] *tert*-butyl (2-aminophenyl)carbamate);[46] *tert*-butyl *N*-(2-{4-[(trifluoroacetamido)methyl]benzamido}phenyl)carbamate (**21a**);[47] *tert*-butyl *N*-{2-[4-(aminomethyl)benzamido]phenyl}carbamate (**21b**);[47] D-phenylalaninol;[48] L-phenylalaninol (**25a**);[49] (2*R*)-2-amino-2-phenylethan-1-ol;[48] (2*S*)-2-amino-2-phenylethan-1-ol (**25c**);[31] (4*S*)-4-benzyl-1,3-thiazolidine-2-thione (**26a**).[50] (4*S*)-4-phenyl-1,3-thiazolidine-2-thione (**26c**).[32]

***N*-(2-Amino-4-bromophenyl)-4-({[4-(pyridin-3-yl)pyrimidin-2-yl]amino}methyl)benzamide (8c)**. To a solution of iron(II) sulfate heptahydrate (1.87 g, 6.74 mmol) in water (4 mL) and 30% aqueous ammonia (4 mL) heated to 60 °C was added a suspension of *N*-(4-bromo-2-nitrophenyl)-4-({[4-(pyridin-3-yl)pyrimidin-2-yl]amino}methyl)benzamide (0.28 g, 0.56 mmol) in ethanol (4 mL). The mixture was stirred at 60 °C for 4 h then cooled to 20 °C, filtered through celite and the celite washed with 1:9 methanol:dichloromethane (100 mL) and dichloromethane 100 mL**).** The combined filtrates were washed with water (100 mL) and then with brine (100 mL), dried over $MgSO_4$, filtered and evaporated to give a pale yellow solid (0.24 g) that was purified by column chromatography on silica gel (5% methanol in chloroform) to give **8c** as a white solid (0.164 g, 61%), mp 206-208 °C; ν_{max} (cm^{-1}) 3247, 3055, 1633, 1608; ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.64 (2H, d, *J*=6.2 Hz), 5.19 (2H, s), 6.69 (1H, dd, *J*=8.4, 2.0 Hz), 6.93 (1H, d, *J*=2.0 Hz), 7.09 (1H, d, *J*=8.4 Hz), 7.24 (1H, d, *J*=5.2 Hz), 7.44 - 7.55 (3H, m), 7.90 (2H, d, *J*=8.2 Hz), 7.97 (1H, t, *J*=6.2 Hz), 8.40 (2H, d, *J*=5.2 Hz), 8.67 (1H, d, *J*=3.6 Hz), 9.23 (1H, br. s), 9.53 (1H, s); ^{13}C NMR (150 MHz, DMSO-d_6) δ 44.0, 106.0, 117.7, 118.3, 118.8, 122.5,

123.9, 126.9, 127.9, 128.6, 132.4, 132.8, 134.2, 144.3, 145.1, 148.1, 151.3, 159.1, 159.6, 162.4, 165.4; *m/z* (ES^+, %) 475 (53), 307 (100); HRMS $C_{23}H_{20}BrN_6O$ calcd. for $[M+H]^+$ 475.0882, found: 475.0866.

***N*-(2-Amino-4-methoxyphenyl)-4-({[4-(pyridin-3-yl)pyrimidin-2-yl]amino}methyl)benzamide (8d)**. To a solution of acid **5** (0.306 g, 1.0 mmol), 4-methoxy-1,2-phenylenediamine (0.531 g, 1.1 mmol) and (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) (0.531 g, 1.2 mmol) in DMF (10 mL) was added triethylamine (0.28 mL, 2.0 mmol). The mixture was stirred under a blanket of nitrogen at 20 °C for 27 h then diluted with ethyl acetate (50 mL), washed with brine (3 x 50 mL) and then with saturated aqueous sodium hydrogen carbonate (25 mL). The combined aqueous layers were extracted with ethyl acetate (25 mL) and the combined organic layers were washed with brine (2 x 25 mL), dried over Na_2SO_4 and evaporated. Column chromatography on silica gel (ethyl acetate) gave **8d** as a cream solid (0.21 g, 49%), mp 176-178 °C; ν_{max} (cm^{-1}) 3435, 3249, 1628; ^1H NMR (400 MHz, DMSO-d_6) δ 3.67 (3H, s), 4.65 (2H, d, *J*=5.9 Hz), 4.91 (2H, br. s), 6.18 (1H, dd, *J*=8.5, 2.5 Hz), 6.35 (1H, d, *J*=2.5 Hz), 6.99 (1H, d, *J*=8.5 Hz), 7.27 (1H, d, *J*=5.0 Hz), 7.41-7.60 (3H, m), 7.92 (2H, d, *J*=8.0 Hz), 8.02 (1H, t, *J*=5.9 Hz), 8.42 (2H, d, *J*=5.3 Hz), 8.69 (1H, d, *J*=3.3 Hz), 9.25 (1H, br. s), 9.49 (1H, br. s); ^{13}C NMR **(**150 MHz, DMSO-d_6) δ 44.0, 54.9, 100.7, 101.9, 106.1, 116.6, 123.9, 126.9, 127.7, 127.9, 132.4, 133.0, 134.2, 144.1, 144.6, 148.1, 151.3, 158.2, 159.2, 159.6, 162.4, 165.3; *m/z* (ES^-, %) 425 (100); HRMS calcd. for $C_{24}H_{21}N_6O_2$ $[M-H]^-$ 425.1731, found: 425.1732.

(±)-1-Acetyl-5-benzyl-2-(methylsulfanyl)-4,5-dihydro-1*H*-imidazol-4-one (11a). To a stirred mixture of the thiohydantoin **10a** (0.70 g, 2.82 mmol) and anhydrous potassium carbonate (0.468 g, 3.38 mmol) in acetonitrile (35 mL) under nitrogen at 20 °C was added iodomethane (0.263 mL, 4.23 mmol), dropwise. The mixture was stirred vigorously at 20 °C

under nitrogen for 3.5 h, then filtered and the filtrate evaporated. The resulting cream solid was purified by column chromatography (1:9 to 2:1 ethyl acetate:hexane) to give **11a** as a white solid (0.203 g, 27%), mp 142-143 °C; ν_{max} (cm^{-1}) 1725, 1698; ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ 2.24 (3H, s), 2.39 (3H, s), 3.17 (1H, dd, *J*=14.3, 2.7 Hz), 3.35 (1H, dd, *J*=14.3, 6.0 Hz), 4.96 (1H, dd, *J*=6.0, 2.8 Hz), 6.97 - 7.06 (2H, m), 7.22 (3H, s); ^{13}C NMR (125 MHz, DMSO-*d*$_6$) δ 15.7, 23.6, 36.1, 64.1, 127.1, 128.2, 129.4, 134.0, 168.5, 184.5, 185.4; *m/z* (EI^+, %) 262 (12); HRMS calcd. for $C_{13}H_{14}N_2O_2S$ M^+ 262.0771, found: 262.0765.

(±)-1-Acetyl-5-(1*H*-indol-3-ylmethyl)-2-(methylsulfanyl)-4,5-dihydro-1*H*-imidazol-4-one (11b). The thiohydantoin **10b** (0.907 g, 3.38 mmol) and anhydrous potassium carbonate (0.56 g, 4.05 mmol) in acetonitrile (40 mL) under nitrogen at 20 °C was added iodomethane (0.32 mL, 5.06 mmol), dropwise. The mixture was stirred vigorously at 20 °C under nitrogen for 2.5 h, then filtered and the filtrate evaporated. Purification of the residue by column chromatography (1:1 then 2:1 ethyl acetate:hexane) gave **11b** as a cream solid (0.61 g, 60%), mp 187-190 °C; ν_{max} (cm^{-1}) 3234, 1725, 1698; ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ 2.22 (3H, s), 2.39 (3H, s), 3.35 (1H, dd, *J*=15.4, 2.9 Hz), 3.47 (1H, dd, *J*=15.4, 5.8 Hz), 4.94 (1H, dd, *J*=5.8, 3.0 Hz), 6.93 (1H, t, *J*=7.5 Hz), 6.96 (1H, d, *J*=2.4 Hz), 7.03 (1H, t, *J*=7.5 Hz), 7.29 (1H, d, *J*=8.0 Hz), 7.40 (1H, d, *J*=8.0 Hz), 10.90 (1H, br. s); ^{13}C NMR (125 MHz, DMSO-*d*$_6$) δ 15.6, 23.6, 26.4, 64.1, 106.4, 111.3, 118.3, 118.5, 121.0, 124.1, 127.2, 135.7, 168.5, 185.1, 185.3; *m/z* (EI^+, %) 301 (10), 130 (100); HRMS calcd. for $C_{15}H_{15}N_3O_2S$ M^+ 301.0879, found: 301.0878.

4-({[(±)-1-Acetyl-5-benzyl-4-oxo-4,5-dihydro-1*H*-imidazol-2-yl]amino}methyl)benzoic acid (12a) To a solution of the imidazolin-4-one **11a** (179 mg, 0.682 mmol) in ethanol (8 mL) was added 4-(methylamino)benzoic acid (103 mg, 0.682 mmol). The mixture was stirred at reflux under nitrogen for 18 h. The solution was cooled to 20 °C, then cooled in a refrigerator, filtered and the solid dried under vacuum to give **12a** as a cream solid (122 mg,

49%), mp 232-234 °C; ν_{max} (cm^{-1}) 3263, 2938, 1688; ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ 2.42 (3H, s), 3.14 (1H, dd, *J*=14.2, 1.9 Hz), 3.30 (1H, dd, *J*=14.2, 5.7 Hz), 4.37 (1H, dd, *J*=15.8, 6.0 Hz), 4.52 (1H, dd, *J*=15.8, 6.6 Hz), 4.77 (1H, dd, *J*=5.7, 1.9 Hz), 6.91 (2H, d, *J*=8.2 Hz), 6.99 - 7.08 (2H, m), 7.18 - 7.32 (3H, m), 7.77 (2H, d, *J*=8.2 Hz), 9.28 (1H, t, *J*=6.3 Hz); ^{13}C NMR (125 MHz, DMSO-*d*$_6$) δ 24.2, 35.7, 45.4, 63.1, 126.6, 126.8, 128.1, 129.2, 129.6, 134.4, 142.9, 167.2, 167.6, 171.2, 183.4; *m/z* (ES, %) 366 ([M+H]$^+$100); HRMS calcd. for $C_{20}H_{20}N_3O_4$ [M+H]$^+$ 366.1448, found: 366.1448.

4-({[(±)-1-Acetyl-5-(1*H*-indol-3-ylmethyl)-4-oxo-4,5-dihydro-1*H*-imidazol-2-yl]amino}methyl)benzoic acid (12b). The imidazolin-4-one **11b** (0.60 g, 1.99 mmol) was reacted with 4-(methylamino)benzoic acid according to the above procedure for **12a**. Filtration gave **12b** as a tan solid (0.295 g, 37%); the mother liquor was evaporated and the residue recrystallized from ethanol to give further **12b** as a tan solid (0.13 g, 16%), overall yield 53%, mp 163-166 °C; ν_{max} (cm^{-1}) 3315, 1728, 1674, 1663; ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ 2.46 (3H, s), 3.31 (1H, dd, *J*=15.1, 2.2 Hz), 3.45 (1H, dd, *J*=15.1, 5.4 Hz), 4.32 (1H, dd, *J*=15.8, 5.7 Hz), 4.49 (1H, dd, *J*=15.8, 6.9 Hz), 4.75 (1H, dd, *J*=5.4, 2.2 Hz), 6.74 (2H, d, *J*=8.0 Hz), 6.92 (1H, t, *J*=7.3 Hz), 6.99 (1H, d, *J*=2.0 Hz), 7.04 (1H, t, *J*=7.4 Hz), 7.36 (1H, d, *J*=8.2 Hz), 7.41 (1H, d, *J*=7.9 Hz), 7.74 (2H, d, *J*=8.2 Hz), 9.20 (1H, t, *J*=6.3 Hz), 11.00 (1H, br. s); ^{13}C NMR (125 MHz, DMSO-*d*$_6$) δ 24.2, 26.2, 45.3, 63.3, 106.6, 111.3, 118.3, 118.5, 120.8, 124.3, 126.2, 127.5, 129.3, 129.3, 135.9, 142.9, 167.2, 167.8, 171.2, 184.1; *m/z* (ES$^-$, %) 403 ([M-H]$^-$, 95), HRMS calcd. for $C_{22}H_{19}N_4O_4$ [M-H]$^-$ 403.1412, found: 403.1412.

4-({[(±)-1-Acetyl-5-benzyl-4-oxo-4,5-dihydro-1*H*-imidazol-2-yl]amino}methyl)-*N*-(2-aminophenyl)benzamide (13a). To a solution of acid **12a** (114 mg, 0.312 mmol) and (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) (165 mg, 0.374 mmol) in anhydrous DMF (1.4 mL) was added triethylamine (174 μl, 1.25 mmol)

and the mixture stirred at 20 °C under nitrogen for 15 min. Phenylene-1,2-diamine (40 mg, 0.374 mmol) was then added and the solution stirred at 20 °C under nitrogen for 3.5 h. The mixture was diluted with ethyl acetate (50 mL) then washed with water (25 mL), saturated aqueous sodium hydrogen carbonate (25 mL), and lastly with brine (25 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to give an orange solid. Purification by column chromatography (4% methanol in dichloromethane) gave **13a** as a white solid (21 mg, 15%), mp 235-236 °C; ν_{max} (cm^{-1}) 3283, 1720, 1671; ^{1}H NMR (600 MHz, DMSO-*d*6) δ 2.44 (3H, s), 3.16 (1H, dd, *J*=14.0, 2.3 Hz), 3.31 (1H, dd, *J*=14.0, 5.6 Hz), 4.40 (1H, dd, *J*=15.6, 5.6 Hz), 4.54 (1H, dd, *J*=15.6, 6.6 Hz), 4.80 (1H, dd, *J*=5.6, 2.3 Hz), 4.91 (2H, br. s), 6.61 (1H, t, *J*=7.3 Hz), 6.79 (1H, d, *J*=7.2 Hz), 6.94 - 7.00 (3H, m), 7.07 (2H, d, *J*=7.2 Hz), 7.17 (1H, d, *J*=7.5 Hz), 7.28 (2H, t, *J*=7.5 Hz), 7.32 (1H, t, *J*=7.2 Hz), 7.84 (2H, d, *J*=7.5 Hz), 9.30 (1H, t, *J*=6.4 Hz), 9.64 (1H, s); ^{13}C NMR (150 MHz, DMSO-*d*6) δ 24.3, 35.8, 45.4, 63.1, 116.1, 116.3, 123.3, 126.5, 126.6, 126.8, 127.0, 127.7, 128.3, 129.6, 133.2, 134.4, 141.4, 143.2, 165.1, 167.6, 171.3, 183.5; *m/z* (ES, %) 456 (M^+, 80); HRMS calcd. for $C_{26}H_{26}N_5O_3$ M^+ 456.2030, found: 456.2017.

4-({[(±)-1-Acetyl-5-(1*H*-indol-3-ylmethyl)-4-oxo-4,5-dihydro-1*H*-imidazol-2-yl]amino}methyl)-*N*-(2-aminophenyl)benzamide (13b). Acid **12b** (240 mg, 0.593 mmol) was reacted with phenylene-1,2-diamine according to the above procedure for **13a**. Column chromatography of the residue (6% methanol in dichloromethane) afforded a solid that was recrystallized from methanol-dichloromethane to give **13b** as a cream crystalline solid (182 mg, 62%), mp 168-170 °C; ν_{max} (cm^{-1}) 3267, 1720, 1676; ^{1}H NMR (500 MHz, DMSO-*d*6) δ 2.45 (3H, s), 3.31 (1H, dd, *J*=15.1, 2.4 Hz), 3.46 (1H, dd, *J*=15.1, 5.4 Hz), 4.36 (1H, dd, *J*=15.6, 6.0 Hz), 4.49 (1H, dd, *J*=15.6, 6.8 Hz), 4.76 (1H, dd, *J*=5.4, 2.4 Hz), 4.90 (2H, br. s), 6.61 (1H, t, *J*=8.0 Hz), 6.79 (1H, dd, *J*=8.0, 1.1 Hz), 6.84 (2H, d, *J*=8.0 Hz), 6.92 - 7.00 (3H, m), 7.05 (1H, t, *J*=8.0 Hz), 7.19 (1H, d, *J*=7.7 Hz), 7.38 (1H, d, *J*=8.0 Hz), 7.43 (1H, d, *J*=8.0 Hz), 7.79 (2H, d, *J*=8.0 Hz), 9.22 (1H, t, *J*=6.4 Hz), 9.55 (1H, s), 10.96 (1H, br. d,

J=1.6 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 24.2, 26.2, 45.3, 63.3, 106.6, 111.4, 116.1, 116.3, 118.3, 118.5, 120.9, 123.4, 124.2, 126.2, 126.5, 126.6, 127.5, 127.7, 133.2, 135.8, 141.3, 143.1, 165.2, 167.7, 171.2, 184.1; *m/z* (ES^+, %) 495 ($[M+H]^+$, 100); HRMS calcd. for $C_{28}H_{27}N_6O_3$ $[M+H]^+$ 495.2139, found: 495.2129.

(5*S*)-1-(*tert*-Butoxycarbonyl)-5-(1*H*-indol-3-ylmethyl)-2-thiohydantoin (15b). To a stirred solution of *N*-(*tert*-butoxycarbonyl)-L-tryptophan (**14b)** (2.11 g, 6.93 mmol) in acetonitrile (155 mL) was added ethoxycarbonyl isothiocyanate (0.98 mL, 8.3 mmol) and pyridine (0.67 mL, 8.3 mmol). The mixture was stirred at 20 °C for 17 h, evaporated and the residue purified by column chromatography (1:2 ethyl acetate:hexane) to give **15b** as a white solid (1.98 g, 82%), mp 85-87 °C; ν_{max} (cm^{-1}) 3354, 1736; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.64 (9H, s), 3.53 (1H, dd, *J*=15.0, 2.8 Hz), 3.63 (1H, dd, *J*=15.1, 5.3 Hz), 4.81 (1H, dd, *J*=5.3, 2.9 Hz), 6.93 (1H, d, *J*=2.0 Hz), 7.10 (1H, t, *J*=7.2 Hz), 7.17 (1H, t, *J*=7.2 Hz), 7.31 (1H, d, *J*=8.0 Hz), 7.56 (1H, d, *J*=8.0 Hz), 8.12 (1H, br. s), 8.35 (1H, br. s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 26.1, 28.2, 64.4, 85.5, 107.1, 111.3, 118.7, 120.0, 122.4, 123.7, 127.4, 135.9, 148.8, 171.5, 178.6; *m/z* (EI^+, %) 345 (M^+, 12), 130 (97); HRMS calcd. for $C_{17}H_{19}N_3O_3S$ $[M+H]^+$ 345.1142, found: 345.1144.

(5*S*)-1-(*tert*-Butoxycarbonyl)-5-phenyl-2-thiohydantoin (15c). To a stirred solution of *N*-Boc D-phenylglycine, **(14c)** (2.50 g, 9.95 mmol) in acetonitrile (200 mL) was added ethoxycarbonyl isothiocyanate (1.4 mL, 12.0 mmol) and pyridine (0.96 mL, 12.0 mmol). The mixture was stirred at 20 °C for 17 h, evaporated and the residue purified by column chromatography (1:3 ethyl acetate:hexane) to give a sticky mass that was recrystallized from chloroform to give **15c** as a white solid (1.71 g, 59%), mp 172-173 °C; ν_{max} (cm^{-1}) 3111, 1740; ^{1}H NMR (500 MHz, *DMSO-d_6*) δ 1.17 (9H, s), 5.62 (1H, s), 7.23 (2H, d, *J*=6.9 Hz), 7.34 - 7.47 (3H, m), 12.63 (1H, br. s); ^{13}C NMR (125 MHz, *DMSO-d_6*) δ 27.2, 66.6, 83.3,

126.4, 128.7, 129.0, 134.9, 147.5, 171.3, 180.9 (HNC=O); *m/z* (EI^+, %) 292 (M^+, 3%), 192 (100); HRMS calcd. for $C_{14}H_{16}N_2O_3S$ M^+ 292.0876, found: 292.0870.

(±)-1-(*tert*-Butoxycarbonyl)-5-benzyl-2-(methylsulfanyl)-4,5-dihydro-1*H*-imidazol-4-one (16a). To a vigorously stirred mixture of the thiohydantoin **15a** (0.90 g, 2.9 mmol) and anhydrous potassium carbonate (0.481 g, 3.48 mmol) in acetonitrile (35 mL) under nitrogen at 20 °C was added iodomethane (0.27 mL, 4.35 mmol), dropwise. The mixture was stirred at 20 °C under nitrogen for 2.5 h, then filtered and the filtrate evaporated. Purification by column chromatography (1:2 ethyl acetate:hexane) gave **16a** as a white solid (0.586 g, 63%), mp 105-107 °C; ν_{max} (cm^{-1}) 2979, 2933, 1722; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.61 (9H, s), 2.37 (3H, s), 3.32 (1H, dd, *J*=13.9, 2.5 Hz), 3.42 (1H, dd, *J*=13.9, 6.0 Hz), 4.52 (1H, dd, *J*=6.0, 2.5 Hz), 7.02 - 7.10 (2H, m), 7.18 - 7.25 (3H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 16.2, 28.2, 36.1, 64.5, 85.7, 127.4, 128.6, 129.5, 133.8, 148.4, 184.7, 186.0; *m/z* (ES, %) 321 ($[M+H]^+$, 100); HRMS calcd. for $C_{16}H_{21}N_2O_3S$ $[M+H]^+$ 321.1267, found: 321.1273.

(±)-1-(*tert*-Butoxycarbonyl)-5-(1*H*-indol-3-ylmethyl)-2-(methylsulfanyl)-4,5-dihydro-1*H*-imidazol-4-one (16b). To a vigorously stirred mixture of the thiohydantoin **15b** (1.85 g, 5.36 mmol) and anhydrous potassium carbonate (0.89 g, 6.4 mmol) in acetonitrile (65 mL) under nitrogen at 20 °C was added iodomethane (0.50 mL, 8.04 mmol), dropwise. The mixture was stirred vigorously at 20 °C under nitrogen for 2.5 h, then filtered and the filtrate evaporated. Purification of the residue by column chromatography (2:3 then 1:1 ethyl acetate:hexane) gave **16b** as a cream solid (1.35 g, 70%), mp 96-98 °C; ν_{max} (cm^{-1}) 3312, 1720; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.60 (9H, s), 2.33 (3H, s), 3.49 (1H, dd, *J*=15.1, 3.0 Hz), 3.64 (1H, dd, *J*=15.0, 5.5 Hz), 4.60 (1H, dd, *J*=5.5, 3.0 Hz), 6.95 (1H, d, *J*=2.4 Hz), 7.07 (1H, t, *J*=7.5 Hz), 7.14 (1H, t, *J*=7.6 Hz), 7.30 (1H, d, *J*=8.0 Hz), 7.53 (1H, d, *J*=8.0 Hz), 8.26 (1H, br. s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 16.2, 26.0, 28.2, 64.8, 85.8, 107.9, 111.2, 118.5, 119.6, 122.0, 123.5, 127.8, 135.9, 148.5, 185.4, 186.1; *m/z* (ES, %) 382

([M+Na]$^+$, 90); HRMS calcd. for $C_{18}H_{21}N_3NaO_3S$ [M+Na]$^+$ 382.1196, found: 382.1188.

(±)-1-(*tert*-Butoxycarbonyl)-5-phenyl-2-(methylsulfanyl)-4,5-dihydro-1*H*-imidazol-4-one (16c). To a vigorously stirred mixture of the imidazol-4-one **15c** (0.25 g, 0.855 mmol) and anhydrous potassium carbonate (0.141 g, 1.03 mmol) in acetonitrile (10 mL) at 20 °C was added iodomethane (80 μL, 1.28 mmol) dropwise. The mixture was stirred vigorously at 20°C under nitrogen for 2.5 h, then evaporated. Ethyl acetate (20 mL) was added to the residue and the mixture was stirred and filtered. The filtrate was evaporated to give **16c** as a white solid (0.26 g, 99%), mp 145-147 °C; ν_{max} (cm^{-1}) 1737, 1713; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.26 (9H, s), 2.67 (3H, s), 5.18 (1H, s), 7.15 - 7.23 (2H, m), 7.30 - 7.39 (3H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 16.4, 27.7, 67.6, 85.4, 126.4, 128.7, 129.0, 134.5, 148.4, 183.0, 186.6; *m/z* (EI$^+$, %) 306 (M$^+$, 3), 205 (100); HRMS calcd. for $C_{15}H_{18}N_2O_3S$ [M+H]$^+$ 306.1033, found: 306.1036.

4-({[(±)-1-(*tert*-Butoxycarbonyl)-5-benzyl-4-oxo-4,5-dihydro-1*H*-imidazol-2-yl]amino}methyl)benzoic acid (17a). To a stirred solution of the imidazol-4-one **16a** (0.550 g, 1.72 mmol) in ethanol (15 mL) was added 4-(methylamino)benzoic acid (0.260 g, 1.72 mmol). The mixture was stirred at reflux under nitrogen for 18 h. The solvent was evaporated and the residue shaken with ethyl acetate (50 mL). The organic layer was washed with water (50 mL) then with brine (50 mL), dried over $MgSO_4$, filtered and evaporated. Purification of the residue by column chromatography (ethyl acetate) gave **17a** as a cream solid (0.238 g, 33%), mp 137-140 °C; ν_{max} (cm^{-1}) 3386, 2975, 1702; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.62 (9H, br. s), 3.22 - 3.40 (2H, m), 4.36 (1H, m), 4.49 (1H, br. s), 4.77 (1H, dd, *J*=14.8, 6.6 Hz), 6.96 (2H, d, *J*=7.3 Hz), 7.10 (2H, d, *J*=6.3 Hz), 7.15 - 7.34 (3H, m), 7.94 (2H, d, *J*=7.3 Hz), 8.43 (1H, br. s), 8.95 (1H, br. s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 28.3, 35.8, 46.4, 63.5, 85.7, 127.2, 127.4, 128.6, 128.9, 129.7, 130.6, 134.1, 142.2, 167.8, 170.5, 174.1, 184.3; *m/z* (ES, %) 422 ([M-H]$^-$, 100), HRMS calcd. for $C_{23}H_{24}N_3O_5$ [M-H]$^-$

422.1721, found: 422.1707.

4-({[(±)-1-(*tert*-Butoxycarbonyl)-5-(1*H*-indol-3-ylmethyl)-4-oxo-4,5-dihydro-1*H*-imidazol-2-yl]amino}methyl)benzoic acid (17b). To a solution of the imidazol-4-one **16b** (1.30 g, 3.62 mmol) in ethanol (30 mL) was added 4-(methylamino)benzoic acid (0.547 g, 3.62 mmol). The mixture was stirred at reflux under nitrogen for 4 h. The solvent was evaporated and the resulting yellow solid purified by column chromatography (6% methanol in dichloromethane) to give **17b** as a cream solid (1.0 g, 60%), mp 187-190 °C; ν_{max} (cm^{-1}) 3271, 1746, 1697, 1693; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.58 (9H, s), 3.23 (1H, dd, *J*=14.7, 2.3 Hz), 3.44 (1H, dd, *J*=14.7, 5.3 Hz), 4.31 (1H, dd, *J*=15.8, 5.8 Hz), 4.45 - 4.52 (2H, m), 6.76 (2H, d, *J*=8.2 Hz), 6.88 (1H, d, *J*=2.2 Hz), 6.92 (1H, t, *J*=7.5 Hz), 7.04 (1H, t, *J*=7.5 Hz), 7.36 (1H, d, *J*=8.0 Hz), 7.44 (1H, d, *J*=8.0 Hz), 7.74 (2H, d, *J*=8.2 Hz), 8.72 (1H, br. t, *J*=5.7, 5.7 Hz), 10.97 (1H, br. d, *J*=2.2 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 25.2, 27.7, 45.3, 63.2, 84.0, 107.0, 111.3, 118.2, 118.4, 120.8, 123.8, 126.2, 127.6, 129.2, 135.8, 150.2, 167.2, 184.0; *m/z* (ES, %) 463 ($[M+H]^+$, 10), HRMS calcd. for $C_{25}H_{27}N_4O_5$ $[M+H]^+$ 463.1976, found: 463.1993.

4-(([(±)-1-(*tert*-Butoxycarbonyl)-5-phenyl-4-oxo-4,5-dihydro-1*H*-imidazol-2-yl]amino)methyl)benzoic acid (17c). To a solution of the imidazol-4-one **16c** (0.25 g, 0.817 mmol) in ethanol (5 mL) was added 4-(aminomethyl)benzoic acid (0.124 g, 0.817 mmol). The mixture was heated under reflux for 4 h with stirring. After cooling to 20 °C, the mixture was placed in the refrigerator (1 h), and then filtered. The filtrate was evaporated and the residue was purified by column chromatography (4:1 ethyl acetate:hexane) to give **17c** as a white solid (0.131 g, 39%), mp 166-168 °C; ν_{max} (cm^{-1}) 3315, 1702; ^{1}H NMR (500 MHz, methanol-d_4) δ 1.24 (9H, s), 4.76 - 4.84 (2H, m), 5.28 (1H, s), 7.24 (2H, d, *J*=7.3 Hz), 7.32 - 7.43 (3H, m), 7.52 (2H, d, *J*=8.2 Hz), 8.03 (2H, d, *J*=8.5 Hz); ^{13}C NMR (125 MHz, methanol-d_4) δ 27.9, 47.5, 68.1, 86.1, 127.8, 128.5, 129.5, 129.9, 131.2, 131.4, 137.2, 143.9,

151.7, 169.4, 169.5, 185.7; *m/z* (ES, %) 410 ($[M+H]^+$, 8); HRMS calcd. for $C_{22}H_{24}N_3O_5$ $[M+H]^+$ 410.1710, found: 410.1704.

1-*tert*-Butyloxycarbonyl (±)-2-[({4-[(2-aminophenyl)carbamoyl]phenyl}methyl)amino]-5-benzyl-4-oxo-4,5-dihydro-1*H*-imidazole (18a). To a solution of acid **17a** (230 mg, 0.543 mmol) and (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) (0.287 g, 0.65 mmol) in anhydrous DMF (2.5 mL) was added triethylamine (0.30 ml, 2.16 mmol) and the mixture stirred at 20 °C under nitrogen for 30 min. Phenylene-1,2-diamine (70 mg, 0.65 mmol) was then added and the solution stirred at 20 °C under nitrogen for 5.5 h. The mixture was diluted with water (50 mL) then extracted with ethyl acetate (100 mL, then 50 mL). The combined organic layers were washed with saturated aqueous sodium hydrogen carbonate (50 mL), and lastly with brine (50 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to give an orange solid. Purification by column chromatography (2% methanol in dichloromethane) gave **18a** as a cream solid (154 mg, 55%), mp 121-124 °C; ν_{max} (cm^{-1}) 3304, 1699; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.65 (9H, s), 3.29 - 3.39 (2H, m), 3.92 (2H, br. s), 4.40 (1H, dd, *J*=15.4, 5.4 Hz), 4.44- 4.50 (1H, m), 4.73 (1H, dd, *J*=15.4, 7.2 Hz), 6.81 - 6.89 (2H, m), 7.02 (2H, d, *J*=8.0 Hz), 7.07 - 7.14 (3H, m), 7.17 - 7.40 (4H, m), 7.81 (2H, d, *J*=8.0 Hz), 8.15 (1H, br. s), 8.41 (1H, br. s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 28.3, 35.8, 46.2, 63.4, 85.6, 118.4, 119.8, 124.7, 125.4, 127.3, 127.3, 127.6, 127.9, 128.6, 129.7, 133.6, 134.2, 140.6, 140.8, 151.1, 165.5, 167.8, 184.1; *m/z* (ES, %) 514 ($[M+H]^+$, 10), 536 ($[M+Na]^+$, 19); HRMS calcd. for $C_{29}H_{31}N_5NaO_4$ $[M+Na]^+$ 536.2268, found: 536.2277.

1-*tert*-Butyloxycarbonyl (±)-2-[({4-[(2-aminophenyl)carbamoyl]phenyl}methyl)amino]-5-(1*H*-indol-3-ylmethyl)-4-oxo-4,5-dihydro-1*H*-imidazole (18b). To a solution of acid **17b** (0.97 g, 2.10 mmol) and (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) (1.12 g, 2.52 mmol) in anhydrous DMF (10 mL) was added

triethylamine (1.17 ml, 8.4 mmol) and the mixture stirred at 20 °C under nitrogen for 15 min. Phenylene-1,2-diamine (0.272 g, 2.52 mmol) was then added and the solution stirred at 20 °C under nitrogen for 5 h. The mixture was diluted with ethyl acetate (240 mL), washed with water (90 mL), then with brine (50 mL), dried over $MgSO_4$, filtered and evaporated to give an orange solid. Purification by column chromatography (6% methanol in chloroform) gave **18b** as a cream solid (0.398 g, 34%), mp 185-186 °C; ν_{max} (cm^{-1}) 3288, 1732, 1699; ^1H NMR (500 MHz, $CDCl_3$) δ 1.66 (9H, s), 3.30 (1H, dd, *J*=14.8, 2.4 Hz), 3.49 (1H, dd, *J*=14.8, 3.7 Hz), 3.98 (2H, br. s), 4.12 (1H, dd, *J*=15.8, 5.2 Hz), 4.43 (1H, m), 4.58 (1H, dd, *J*=15.8, 7.7 Hz), 6.52 (2H, d, *J*=8.0 Hz), 6.67 (1H, d, *J*=1.7 Hz), 6.75 - 6.82 (2H, m), 6.99 (1H, t, *J*=7.5 Hz), 7.03 - 7.12 (2H, m), 7.34 (1H, d, *J*=7.7 Hz), 7.41 (1H, d, *J*=8.4 Hz), 7.44 (1H, d, *J*=8.2 Hz), 7.66 (2H, d, *J*=7.7 Hz), 8.31 (1H, br. s), 8.71 (1H, br. s), 9.11 (1H, br. s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.7, 28.3, 45.9, 64.2, 85.7, 107.1, 111.8, 117.8, 118.1, 119.3, 119.4, 121.7, 124.2, 124.6, 125.8, 126.5, 127.2, 127.9, 128.0, 133.2, 136.0, 140.3, 141.2, 151.0, 166.0, 168.0, 185.4; *m/z* (ES, %) 553 ($[M+H]^+$, 15), 575 ($[M+Na]^+$, 8); HRMS calcd. for $C_{31}H_{32}N_6NaO_4$, $[M+Na]^+$ 575.2377, found: 575.2389.

1-*tert*-Butyloxycarbonyl (±)-2-[({4-[(2-aminophenyl)carbamoyl]phenyl}methyl)amino]-5-phenyl-4-oxo-4,5-dihydro-1*H*-imidazole (18c). To a solution of acid **17c** (0.115 g, 0.281 mmol), (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) (0.143 g, 0.323 mmol) and phenylene-1,2-diamine (0.061 g, 0.562 mmol) in DMF (3 mL) was added triethylamine (0.075 mL, 0.562 mmol). The mixture was stirred at 20 °C for 4 h. Some DMF was removed *in vacuo* and the residual oil was diluted with ethyl acetate (50 mL) and the organic layer washed with saturated aqueous lithium chloride (20 mL), then with brine (2 x 20 mL). Evaporation gave a residue that was purified by column chromatography on silica gel (ethyl acetate) to give **18c** as a cream solid (0.14 g, 98%), mp 178-180 °C; ν_{max} (cm^{-1}) 3318, 1736, 1700, 1596; ^1H NMR (500 MHz, $CDCl_3$) δ 1.22 (9H, s), 4.02 (2H, br. s), 4.71 (1H, d, *J*=15.1 Hz), 4.76 (1H, d, *J*=15.1 Hz), 5.10 (1H, s), 6.78 (2H, d,

J=7.3 Hz), 7.03 (1H, t, *J*=7.6 Hz), 7.17 (2H, d, *J*=7.3 Hz), 7.24 (1H, d, *J*=7.6 Hz), 7.27 - 7.39 (5H, m), 7.82 (2H, d, *J*=7.3 Hz), 8.44 (1H, br. s), 8.79 (1H, br. s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 27.7, 46.8, 66.7, 85.5, 118.5, 119.9, 124.6, 125.7, 126.5, 127.3, 128.0, 128.2, 128.6, 128.9, 133.8, 135.2, 140.4, 140.5, 150.8, 165.8, 168.0, 183.1; *m/z* (ES, %) 522 ($[M+Na]^+$, 45); HRMS calcd. for $C_{28}H_{29}N_5NaO_4$ $[M+Na]^+$ 522.2112, found: 522.2103.

(±)-*N*-(2-Aminophenyl)-4-({5-benzyl-4-oxo-4,5-dihydro-1*H*-imidazol-2-yl]amino}methyl)benzamide (19a). To a solution of the imidazole **18a** (100 mg, 0.195 mmol) in dichloromethane (1 mL) was added trifluoroacetic acid (1 mL). The solution was stirred at 20 °C for 3 h, then evaporated and toluene (3 mL) added to the residue. Evaporation of the solvent afforded a pink solid that was partitioned between saturated aqueous sodium hydrogen carbonate (50 mL) and ethyl acetate (50 mL). The organic layer was washed with brine, dried over $MgSO_4$, filtered and evaporated to give a solid that was purified by column chromatography (10% methanol in dichloromethane) to give **19a** as a pale yellow solid (58 mg, 72%), mp 157-160 °C; ν_{max} (cm^{-1}) 3197, 1699, ^{1}H NMR (2:1 mixture of tautomers) (600 MHz, DMSO-*d*6) δ 2.76 (1H, dd, *J*=13.6, 6.4 Hz), 2.97 (1H, m, minor tautomer), 3.03 (1H, dd, *J*=13.4, 3.2 Hz, major tautomer), 4.06 (1H, app. t, *J*=5.1 Hz), 4.28 - 4.52 (2H, m), 4.91 (2H, br. s), 6.60 (1H, t, *J*=7.3 Hz), 6.79 (1H, d, *J*=7.9 Hz), 6.97 (1H, t, *J*=7.5 Hz), 7.10 - 7.31 (8H, m), 7.68 (2H, br. s, major tautomer) 7.89 (2H, d, *J*=7.2 Hz, major tautomer), 7.95 (2H, br. d, *J*=7.2 Hz, minor tautomer), 8.28 (2H, br. s, minor tautomer), 9.67 (1H, br. s); ^{13}C NMR (150 MHz, DMSO-*d*6) δ 37.2, 43.7, 44.7, 61.2, 116.1, 116.3, 123.3, 126.3, 126.5, 126.7, 126.7, 126.9, 127.8, 128.0, 128.1, 129.5, 129.6, 133.2, 133.3, 136.9, 137.3, 141.8, 142.8, 143.2, 165.1, 170.8, 171.5, 187.6, 188.1(tautomers); *m/z* (CI, %) 414 ($[M+H]^+$, 15); HRMS calcd. for $C_{24}H_{24}N_5O_2$ $[M+H]^+$ 414.1925, found: 414.1935.

(±)-*N*-(2-Aminophenyl)-4-({[5-(1*H*-indol-3-ylmethyl)-4-oxo-4,5-dihydro-1*H*-imidazol-2-yl]amino}methyl)benzamide (19b). To a solution of the imidazole **18b** (300 mg, 0.543

mmol) in dichloromethane (3 mL) was added trifluoroacetic acid (1.5 mL). The solution was stirred at 20 °C for 3 h, then evaporated and toluene (3 mL) added to the residue. Evaporation of the solvent afforded a solid that was partitioned between saturated aqueous sodium hydrogen carbonate (100 mL) and ethyl acetate (150 mL). The organic layer was washed with brine, dried over $MgSO_4$, filtered and evaporated to give **19b** as a pale yellow solid (0.24 g, 98%), mp 205-207 °C. Recrystallization of 50 mg from dichloromethane-methanol gave **19b** (30 mg) as a cream solid, mp 214-215 °C; ν_{max} (cm^{-1}) 3216, 1689, 1662; ^{1}H NMR (2:1 mixture of tautomers) (400 MHz, DMSO-*d*$_6$) δ 2.86 (1H, dd, *J*=14.8, 7.0 Hz), 3.16 (1H, br. dd, *J*=14.8, 5.3 Hz), 4.06 (1H, m), 4.34 - 4.50 (2H, m), 4.91 (2H, br. s), 6.61 (1H, t, *J*=7.4 Hz), 6.79 (1H, d, *J*=7.5 Hz), 6.90 - 7.39 (8H, m), 7.57 (6H, m), 9.62 (1H, br. s), 10.87 (1H, br. s); ^{13}C NMR (125 MHz, DMSO-*d*$_6$) δ 27.4, 43.7, 44.7, 48.6, 54.9, 61.1, 76.6, 106.8, 109.9, 111.2, 116.1, 116.3, 118.2, 118.6, 120.8, 123.3, 126.5, 126.6, 127.5, 127.8, 133.2, 136.0, 142.8, 143.1, 165.1, 171.7, 178.1, 184.9, 188.6 (tautomers); *m/z* (ES^+, %) 453 ($[M+H]^+$, 100); HRMS calcd. for $C_{26}H_{25}N_6O_2$ $[M+H]^+$ 453.2034, found: 453.2023.

(±)-*N*-(2-Aminophenyl)-4-(4-oxo-5-phenyl-4,5-dihydro-1*H*-imidazol-2-yl]amino)methyl)benzamide (19c). To a solution of the imidazole **18c** (0.135 g, 0.27 mmol) in dichloromethane (1.5 mL) was added trifluoroacetic acid (0.75 mL). The solution was stirred at 20 °C for 3 h, then evaporated and toluene (3 mL) added to the residue. The solvent was evaporated and the process repeated three more times. The residue was then dissolved in methanol (10 mL), and stirred with saturated aqueous sodium hydrogen carbonate (10 mL) for 30 min. The methanol was then evaporated and then water (10 mL) was added. The suspension was sonicated for 5 min. The precipitate was filtered, washed with water then with diethyl ether, and dried under vacuum to give **19c** as a cream solid (75 mg, 70%), mp 221-223 °C; ν_{max} (cm^{-1}) 3263, 3062, 1706, 1654; ^{1}H NMR (500 MHz, *DMSO-d*$_6$) (3:2 mixture of NH tautomers) δ 4.47 - 4.63 (2H, m), 4.85 (1H, s), 4.89 (2H, br. s), 6.59 (1H, t, *J*=7.1 Hz), 6.77 (1H, d, *J*=7.9 Hz), 6.96 (1H, t, *J*=7.1 Hz), 7.12 - 7.20 (2H, m), 7.20 - 7.30

(2H, m), 7.30 - 7.39 (2H, m), 7.41 - 7.49 (2H, m), 7.92 - 8.02 (2H, m), 8.12 - 8.24 (1H, m, major tautomer), 8.72 (1H, br. s, minor tautomer), 9.63 (1H, br. s); ^{13}C NMR (150 MHz, DMSO-d_6) δ 43.4, 43.8, 45.0, 45.1, 63.8, 116.2, 116.5, 123.2, 123.3, 125.6, 125.8, 126.5, 126.6, 126.7, 127.0, 127.1, 127.2, 127.4, 127.5, 127.5, 127.7, 128.0, 128.2, 128.3, 128.4, 128.6, 133.3, 133.4, 137.7, 141.6, 142.7, 143.0, 143.1, 165.2, 171.7, 186.7 (tautomers); *m/z* (ES, %) 400 ([M+H]$^+$, 100); HRMS calcd. for $C_{23}H_{22}N_5O_2$ [M+H]$^+$ 400.1768, found: 400.1755. Purity by HPLC was determined to be 92%.

***tert*-Butyl *N*-{2-[4-isothiocyanatomethyl)benzamido]phenyl}carbamate (22)**. A previously described protocol for preparing isothiocyanates was used.[51] To a stirred solution of amine **21b** (0.341 g, 1.0 mmol) in dichloromethane (10 mL) was added carbon disulfide (1 mL, 10.4 mmol, CAUTION: FLAMMABLE). The mixture was stirred at 20 °C for 1 min, then triethylamine (0.14 mL, 1.0 mmol) was added and stirring at 20 °C continued for a further 10 min. The mixture was cooled in an ice-bath and *p*-toluenesulfonyl chloride (0.191 g, 1.0 mmol) was added. The mixture was stirred at 0 °C for 10 min, then hydrochloric acid (10 mL, 1M) added. The mixture was extracted with dichloromethane (2 x 10 mL) and the combined organic layers washed with brine (20 mL), dried over Na_2SO_4 and evaporated. Purification of the residue by column chromatography on silica gel (1:3 ethyl acetate:hexane) gave **22** as a white solid (0.26 g, 68%), mp 152-153 °C; ν_{max} (cm^{-1}) 3277, 2086, 1693, 1656; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.54 (9H, s), 4.81 (2H, s), 6.87 (1H, s), 7.12 - 7.26 (3H, m), 7.43 (2H, d, *J*=8.3 Hz), 7.81 (1H, d, *J*=7.8 Hz), 8.01 (2H, d, *J*=8.3 Hz), 9.35 (1H, br. s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 28.4, 48.4, 81.6, 124.6, 125.9, 126.1, 126.1, 127.0, 128.2, 129.9, 130.9, 133.6, 134.5, 138.2, 154.8, 164.9; *m/z* (ES, %) 406 ([M+Na]$^+$, 93), 284 (20); HRMS calcd. for $C_{20}H_{21}N_3NaO_3S$ [M+Na]$^+$ 406.1196, found: 406.1198.

Synthesis of thioureas: general procedure A. To a solution of the amino alcohol (1 equiv.) in acetonitrile (4 mL/mmol) was added isothiocyanate **22** (1 equiv.) and the resulting

suspension was stirred at 20 °C for 16 h, then evaporated, and either purified or used directly, as described below.

***tert*-Butyl *N*-(2-{4-[({[(*1S*)-2-hydroxy-1-phenylethyl]carbamothioyl}amino)methyl] benzamido} phenyl)carbamate (23a)**. To a solution of *(S)*-2-phenylglycinol (0.165 g, 1.2 mmol) in acetonitrile (4 mL) was added **22** (0.384 g, 1.0 mmol). The resulting suspension was stirred at 20 °C for 16.5 h, then evaporated. The residue was purified by column chromatography on silica gel (3:2 then 2:1 ethyl acetate:hexane) to give **23a** as a white solid (0.464 g, 89%), mp 108-111 °C; ν_{max} (cm^{-1}) 3285, 1691, 1646; ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.46 (9H, s), 1.84 (1H, s), 3.45 (1H, br. s), 3.61 - 3.69 (1H, m), 3.77 (1H, m), 4.53 - 4.81 (2H, m), 5.19 (1H, br. s), 7.08 (2H, br. s), 7.11 - 7.18 (3H, m), 7.20 (2H, d, *J*=7.2 Hz), 7.24 - 7.32 (5H, m), 7.59 (1H, d, *J*=6.8 Hz), 7.67 (2H, d, *J*=7.5 Hz), 9.33 (1H, br. s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 28.4, 48.4, 60.2, 66.5, 81.7, 124.9, 125.9, 126.0, 126.5, 126.9, 127.4, 127.8, 128.3, 129.1, 130.4, 130.7, 132.7, 138.2, 142.3, 154.9, 166.2, 182.7; *m/z* (ES, %) 521 ($[M+H]^+$, 20), 543 (50); HRMS calcd. for $C_{28}H_{31}N_4O_4S$ $[M-H]^-$ 519.2071, found: 519.2094.

***tert*-Butyl *N*-(2-{4-[({[(*1R*)-2-hydroxy-1-phenylethyl]carbamothioyl}amino)methyl]benzamido} phenyl)carbamate (23b)**. (*R*)-2-phenylglycinol (0.112 g, 0.82 mmol) was reacted according to general procedure A and the product purified by column chromatography on silica gel (3:2 then 2:1 ethyl acetate:hexane) to give **23b** as a white solid (0.37 g, 96%), mp 107-109 °C; ν_{max} (cm^{-1}) 3278, 1692, 1648; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.49 (9H, s), 1.88 (1H, br. s), 3.49 (1H, br. s), 3.66 (1H, m), 3.79 (1H, d, *J*=8.8 Hz), 4.69 (2H, d, *J*=2.8 Hz), 5.20 (1H, br. s), 6.97 - 7.39 (11H, m), 7.59 (1H, d, *J*=6.8 Hz), 7.69 (2H, d, *J*=8.0 Hz), 9.35 (1H, br. s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 28.4, 48.4, 60.2, 66.5, 81.7, 124.9, 125.9, 126.0, 126.5, 126.9, 127.4, 127.8, 128.3, 129.1, 130.4, 130.7, 132.7, 138.2, 142.3, 154.9, 166.2, 182.7; *m/z* (ES, %) 521 ($[M+H]^+$, 80), 421 (100); HRMS calcd. for $C_{28}H_{33}N_4O_4S$ $[M+H]^+$ 521.2217, found: 521.2195.

(*S*)-*tert*-Butyl *N*-(2-(4-((3-(2-hydroxy-1-(4-hydroxyphenyl)ethyl)thioureido)methyl)benzamido)phenyl)carbamate (23c). An oven-dried three-necked flask was charged with a stirrer bar, sodium borohydride (0.756 g, 20.0 mmol) and anhydrous THF (20 mL) then cooled in an ice bath. To this stirring suspension was added a solution of iodine (2.54 g, 10.0 mmol) in anhydrous THF (10 mL) dropwise over 15 min (CAUTION: hydrogen gas evolved). After evolution of gas had ceased, 4-hydroxy-L-phenylglycine (1.67 g, 10.0 mmol) was added and the mixture was heated at reflux for 14 h. After cooling to 20 °C, methanol (~ 5 mL) was added cautiously until a clear solution formed; after stirring for 30 min the mixture was evaporated and the resulting white paste was dissolved in aqueous potassium hydroxide (20%, 20 mL). The mixture was stirred at 20 °C for 4 h then neutralised to pH 8 – 9. The product could not be extracted into an organic solvent and so the water was removed using a freeze drier. The resulting hygroscopic white solid was stirred with ethanol:chloroform (1:1, 200 mL) for 1 h then filtered and the filtrate evaporated. The residue was suspended in ethanol:chloroform (1:9, 200 mL), stirred for 1 h then filtered. The filtrate was dried over $MgSO_4$ and evaporated to give **(*S*)-4-(1-amino-2-hydroxyethyl)phenol** as a hygroscopic white solid (1.50 g, 98%) which was used without further purification.

To a suspension of the crude (*S*)-4-(1-amino-2-hydroxyethyl)phenol (0.536 g, 3.5 mmol) in acetonitrile (12 mL) was added **22** (0.384 g, 1.0 mmol). The resulting mixture was stirred vigorously at 25 °C for 17.5 h, then evaporated, and the residue partitioned between ethyl acetate (100 mL) and aqueous hydrochloric acid (1 M, 50 mL). The organic layer was washed with water (50 mL), brine (50 mL), dried over $MgSO_4$ and evaporated. Purification of the yellow solid by column chromatography on silica gel (3:1 ethyl acetate:hexane) gave **23c** as a white solid (0.359 g, 67%); mp 128-132 °C; ν_{max} (cm^{-1}) 3250, 1685, 1646; ^{1}H NMR (400 MHz, methanol-d_4) δ 1.51 (9H, s), 3.79 (2H, d, *J*=5.8 Hz), 4.62 - 4.82 (1H, m), 4.86 - 5.00 (1H, m), 5.27 (1H, br. s), 6.79 (2H, d, *J*=8.5 Hz), 7.12 - 7.29 (4H, m), 7.30 - 7.41 (2H,

m), 7.42 - 7.50 (1H, m), 7.58 - 7.66 (1H, m), 7.90 (2H, d, *J*=8.0 Hz); ^{13}C NMR (400 MHz, methanol-d_4) δ 28.7, 60.4, 66.4, 79.5, 81.8, 116.3, 125.6, 126.3, 127.2, 127.5, 128.5, 128.7, 129.3, 131.6, 133.0, 134.0, 144.9, 156.3, 158.0, 168.2, 183.9; *m/z* (ES, %) 535 ([M-H]$^-$, 100); HRMS calcd. for $C_{28}H_{31}N_4O_5S$ [M-H]$^-$ 535.2021, found: 535.2017.

***tert*-Butyl *N*-(2-{4-[({[(2S)-1-hydroxy-3-phenylpropan-2-yl]carbamothioyl}amino)methyl]benzamido}phenyl)carbamate (23d)**. To a suspension of **22** (0.24 g, 0.625 mmol) in toluene (5 mL) was added L-phenylalaninol (**25a**) (0.095 g, 0.625 mmol). The mixture was stirred at 20 °C under an atmosphere of nitrogen for 63 h. Evaporation afforded a residue to which dichloromethane (5 mL) was added, and the mixture then evaporated. The residue was purified by column chromatography on silica gel (2:1 ethyl acetate:hexane) to give **23d** as a white solid (0.29 g, 87%), mp 103-106 °C; ν_{max} (cm^{-1}) 3287, 1693, 1648; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.49 (9H, s), 1.81 (1H, br. s), 2.73 (1H, dd, *J*=13.7, 7.8 Hz), 2.85 (1H, dd, *J*=13.7, 6.0 Hz), 3.20 (1H, br. s), 3.40 (1H, m), 3.62 (1H, br. d, *J*=8.3 Hz), 4.36 - 4.84 (3H, m), 6.61 (1H, br. s), 7.06 - 7.35 (11H, m), 7.63 (1H, m), 7.69 (2H, d, *J*=8.0 Hz), 9.35 (1H, br. s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 28.4, 37.3, 48.0, 57.2, 64.2, 81.8, 124.9, 125.9, 126.1, 126.6, 126.8, 127.5 , 127.8, 128.8, 129.3, 130.4, 130.7, 132.6, 137.5, 142.5, 154.9, 166.3, 182.8; *m/z* (ES, %) 557 ([M+Na]$^+$, 36), 435 (100); HRMS calcd. for $C_{29}H_{34}N_4NaO_4S$ [M+Na]$^+$ 557.2193, found: 557.2192.

***tert*-Butyl *N*-(2-{4-[({[(2*R*)-1-hydroxy-3-phenylpropan-2-yl]carbamothioyl}amino)methyl]benzamido}phenyl)carbamate (23e)**. D-Phenylalaninol (0.126 g, 0.83 mmol) was reacted according to general procedure A and the product purified by column chromatography on silica gel (2:1 ethyl acetate:hexane) to give **23e** as a white solid (0.382 g, 94%), mp 101-103 °C; ν_{max} (cm^{-1}) 3279, 1692, 1648; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.49 (9H, s), 1.88 (1H, s), 2.72 (1H, dd, *J*=13.6, 8.0 Hz), 2.84 (1H, dd, *J*=13.6, 5.8 Hz), 3.37 (2H, br. s), 3.60 (1H, d, *J*=10.5 Hz), 4.28 - 5.00 (3H, m), 6.65 (1H, br. s), 7.06 -

7.37 (10H, m), 7.60 (1H, d, *J*=7.3 Hz), 7.67 (2H, d, *J*=7.8 Hz), 9.38 (1H, br. s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 28.4, 37.3, 48.0, 57.2, 64.1, 81.8, 124.9, 125.9, 126.1, 126.6, 126.8, 127.5, 127.8, 128.8, 129.3, 130.4, 130.7, 132.6, 137.5, 142.5, 154.9, 166.3, 182.8; *m/z* (ES, %) 557 ($[M+Na]^+$, 100); HRMS (CI) calcd. for $C_{29}H_{35}N_4O_4S$ $[M+H]^+$ 535.2374, found: 535.2378.

(*S*)-*tert*-Butyl *N*-(2-(4-((3-(2-hydroxy-2-phenylethyl)thioureido)methyl)benzamido)phenyl)carbamate (23f). (*S*)-(+)-2-Amino-1-phenylethanol (0.125 g, 0.91 mmol) was reacted according to general procedure A. The product was purified by column chromatography on silica gel (3:2 ethyl acetate:hexane), followed by column chromatography on silica gel (1:1 ethyl acetate:hexane) to give **23f** as a white solid (0.272 g, 57%), mp 150-154 °C; ν_{max} (cm^{-1}) 3284, 1682, 1661; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.48 (9H, s), 1.82 (1H, s), 3.48 (1H, br. s), 3.58 - 4.35 (2H, m), 4.69 (3H, br. s), 6.88 (1H, br. s), 7.10 - 7.20 (2H, m), 7.20 - 7.40 (8H, m), 7.53 - 7.62 (1H, m), 7.68 (2H, d, *J*=8.0 Hz), 9.41 (1H, br. s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 28.4, 48.3, 52.0, 74.0, 81.8, 124.9, 125.9, 126.0, 126.1, 126.6, 127.7, 127.8, 128.0, 128.6, 130.4, 130.6, 132.7, 141.6, 142.4, 154.9, 166.2, 183.7; *m/z* (EI, %) 519 ($[M-H]^-$, 35); HRMS (CI) calcd. for $C_{28}H_{33}N_4O_4S$ $[M+H]^+$ 521.2217, found: 521.2208.

(*R*)-*tert*-Butyl *N*-(2-(4-((3-(2-hydroxy-2-phenylethyl)thioureido)methyl)benzamido)phenyl)carbamate (23g). (*R*)-(-)-2-amino-1-phenylethanol (0.137 g, 1.0 mmol) was reacted according to general procedure A to give **23g** as a pale yellow solid (0.519 g, quant.) which was used without purification, mp 153-156 °C; ν_{max} (cm^{-1}) 3287, 1682, 1660; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.48 (9H, s), 1.60 (1H, s), 3.29 - 4.08 (3H, m), 4.57 - 5.01 (3H, m), 6.64 (1H, br. s), 7.06 (1H, br. s), 7.15 - 7.23 (2H, m), 7.24 - 7.36 (7H, m), 7.66 (1H, d, *J*=7.2 Hz), 7.73 (2H, d, *J*=7.2 Hz), 9.36 (1H, br. s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 28.4, 48.5, 52.0, 74.2, 81.9, 125.0, 125.9, 126.2, 126.5, 127.7,

127.9, 128.1, 128.7, 130.4, 130.7, 132.9, 141.6, 142.2, 154.9, 166.0, 183.8; *m/z* (EI, %) 519 ([M-H]$^-$, 50); HRMS (CI) calcd. for $C_{28}H_{33}N_4O_4S$ [M+H]$^+$ 521.2217, found: 521.2195.

***tert*-Butyl *N*-(2-(4-((3-((1*R*,2*S*)-2-hydroxy-1,2-diphenylethyl)thioureido)methyl)benzamido)phenyl)carbamate (23h)**. (1*S*,2*R*)-(+)-2-Amino-1,2-diphenyl ethanol (0.213 g, 1.0 mmol) was reacted according to general procedure A to give **23h** as a pale yellow solid (0.59 g, 99%) which was used without purification, mp 130-133 °C; ν_{max} (cm^{-1}) 3275, 1693, 1650; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.49 (9H, s), 3.21 (1H, br. s), 4.56 (1H, dd, *J*=15.4, 4.9 Hz), 4.76 (1H, d, *J*=15.0 Hz), 5.08 (1H, d, *J*=3.8 Hz), 5.51 (1H, br. s), 6.85 - 7.11 (10H, m), 7.12 - 7.30 (8H, m), 7.58 - 7.74 (3H, m), 9.35 (1H, br. s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 28.4, 48.3, 63.8, 76.7, 81.7, 124.8, 125.9, 126.1, 126.4, 126.6, 127.5, 127.8, 128.0, 128.2, 128.2, 128.3, 130.3, 130.5, 133.0, 136.4, 139.6, 142.1, 154.8, 166.1, 182.4; *m/z* (EI, %) 595 ([M-H]$^-$, 80); HRMS (CI) calcd. for $C_{34}H_{37}N_4O_4S$ [M+H]$^+$ 597.2530, found: 597.2516.

***tert*-Butyl *N*-(2-(4-((3-((1*S*,2*R*)-2-hydroxy-1,2-diphenylethyl)thioureido)methyl)benzamido)phenyl)carbamate (23i)**. (1*R*,2*S*)-(-)-2-Amino-1,2-diphenylethanol (0.213 g, 1.0 mmol) was reacted according to general procedure A to give **23i** as a pale yellow solid (0.59 g, quant.) which was used without purification, mp 135-140 °C; ν_{max} (cm^{-1}) 3273, 1693, 1651; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (9H, s), 3.21 (1H, br. s.), 4.54 (1H, dd, *J*=15.1, 4.1 Hz), 4.72 (1H, br. s.), 5.06 (1H, d, *J*=3.4 Hz), 5.51 (1H, br. s.), 6.87 - 7.10 (10H, m), 7.12 - 7.25 (8H, m), 7.58 - 7.70 (3H, m), 9.33 (1H, br. s.); ^{13}C NMR (150 MHz, $CDCl_3$) δ 28.4, 48.3, 63.8, 76.6, 81.7, 124.8, 125.9, 126.0, 126.4, 126.6, 127.5, 127.8, 128.0, 128.2, 128.2, 128.3, 130.3, 130.5, 133.0, 136.4, 139.6, 142.1, 154.8, 166.1, 182.4; *m/z* (EI, %) 595 ([M-H]$^-$, 60); HRMS (CI) calcd. for $C_{34}H_{37}N_4O_4S$ [M+H]$^+$ 597.2530, found: 597.2524.

Synthesis of 2-aminothiazolines: general procedure B. A solution of the thiourea (1 mmol) in conc. hydrochloric acid (20 mL, 10 M) was heated at 90 °C under reflux with stirring for the given length of time. Aqueous sodium hydroxide (5 M) was then added to pH ~ 9 and the mixture was extracted with ethyl acetate (3 x 50 mL). The combined organic layers were washed with brine (2 x 100 mL), dried over Na_2SO_4 and evaporated. The residue was purified as described below.

***N*-(2-Aminophenyl)-4-({[(4*S*)-4-phenyl-4,5-dihydro-1,3-thiazol-2-yl]amino}methyl)benzamide (24a)**. A solution of **23a** (0.460 g, 0.883 mmol) in conc. hydrochloric acid (15 mL, 10M) was heated at 90 °C under reflux with stirring for 75 min. Aqueous sodium hydroxide (5M) was then added to pH ~ 9; the mixture was extracted with ethyl acetate (100 mL, then 2 x 50 mL). The combined organic layers were washed with brine, dried Na_2SO_4, and evaporated. Purification of the residue by column chromatography on silica gel (7:1 then 8:1 ethyl acetate:hexane) gave a cream solid (257 mg) which was further purified by recrystallization from chloroform to give **24a** as a white solid (85 mg, 24%), mp 150-151 °C; $[\alpha]_D^{20}$ +77.3 (*c* 1.0, methanol); ν_{max} (cm^{-1}) 3190, 1622; ^{1}H NMR (400 MHz, $CDCl_3$) d 3.23 (1H, dd, *J*=10.6, 8.3 Hz), 3.70 (1H, dd, *J*=10.6, 7.7 Hz), 3.89 (2H, br. s), 4.54 (2H, s), 5.28 (1H, app. t, *J*=8.0 Hz), 5.66 (1H, br. s), 6.79 - 6.90 (2H, m), 7.11 (1H, td, *J*=7.7, 1.5 Hz), 7.22 - 7.36 (6H, m), 7.40 (2H, d, *J*=8.0 Hz), 7.84 (2H, d, *J*=8.0 Hz), 8.04 (1H, br. s); ^{13}C NMR (150 MHz, $CDCl_3$) d 42.5, 49.4, 74.4, 118.5, 119.9, 124.7, 125.4, 126.5, 127.3, 127.6, 127.7, 127.8, 128.6, 133.2, 140.9, 143.1, 143.3, 162.3, 165.8; *m/z* (CI, %) 403 ($[M+H]^+$, 21), 385 (100); HRMS calcd. for $C_{23}H_{23}N_4OS$ $[M+H]^+$ 403.1587, found: 403.1585. Anal. Calcd for $C_{23}H_{22}N_4OS$: C, 68.63; H, 5.51; N, 13.92. Found: C, 68.02; H, 5.38; N, 13.85.

***N*-(2-Aminophenyl)-4-({[(4*R*)-4-phenyl-4,5-dihydro-1,3-thiazol-2-yl]amino}methyl)benzamide (24b)**. The thiourea **23b** (0.345 g, 0.663 mmol) was reacted according to general procedure B for 45 min. Purification of the residue by column chromatography on silica gel (8:1 ethyl acetate:hexane) afforded a cream solid (149 mg) which was recrystallized from chloroform to give **24b** as a white solid (62 mg, 23%), mp 155-156 °C; $[\alpha]_D^{20}$ -62.0 (*c* 1.0, methanol); ν_{max} (cm^{-1}) 3281, 1612; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.23 (1H, dd, *J*=10.5, 8.3 Hz), 3.70 (1H, dd, *J*=10.5, 7.5 Hz), 3.90 (2H, br. s), 4.47 - 4.60 (2H, m), 5.28 (1H, t, *J*=7.9 Hz), 5.71 (1H, br. s), 6.78 - 6.91 (2H, m), 7.11 (1H, td, *J*=7.6, 1.0 Hz), 7.20 - 7.36 (6H, m), 7.40 (2H, d, *J*=8.0 Hz), 7.85 (2H, d, *J*=7.8 Hz), 8.05 (1H, br. s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 42.5, 49.3, 74.5, 118.5, 119.9, 124.7, 125.4, 126.5, 127.3, 127.6, 127.7, 127.8, 128.6, 133.2, 140.8, 143.1, 143.3, 162.2, 165.7; *m/z* (ES, %) 403 ([M+H]$^+$, 100); HRMS (CI) calcd. for $C_{23}H_{23}N_4OS$ [M+H]$^+$ 403.1587, found: 403.1570.

(*S*)-*N*-(2-Aminophenyl)-4-(((4-(4-hydroxyphenyl)-4,5-dihydrothiazol-2-yl)amino)methyl)benzamide (24c). To a solution of the thiourea **23c** (0.35 g, 0.65 mmol) in anhydrous THF (10 mL) was added triphenylphosphine (0.26 g, 0.98 mmol) and then a solution of diethyl azodicarboxylate (0.155 mL, 0.98 mmol) in anhydrous THF (5.0 mL) was added dropwise by syringe over 5 min. The solution was stirred for 75 min at 20 °C then HCl in diethyl ether (2M, 5.0 mL) was added and the mixture stirred for 5 h at 20 °C. The suspension was kept at 4 °C for 3 days, then filtered, and the hygroscopic white solid was partitioned between 5% methanol:dichloromethane (25 mL) and saturated aqueous sodium hydrogen carbonate (20 mL). The aqueous layer was extracted with 5% methanol:dichloromethane (2 x 25 mL). The combined organic layers were washed with brine (20 mL), dried over Na_2SO_4 and evaporated. Purification of the residue by column chromatography on silica gel (8% methanol:dichloromethane) gave **24c** as a white solid (0.128 g, 47%), mp 128-131 °C; $[\alpha]_D^{20}$ -63.8 (*c* 1.0, methanol); ν_{max} (cm^{-1}) 3250, 1607; ^{1}H

NMR (400 MHz, methanol-d_4) δ 3.15 (1H, dd, *J*=10.5, 7.5 Hz), 3.66 (1H, dd, *J*=10.7, 7.7 Hz), 4.53 (1H, m), 4.61 (1H, m), 5.21 (1H, t, *J*=7.4 Hz), 6.74 (2H, d, *J*=8.5 Hz), 6.80 (1H, t, *J*=7.5 Hz), 6.93 (1H, d, *J*=7.8 Hz), 7.03 - 7.16 (3H, m), 7.21 (1H, d, *J*=7.8 Hz), 7.50 (2H, d, *J*=8.2 Hz), 7.98 (2H, d, *J*=8.2 Hz); ^{13}C NMR (400 MHz, methanol-d_4) δ 42.8, 49.9, 54.9, 75.4, 116.1, 118.8, 119.8, 125.4, 127.7, 128.6, 128.7, 129.1, 134.2, 135.4, 143.7, 144.8, 157.9, 165.0, 168.7; *m/z* (CI, %) 419 ([M+H]$^+$, 85); HRMS calcd. for $C_{23}H_{23}N_4O_2S$ [M+H]$^+$ 419.1536, found: 419.1525.

***N*-(2-Aminophenyl)-4-(([(4S)-4-benzyl-4,5-dihydro-1,3-thiazol-2-yl]amino)methyl)benzamide (24d)**. A solution of **23d** (0.135 g, 0.252 mmol) in conc. hydrochloric acid (10M, 5 mL) was heated at 90 °C under reflux with stirring for 15 min. Aqueous sodium hydroxide (5 M) was then added to pH ~ 9, and the mixture extracted with ethyl acetate (3 x 30 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, and evaporated. Purification of the residue by column chromatography on silica gel (1% methanol in ethyl acetate) gave a cream solid (68 mg) which was further purified by recrystallization from chloroform to give **24d** as a white solid (10 mg, 10%), mp 144-145 °C; $[\alpha]_D^{20}$ -9.2 (*c* 1.0, methanol); ν_{max} (cm^{-1}) 3162, 1636, 1614; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.72 (1H, dd, *J*=13.4, 8.8 Hz), 3.00 - 3.10 (2H, m), 3.24 (1H, dd, *J*=10.6, 7.2 Hz), 3.94 (3H, br. s), 4.34 - 4.42 (1H, m), 4.47 (2H, m), 6.74 - 6.86 (2H, m), 7.07 (1H, app. t, *J*=7.5 Hz), 7.19 (2H, d, *J*=7.4 Hz), 7.22 (1H, d, *J*=7.1 Hz), 7.25 - 7.31 (3H, m), 7.34 (2H, d, *J*=7.7 Hz), 7.80 (2H, d, *J*=7.7 Hz), 8.13 (1H, br. s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 38.3, 41.3, 49.6, 71.9, 118.5, 119.8, 124.6, 125.5, 126.6, 127.3, 127.7, 127.8, 128.6, 129.4, 133.2, 138.8, 140.9, 143.2, 161.5, 165.8; *m/z* (ES, %) 417 ([M+H]$^+$, 100); HRMS calcd. for $C_{24}H_{25}N_4OS$ [M+H]$^+$ 417.1744, found: 417.1738.

***N*-(2-Aminophenyl)-4-(([(4*R*)-4-benzyl-4,5-dihydro-1,3-thiazol-2-yl]amino)methyl)benzamide (24e)**. The thiourea **23e** (0.355 g, 0.664 mmol) was reacted

according to general procedure B for 15 min. Purification of the residue by column chromatography on silica gel (3:2 then 2:1 ethyl acetate:hexane) gave a cream solid (117 mg) which was recrystallized from chloroform to give **24e** as a white solid (62 mg, 22%), mp 143-144 °C; $[\alpha]_D^{20}$ +9.7 (*c* 1.0, methanol); ν_{max} (cm^{-1}) 3164, 1662, 1615; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.75 (1H, dd, *J*=13.3, 8.8 Hz), 2.98 - 3.17 (2H, m), 3.28 (1H, dd, *J*=10.7, 7.2 Hz), 3.90 (2H, br. s), 4.38 - 4.47 (1H, m), 4.47 - 4.59 (2H, m), 5.16 (1H, br. s), 6.76 - 6.93 (2H, m), 7.11 (1H, td, *J*=7.7, 1.0 Hz), 7.17 - 7.35 (6H, m), 7.40 (2H, d, *J*=8.0 Hz), 7.85 (2H, d, *J*=8.0 Hz), 8.05 (1H, br. s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 38.5, 41.3, 49.6, 72.1, 118.5, 119.9, 124.7, 125.3, 126.5, 127.3, 127.7, 127.8, 128.6, 129.4, 133.2, 138.8, 140.8, 143.3, 161.0, 165.7; *m/z* (ES, %) 417 ($[M+H]^+$, 100); HRMS (CI) calcd. for $C_{24}H_{25}N_4OS$ $[M+H]^+$ 417.1744, found: 417.1723.

(*R*)-*N*-(2-Aminophenyl)-4-(((5-phenyl-4,5-dihydrothiazol-2-yl)amino)methyl)benzamide (24f). The thiourea **23f** (0.25 g, 0.48 mmol) was reacted according to general procedure B for 45 min. The residue was purified by column chromatography on silica gel (5% methanol:dichloromethane) and then recrystallized from aqueous ethanol to give **24f** as a white solid (41 mg, 21%), mp 161-163 °C; $[\alpha]_D^{20}$ +38.4 (*c* 1.0, methanol); ν_{max} (cm^{-1}) 3250, 1610; ^{1}H NMR (400 MHz, methanol-d_4) δ 4.00 (1H, dd, *J*=13.2, 5.3 Hz), 4.27 (1H, dd, *J*=13.2, 7.6 Hz), 4.57 (2H, s), 5.04 (1H, dd, *J*=7.6, 5.3 Hz), 6.80 (1H, td, *J*=7.7, 1.3 Hz), 6.93 (1H, dd, *J*=8.0, 1.3 Hz), 7.10 (1H, m), 7.21 (1H, dd, *J*=7.9, 1.1 Hz), 7.27 (1H, m), 7.30 - 7.36 (2H, m), 7.37 - 7.42 (2H, m), 7.51 (2H, d, *J*=8.2 Hz), 7.99 (2H, d, *J*=8.2 Hz); ^{13}C NMR (400 MHz, methanol-d_4) δ 48.5, 56.3, 68.3, 118.8, 119.7, 125.4, 127.7, 128.1, 128.4, 128.5, 128.7, 129.0, 129.7, 134.3, 143.1, 143.8, 144.7, 164.4, 168.7; *m/z* (CI, %) 403 ($[M+H]^+$, 100); HRMS calcd. for $C_{23}H_{23}N_4OS$ $[M+H]^+$, 403.1587, found: 403.1566.

(*S*)-*N*-(2-Aminophenyl)-4-(((5-phenyl-4,5-dihydrothiazol-2-yl)amino)methyl)benzamide (24g). The thiourea **23g** (0.50 g, 0.96 mmol) was reacted according to general procedure B

for 30 min. Purification by column chromatography on silica gel (7% methanol:dichloromethane) gave a residue that was twice recrystallized from ethanol and water to give **24g** as a white solid (0.19 g, 49%), mp 159-164 °C; $[\alpha]_D^{20}$ -35.3 (*c* 1.0, methanol); ν_{max} (cm^{-1}) 3328, 1633, 1620; ^{1}H NMR (400 MHz, methanol-d_4) δ 3.98 (1H, dd, *J*=13.2, 5.3 Hz), 4.25 (1H, dd, *J*=13.2, 7.7 Hz), 4.55 (2H, br. s), 5.03 (1H, m), 6.78 (1H, t, *J*=7.5 Hz), 6.91 (1H, d, *J*=7.9 Hz), 7.08 (1H, t, *J*=7.5 Hz), 7.19 (1H, d, *J*=7.5 Hz), 7.25 (1H, m), 7.28 - 7.40 (4H, m), 7.48 (2H, d, *J*=7.9 Hz), 7.97 (2H, d, *J*=7.9 Hz); ^{13}C NMR (150 MHz, methanol-d_4) δ 48.6, 56.3, 68.0, 118.9, 119.9, 125.5, 127.8, 128.3, 128.6, 128.7, 128.9, 129.2, 129.9, 134.5, 143.0, 143.9, 144.6, 164.9, 168.8; *m/z* (ES, %) 401 ([M-H]$^-$, 100); HRMS calcd. for $C_{23}H_{21}N_4OS$ [M-H]$^-$ 401.1442, found: 401.1446.

***N*-(2-Aminophenyl)-4-((((4*R*,5*R*)-4,5-diphenyl-4,5-dihydrothiazol-2-yl)amino)methyl)benzamide (24h)**. The thiourea **23h** (0.570 g, 0.96 mmol) was reacted according to general procedure B for 10 min. The resulting off-white solid was recrystallized from ethyl acetate and hexane to give **24h** as a white solid (0.165 g, 36%), mp 166-168 °C; $[\alpha]_D^{20}$ +80.3 (*c* 1.0, methanol); ν_{max} (cm^{-1}) 3272, 1610; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.87 (2H, br. s), 4.49 (2H, s), 4.75 (1H, d, *J*=6.7 Hz), 5.24 (1H, d, *J*=6.7 Hz), 6.77 - 6.85 (2H, m), 7.08 (1H, t, *J*=7.7 Hz), 7.10 - 7.17 (2H, m), 7.18 - 7.40 (11H, m), 7.80 (2H, d, *J*=7.5 Hz), 8.20 (1H, br. s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 49.2, 64.0, 82.7, 118.4, 119.8, 124.7, 125.5, 126.5, 127.3, 127.7, 127.7, 127.8, 127.9, 128.0, 128.5, 128.9, 133.2, 140.2, 140.9, 142.1, 143.3, 161.3, 165.9; *m/z* (ES, %) 477 ([M-H]$^-$, 100); HRMS calcd. for $C_{29}H_{25}N_4OS$ [M-H]$^-$ 477.1755, found: 477.1770.

***N*-(2-Aminophenyl)-4-((((4*S*,5*S*)-4,5-diphenyl-4,5-dihydrothiazol-2-yl)amino)methyl)benzamide (24i)**. The thiourea **23i** (0.572 g, 0.96 mmol) was reacted according to general procedure B for 10 min. The resulting off-white solid was recrystallized twice from ethyl acetate and hexane to give **24i** as a white solid (0.151 g, 33%), mp 162-164

°C; $[\alpha]_D^{20}$ -79.3 (*c* 1.0, methanol); ν_{max} (cm^{-1}) 3239, 1610; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.31 (2H, br. s), 4.50 (2H, s), 4.75 (1H, d, *J*=6.8 Hz), 5.25 (1H, d, *J*=6.8 Hz), 6.77 - 6.85 (2H, m), 7.08 (1H, t, *J*=7.7 Hz), 7.10 - 7.16 (2H, m), 7.20 - 7.34 (9H, m), 7.36 (2H, d, *J*=7.9 Hz), 7.82 (2H, d, *J*=7.9 Hz), 8.17 (1H, br. s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 49.3, 64.0, 82.6, 118.5, 119.8, 124.6, 125.5, 126.5, 127.4, 127.8, 127.8, 127.9, 128.1, 128.6, 128.9, 133.2, 140.1, 140.9, 142.0, 143.2, 161.5, 165.8; *m/z* (ES, %) 477 ($[M-H]^-$, 100); HRMS calcd. for $C_{29}H_{25}N_4OS$ $[M-H]^-$ 477.1755, found: 477.1761.

(2*S*)-2-Amino-3-(1*H*-indol-3-yl)propan-1-ol (25b).

To an oven-dried three-neck flask equipped with a magnetic stirrer and a reflux condenser was added sodium borohydride (0.756 g, 20.0 mmol) and L-tryptophan (2.04 g, 10.0 mmol), followed by dry THF (20 mL). A dropping funnel was attached to the flask, and the mixture was then cooled to 0 °C in an ice bath. A solution of iodine (2.54 g, 10.0 mmol) in THF (10 ml) was added dropwise over 20 min. When the vigorous evolution of gas had ceased, the mixture was heated at reflux for 17 h. After cooling to 20 °C, methanol was added slowly until solution became clear, and the mixture was then stirred for 30 min. Evaporation gave a white paste which was dissolved in aqueous potassium hydroxide (20%, 20 mL), stirred for 20 h, then adjusted to pH 11 with hydrochloric acid (2 M). The solution was extracted with dichloromethane (3 × 20 mL) and then with a mixture of 1:4 ethanol:chloroform (4 × 25 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and evaporated to give **25b** as a yellow oil (1.38 g, 73%); ν_{max} (cm^{-1}) 3401, 3251, 3049; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.73 (3H, br. s), 2.74 (1H, dd, *J*=14.4, 8.4 Hz), 2.97 (1H, dd, *J*=14.4, 5.1 Hz), 3.29 (1H, m), 3.46 (1H, dd, *J*=10.5, 7.2 Hz), 3.72 (1H, dd, *J*=10.5, 4.0 Hz), 7.08 (1H, d, *J*=2.0 Hz), 7.15 (1H, t, *J*=7.3 Hz), 7.24 (1H, t, *J*=7.2 Hz), 7.40 (1H, d, *J*=8.0 Hz), 7.64 (1H, d, *J*=8.0 Hz), 8.09 (1H, br. s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 30.5, 53.0, 66.9, 111.3, 112.8, 119.0, 119.6, 122.3, 122.7, 127.7, 136.5.

(4*S*)-4-(1*H*-Indol-3-ylmethyl)-1,3-thiazolidine-2-thione (26b). To a mixture of amino alcohol **25b** (0.34 g, 1.79 mmol) in aqueous potassium hydroxide (9 mL, 1 M) was added carbon disulfide (0.54 mL, 8.95 mmol, CAUTION: FLAMMABLE). The mixture was stirred at reflux for 18 h then cooled to 20 °C and extracted with dichloromethane (3 x 10 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica gel (1:1 ethyl acetate:hexane) to give **26b** as a white solid (0.161 g, 36%), mp 73-75 °C; ν_{max} (cm^{-1}) 3310, 3146; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.12 (1H, dd, *J*=14.3, 6.1 Hz), 3.16 (1H, dd, *J*=14.3, 7.7 Hz), 3.35 (1H, dd, *J*=11.1, 6.6 Hz), 3.61 (1H, dd, *J*=11.1, 7.8 Hz), 4.56 (1H, m), 7.08 (1H, d, *J*=2.4 Hz), 7.16 (1H, td, *J*=7.4, 0.9 Hz), 7.24 (1H, td, *J*=7.7, 0.9 Hz), 7.41 (1H, d, *J*=8.2 Hz), 7.52 - 7.61 (2H, m), 8.22 (1H, br. s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 30.1, 38.5, 64.4, 110.3, 111.7, 118.4, 120.1, 122.7, 123.1, 126.9, 136.4, 200.9; *m/z* (EI, %) 248 (M^+, 100); HRMS calcd. for $C_{12}H_{12}N_2S_2$ M^+ 248.0436, found: 248.0438.

***tert*-Butyl *N*-(2-([4-(chloromethyl)benzoyl]amino)phenyl)carbamate (28a)**. To a solution of *tert*-butyl (2-aminophenyl)carbamate)[42] (2.55 g, 12.2 mmol) in dichloromethane (50 mL) cooled to -10 °C, was added triethylamine (1.86 mL, 13.4 mmol) followed by a solution of 4-(chloromethyl)benzoyl chloride (**27**) (2.54 g, 13.4 mmol) in dichloromethane (20 mL), added dropwise. The stirred solution was allowed to warm to 20 °C and then stirred for 1 h. Evaporation gave a solid that was dissolved in dichloromethane (200 mL) and the solution washed successively with saturated aqueous ammonium chloride (200 mL), saturated aqueous sodium hydrogen carbonate (200 mL) and brine, then dried over $MgSO_4$, filtered and evaporated to give **28a** as a white solid (4.02 g, 91%), mp 172-173 °C; ν_{max} (cm^{-1}) 3277, 1682, 1655, 1599; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.51 (9H, s), 4.63 (2H, s), 6.83 (1H, br. s), 7.14 (1H, t, *J*=7.6 Hz), 7.21 (2H, t, *J*=7.6 Hz), 7.48 (2H, d, *J*=8.2 Hz), 7.77 (1H, d, *J*=7.9 Hz), 7.96 (2H, d, *J*=8.2 Hz), 9.27 (1H, br. s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 28.4, 45.5, 81.6, 124.6, 125.9, 126.1, 126.1, 127.9, 128.8, 129.9, 130.9, 134.3, 141.3, 154.8, 165.1; *m/z*

(ES, %) 383 ([M+Na]$^+$, 90); HRMS calcd. for $C_{19}H_{21}ClN_2NaO_3$ [M+Na]$^+$ 383.1133, found: 383.1136.

***N*-(2-Aminophenyl)-4-(chloromethyl)benzamide (28b)**. To a solution of the benzyl chloride **28a** (0.361 g, 1.0 mmol) in dichloromethane (2.5 mL) was added trifluoroacetic acid (0.5 mL). The solution was stirred at 20 °C for 2.5 h, then evaporated and toluene (3 mL) added to the residue. The solvent was evaporated and the process repeated. The resulting yellow solid was triturated with diethyl ether three times and the remaining white solid was partitioned between ethyl acetate (30 mL) and saturated aqueous sodium hydrogen carbonate (20 mL). The organic layer was washed with brine, dried over Na_2SO_4, filtered and evaporated to give **28b** as a white solid (0.19 g, 73%), mp 132-133 °C; ν_{max} (cm^{-1}) 3370, 3279, 1645; ^{1}H NMR (500 MHz, *DMSO-d*$_6$) δ 4.83 (2H, s), 4.90 (2H, br. s), 6.59 (1H, t, *J*=7.4 Hz), 6.77 (1H, d, *J*=7.3 Hz), 6.97 (1H, t, *J*=7.6 Hz), 7.16 (1H, d, *J*=7.6 Hz), 7.56 (2H, d, *J*=7.9 Hz), 7.97 (2H, d, *J*=7.9 Hz), 9.67 (1H, s); ^{13}C NMR (125 MHz, *DMSO-d*$_6$) δ 45.5, 116.1, 116.2, 123.2, 126.6, 126.7, 128.1, 128.7, 134.5, 140.8, 143.1, 164.9; *m/z* (EI, %) 260 (M$^+$, 11), 153 (30); HRMS calcd. for $C_{14}H_{13}ClN_2O$ M$^+$ 260.0711, found: 260.0708.

***tert*-Butyl *N*-(2-{[4-({[(4S)-4-benzyl-4,5-dihydro-1,3-thiazol-2-yl]sulfanyl}methyl)benzene]amido}phenyl)carbamate (29)**. To a solution of the benzyl chloride **28a** (0.92 g, 2.55 mmol) in acetone (15 mL) was added sodium iodide (0.765 g, 5.10 mmol). The round-bottomed flask, oil bath and condenser were wrapped in aluminum foil to exclude light and the mixture was stirred at reflux for 2 h. When the mixture had cooled to 20 °C a solution of thiazolidine-2-thione **26a** (0.485 g, 2.32 mmol) in dichloromethane (10 mL) was added followed by potassium carbonate (0.481 g, 3.48 mmol). The resulting mixture was stirred at 20 °C for 28 h (still with exclusion of light). Evaporation afforded as solid that was shaken with ethyl acetate. Filtration and evaporation of the filtrate gave a residue that was purified by column chromatography (dichloromethane then 5% ethyl

acetate in dichloromethane) to give **29** as a white solid (0.75 g, 61%), mp 67-69 °C; ν_{max} (cm^{-1}) 3275, 1693, 1656, 1600; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.50 (9H, s), 2.77 (1H, dd, *J*=13.6, 8.5 Hz), 3.12 - 3.19 (2H, m), 3.35 (1H, dd, *J*=10.7, 7.9 Hz), 4.36 (1H, d, *J*=13.6 Hz), 4.43 (1H, d, *J*=13.6 Hz), 4.70 (1H, m), 6.88 (1H, br. s), 7.10 - 7.28 (6H, m), 7.32 (2H, t, *J*=7.3 Hz), 7.45 (2H, d, *J*=8.2 Hz), 7.74 (1H, d, *J*=7.9 Hz), 7.90 (2H, d, *J*=8.2 Hz), 9.18 (1H, br. s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 28.4, 36.6, 39.4, 40.2, 77.5, 81.5, 124.6, 125.9, 126.0, 126.1, 126.7, 127.7, 128.6, 129.4, 129.4, 130.1, 130.9, 133.4, 138.3, 141.2, 154.7, 165.3; *m/z* (EI, %) 533 (M^+, 35); HRMS calcd. for $C_{29}H_{31}N_3O_3S_2$ M^+ 533.1801, found: 533.1804.

***N*-(2-Aminophenyl)-4-(([(4S)-4-benzyl-4,5-dihydro-1,3-thiazol-2-yl]sulfanyl)methyl)benzamide (30a)**. To a solution of **29** (0.30 g, 0.562 mmol) in dichloromethane (2.5 mL) was added trifluoroacetic acid (0.5 mL). The solution was stirred at 20 °C for 2.5 h, then evaporated and toluene (3 mL) added to the residue. The solvent was evaporated and the process repeated three more times. The resulting solid was dissolved in dichloromethane (15 mL) and the solution was washed with saturated aqueous sodium hydrogen carbonate (15 mL). Additionally, the aqueous layer was extracted with dichloromethane (10 mL), and the combined organic layers were dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica gel (2:3 ethyl acetate:hexane) and the purified solid was recrystallized from dichloromethane and hexane to give **30a** as a white solid (0.135 g, 55%), mp 135-137 °C; $[\alpha]_D^{20}$ -20.6 (*c* 1.0, methanol); ν_{max} (cm^{-1}) 3274, 1635, 1603; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.79 (1H, dd, *J*=13.6, 8.8 Hz), 3.11-3.23 (2H, m), 3.36 (1H, dd, *J*=10.9, 7.9 Hz), 3.84 (2H, br. s), 4.37 (1H, d, *J*=13.6 Hz), 4.42 (1H, d, *J*=13.6 Hz), 4.71 (1H, m, CH), 6.78 -6.91 (2H, m), 7.09 (1H, t, *J*=7.4 Hz), 7.19 -7.39 (6H, m), 7.46 (2H, d, *J*=7.8 Hz), 7.83 (2H, d, *J*=7.5 Hz), 8.06 (1H, br. s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 36.5, 39.5, 40.2, 77.8, 118.6, 120.0, 124.7, 125.4, 126.7, 127.4, 127.7, 128.6, 129.4, 129.5, 133.2, 138.5, 140.5, 141.5, 163.9, 165.6; *m/z* (ES, %) 434 ($[M+H]^+$, 100); HRMS calcd. for $C_{24}H_{24}N_3OS_2$ $[M+H]^+$ 434.1355, found: 434.1360.

_N_-(2-Aminophenyl)-4-({[(4_S_)-4-(1_H_-indol-3-ylmethyl)-4,5-dihydro-1,3-thiazol-2-yl]sulfanyl}methyl)benzamide (30b). To a solution of **26b** (0.229 g, 0.920 mmol) and **28b** (0.2 g, 0.767 mmol) in acetone (4 mL) was added potassium carbonate (0.159 g, 1.15 mmol). The mixture was stirred at reflux for 16.5 h. After allowing to cool, the solvent was evaporated and the residue partitioned between water (25 mL) and ethyl acetate (25 mL). The aqueous layer was extracted with ethyl acetate (10 mL) and the combined organic layers were washed with brine (10 mL), dried over $MgSO_4$, and evaporated to give a tan solid. Purification by column chromatography on silica gel (1:1 ethyl acetate:hexane) gave **30b** as a white solid (0.31 g, 86%), mp 138-140 °C; $[\alpha]_D^{20}$ 16.5 (*c* 1.0, methanol); ν_{max} (cm^{-1}) 3379, 3324, 3209, 1638; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.96 (1H, dd, *J*=14.6, 8.3 Hz), 3.13 - 3.27 (2H, m), 3.34 (1H, dd, *J*=10.8, 8.0 Hz), 3.81 (2H, br. s), 4.30 (1H, d, *J*=13.6 Hz), 4.40 (1H, d, *J*=13.6 Hz), 4.82 (1H, app. quintet, *J*=7.1 Hz), 6.76 - 6.87 (2H, m), 6.91 (1H, s), 7.03 - 7.15 (2H, m), 7.18 (1H, t, *J*=7.5 Hz), 7.26 (1H, d, *J*=7.9 Hz), 7.33 (1H, d, *J*=8.0 Hz), 7.39 (2H, d, *J*=7.9 Hz), 7.64 (1H, d, *J*=7.9 Hz), 7.72 (2H, d, *J*=8.0 Hz), 7.98 (1H, br. s), 8.36 (1H, br. s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 29.8, 36.4, 39.8, 77.1, 111.4, 112.4, 118.4, 118.9, 119.4, 119.8, 122.1, 122.9, 124.4, 125.6, 127.5, 127.6, 127.7, 129.5, 133.1, 136.3, 141.0, 141.6, 163.5, 165.9; *m/z* (EI, %) 472 (M^+, 10), 208 (100), 130 (100); HRMS calcd. for $C_{26}H_{24}N_4OS_2$ M^+ 472.1386, found: 472.1393.

_N_-(2-Aminophenyl)-4-({[(4_S_)-4-phenyl-4,5-dihydro-1,3-thiazol-2-yl]sulfanyl}methyl)benzamide (30c). To a solution of **26c** (83 mg, 0.425 mmol) and **28b** (110 mg, 0.422 mmol) in acetone (4 mL) was added potassium carbonate (88 mg, 0.638 mmol) and the mixture then stirred at reflux for 17 h. After the mixture had cooled it was evaporated and the residue partitioned between water (15 mL) and ethyl acetate (15 mL). The aqueous layer was extracted with ethyl acetate (10 mL) and the combined organic layers were washed with brine (10 mL), dried over Na_2SO_4, and evaporated to give a tan solid.

Purification by column chromatography on silica gel (1:7 ethyl acetate:dichloromethane) afforded **30c** (34 mg) and an impure fraction that was again purified by column chromatography on silica gel (2:3 ethyl acetate:hexane) to give **30c** as a white solid (total 115 mg, 62%), mp 113-114 °C; $[\alpha]_D^{20}$ +75.5 (*c* 1.0, methanol); ν_{max} (cm^{-1}) 3276, 1647, 1614; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.30 (1H, dd, *J*=10.9, 9.0 Hz), 3.63 (2H, br. s.), 3.78 (1H, dd, *J*=10.9, 8.3 Hz), 4.40 (1H, d, *J*=13.7 Hz), 4.46 (1H, d, *J*=13.7 Hz), 5.49 (1H, t, *J*=8.6 Hz), 6.74 - 6.85 (2H, m), 7.08 (1H, t, *J*=7.3 Hz), 7.24 - 7.32 (4H, m), 7.32 - 7.38 (2H, m), 7.46 (2H, d, *J*=7.9 Hz), 7.79 (2H, d, *J*=7.9 Hz), 8.01 (1H, br. s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 36.5, 43.1, 79.6, 118.5, 119.9, 124.6, 125.4, 126.6, 127.4, 127.7, 127.9, 128.7, 129.6, 133.3, 140.8, 141.5, 141.6, 165.2, 165.6; *m/z* (EI^+, %) 419 (M^+, 100); HRMS calcd. for $C_{23}H_{21}N_3OS_2$ M^+, 419.1121, found: 419.1125.

Docking. The PDB code: 4A69 file was first processed using AutoDockTools 1.5.4; chains B-D, acetate (ACT), glycerol (GOL), inositol phosphate (IOP), potassium ions and water molecules were removed. Polar hydrogen atoms and Kollman charges were added, and the charge on the Zn was set to +2.0. Compound **24a** was drawn in ChemBio3D Ultra 2012 and the structure was energy minimised using a MM2 minimize energy calculation, to a minimum RMS gradient of 0.01. Docking was performed with AutoDock Vina (1.1.2) using an exhaustiveness of 1000 and a grid box 18 x 18 x 16 Å, centered at the active site (34.60 (x), 58.97 (y), 27.32 (z)). The receptor was treated as rigid, and the ligand was treated as flexible. The maximum energy difference between the best and the worst binding modes displayed was set to be 2.5 kcal/mol. A maximum of 50 docking modes were output for further analysis. The image representing the best pose was prepared using PyMOL.

***In Vitro* HDAC Inhibition Assay**. Recombinant HDAC isoforms were used. HDAC2 (1-488, His-tag), HDAC3-NCoR1, HDAC6 (His-tag), Fluor-de-Lys SIRT1, Fluor-de-Lys developer II and TSA were purchased from Enzo Life Sciences, and porcine pancreatic

trypsin (type IX-S) from Sigma. HDAC1 (C-Flag) was purchased from Tebu Bio. HDAC8 was expressed in *Escherichia coli* BL21 cells and purified with a His-tag, which was removed prior to final purification by size exclusion chromatography. Boc-Lys(Ac)-7-amino-4-methylcoumarin (MAL) was synthesised according to the literature.[52] The *in vitro* HDAC assay used was based on a homogeneous fluorogenic HDAC assay.[53] Inhibitor solutions were prepared by serial dilution of a 5 mM DMSO stock solution with assay buffer. In a 96-well white NBS microplate purified recombinant HDAC enzyme (HDAC1, 120 ng; HDAC2, 85 ng; HDAC3-NCoR1, 25 ng; HDAC6, 280 ng; HDAC8, 400 ng) in 20 μL of assay buffer comprising 50 mM Tris/Cl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, 1 mg/ml BSA. To each well was added inhibitor solution (10 μL). After incubation at 20-23 °C for the appropriate time (HDAC1: 1 h; HDAC2, HDAC3 and HDAC6: 3 h; HDAC8: 15 min) the fluorescent substrate (for HDAC1, HDAC2, HDAC3-NCoR1, and HDAC8: MAL, (20 μL) to give concentrations of 60, 80, 14 and 200 mM; and for HDAC6, Fluor-de-Lys SIRT1 to give a concentration of 13 mM) was added and the plate was incubated for 60 min at 37 °C. A developer solution (for HDAC1, HDAC2, HDAC3-NCoR1, and HDAC8: 50 μL of 10 mg/mL trypsin and 2 mM TSA in assay buffer; for HDAC6 Fluor-de-Lys developer II and 2 mM TSA in 50 mM Tris/Cl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$) was then added and the plate incubated for 30 min at 20 °C before the fluorescence was measured on a BMG FLUOstar Optima plate reader with excitation at 380 nm and emission at 460 nm.

Determination of IC_{50}values. OriginPro 8 was used to determine IC_{50} values from the sigmoidal line fitted to a graph of log[concentration] against the average percentage inhibition from two independent experiments, with at least 6 different concentrations. The SEM is the calculated standard error in the IC_{50} value of the fitted line.

Calculation and measurement of physicochemical properties. cLog P values were calculated using Daylight software v4.9:

http://www.daylight.com/dayhtml/doc/clogp/index.html. (Accessed 27th June, 2013). Polar surface area (tpsa) was calculated according to a literature method.[36] Chrom log D and Chrom log P values[35] and cell permeability (artificial membrane permeability)[38] were measured using procedures previously described. Solubility was measured using chemiluminescent nitrogen detection.[37] Cytochrome P450 inhibition data were obtained using an assay kit (Invitrogen) according to the protocol described at http://www.invitrogen.com/site/us/en/home/Products-and-Services/Applications/Drug-Discovery/Target-Based-ADME-Tox-Assays/p450-assays/P450-Metabolism-Assays.html, accessed 27th June, 2013), and using substrates sourced in house. The inhibition of isoform 3A4 was determined separately, using two different substrates, Vivid® Red and Vivid® Green, sourced from Invitrogen.

Cell growth inhibition assays. A549, HCT116 and MCF-7 cells were maintained in RPMI 1640 (Invitrogen) supplemented with 10% (v/v) FCS, 5 mM glutamine (Invitrogen), 1 x non-essential amino acids (Invitrogen) and 1 x sodium pyruvate (Invitrogen). DU145 cells were grown in similarly supplemented DMEM media (Sigma). Cells were detached using trypsin EDTA solution (Invitrogen), then 90 μL of cells (10,000 cells per well) were plated into 96-well plates (Greiner Microclear) in growth medium containing penicillin-streptomycin solution (Invitrogen) and cultured overnight at 37 °C in an incubator.

Immediately prior to addition of compounds, one plate of each cell type was removed from the incubator and equilibrated at 20 °C for 1 h before assaying for ATP content by the addition of an equal volume of Cell Titre Glo solution (Promega). The resulting luminescence was measured using a Perkin Elmer Envision 2104 Multilabel reader in order to determine the zero time-point value.

Test compounds (10 μL, in quadruplicate) were added to the cell plates. Final concentrations of compounds in the range 1.5 nM - 50 μM were used in assay medium

containing 0.5% DMSO. Plates were cultured for a further 72 h at 37 °C, after which cellular ATP levels were assayed, as described above.

For each cell line, 72 h Cell Titre Glo data were expressed as the percentage of the zero time-point value. IC_{50} values were generated by nonlinear regression of the data from three independent experiments using GraphPad Prism (v5).

Histone H3 acetylation assay and analysis of cell cycle phases and apoptosis.

Cell culture and application of compounds. Human HeLa (epithelial adenocarcinoma) and human Jurkat (T cell leukemia) cell lines were cultured in a humidified incubator at 37 °C and 5% CO_2. HeLa cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% v/v fetal bovine serum (Life Technologies) and L-glutamine-penicillin-streptomycin solution (Sigma). The Jurkat cell line was maintained in RPMI 1640 medium supplemented 10% v/v fetal bovine serum (Life Technologies) and L-glutamine-penicillin-streptomycin solution (Sigma). For both cell lines, cell density was adjusted to 1 x 10^6 cells in culture medium (10 mL) in 10 cm dishes for HeLa cells, or 25 mL flasks for Jurkat cells, prior to compound addition. Using a 10 mM stock solution in DMSO, all HDAC inhibitors were dissolved sequentially at either 1 μM or 10 μM final concentration in fresh culture medium (500 μL), added dropwise to the adherent HeLa plates and Jurkat cell suspensions, mixed gently and left to incubate for 24 h. An equivalent volume of DMSO was added to control cell cultures.

Cell cycle analysis by flow cytometry. The method of Lea *et al.*[54] was used. Briefly, samples of 1 x 10^5 cells were taken following 24 h incubation with the compound and fixed in ethanol (400 μL, 70% v/v) at -20 °C. Following centrifugation at 400 g for 8 min, the pellet was resuspended in FITC/PI cell cycle stain (400 μL) consisting of PI (40 μg, to stain for DNA content), FITC (5 μg/mL, protein staining) and RNAse1 (1 μg/mL). The mixture

was incubated at 37 °C for 30 min, then analysed on a FACSCanto (Beckton Dickenson) flow cytometer. The FlowJo v10.0.6 program (Tree Star Inc.) was used to plot PI (FL2-A) against FITC (FL-1A) (x- and y-axes respectively) to determine the percentage of cells in each cell cycle phase. Plotting (FL2-W) against (FL2-H) created a doublet discriminator gate to exclude doublets from the analysis. The percentage of cells in each cycle phase was calculated by applying gates manually around cell populations for the cell cycle phases indicated, as well as cell with sub-G_1 DNA content.

Immunoblotting. HeLa and Jurkat cell pellets, containing 1 x 10^6 cells were resuspended in 1 x SDS loading buffer (50 μL), vortexed and heated for 5 min at 100 °C to denature the proteins. Samples were diluted 1:50 in SDS loading buffer and resolved by electrophoresis through polyacrylamide (4-12% w/v), Bis-Tris gels (Novex, Life Technologies). Proteins in each gel were transferred to a Hybond-C extra nitrocellulose membrane by electrophoresis at 25 V, 160 mA for 75 min. The nitrocellulose membrane was blocked with dried skimmed milk (Marvel, 5% w/v) diluted in PBS-Tween 20 (0.05% v/v) for 1 h. Following a PBS-Tween-20 rinse, the membrane was immunoblotted with primary antibodies against histone H3 (DIH2), acetylated H3 (Lys 9) (C5BII, Cell Signaling Technologies) and PARP (Sc-8007). These were detected with anti-mouse or anti-rabbit horse radish peroxidase conjugated secondary antibodies (Dako or Santa Cruz), as appropriate, and ECL-plus detection reagent (GE Healthcare). Images were acquired by exposure to X-ray film (GE Healthcare).

Supporting Information Available: Synthesis and characterization of **2b**, **3**, **4**, **8a**, **8b**, **8e-8i** and **31**. Experimental procedure for determination of the Michaelis-Menten constant K_m for MAL. Profiles of the pre-incubation of HDAC3-NCoR1 and HDAC8 with Mocetinostat. HDAC inhibition assay using a progression method, and results. This material is available free of charge via the Internet at http://pubs.acs.org.

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Abbreviations used. A549, adenocarcinomic human alveolar cell line; Bis-tris, 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)-1,3-propanediol); BOP, benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate; DMEM, Dulbecco's modified eagle medium; DU145, human prostate cancer cell line; ECL, electrochemiluminescence; FACS, fluorescence-activated cell sorting; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; H3K9, histone H3 lysine 9; HCT116, human colorectal carcinoma cell line; MAL, Boc-Lys(Ac)-7-amino-4-methylcoumarin; MCF-7, human breast cancer cell line; Mocetinostat, MGCD0103, *N*-(2-aminophenyl)-4-({[4-(pyridin-3-yl)pyrimidin-2-yl]amino}methyl)benzamide; NBS, non-binding surface; NCoR1, nuclear receptor co-repressor 1; Pd/C, palladium on activated carbon; PARP, poly□ADP ribose polymerase; PI, propidium iodide; SEM, standard error of the mean; TSA, trichostatin A; Vorinostat, suberoylanilide hydroxamic acid.

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Table 1. *In Vitro* Inhibition of Histone Deacetylase Isoforms by Substituted Benzamides

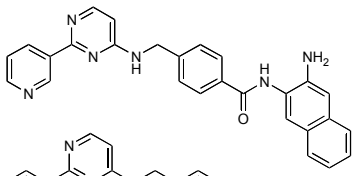
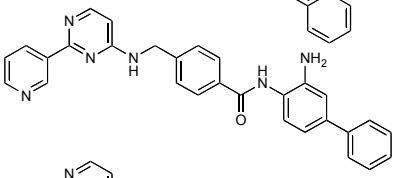
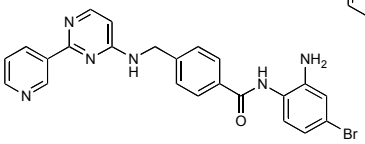
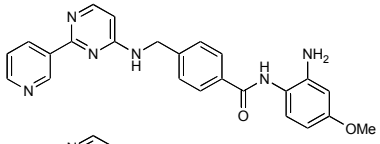
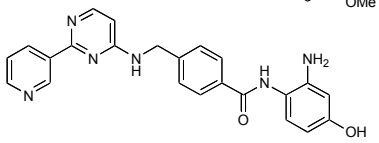
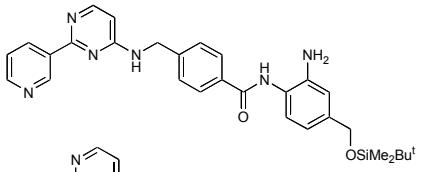
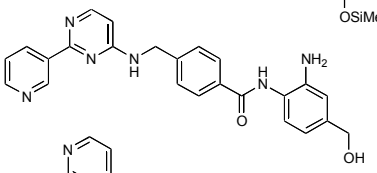
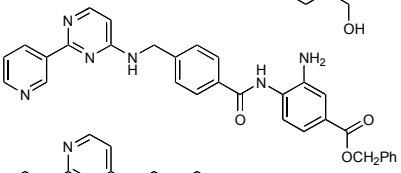
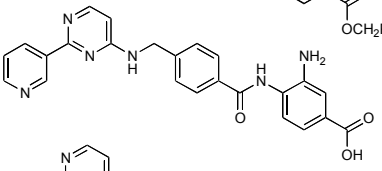
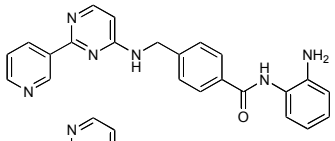
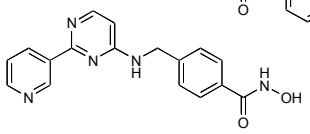
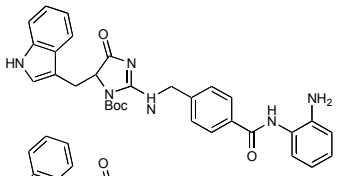
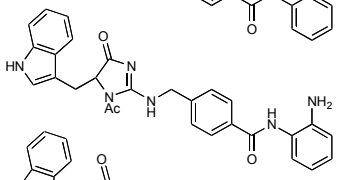
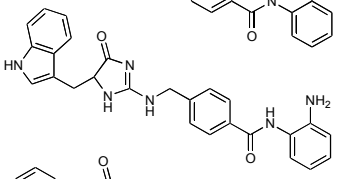
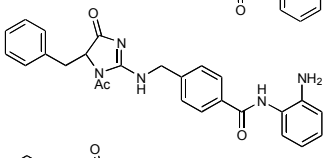
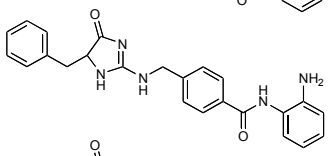
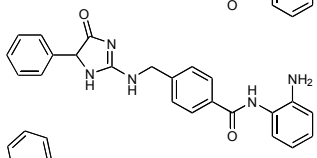
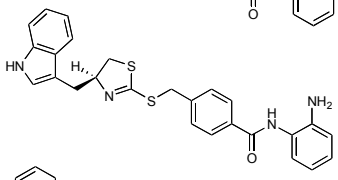
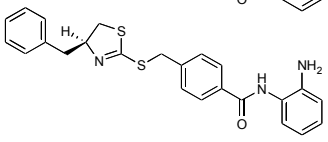
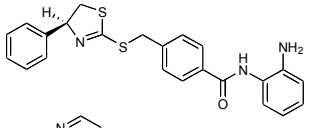
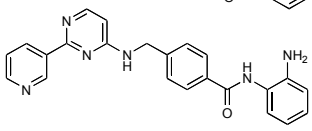
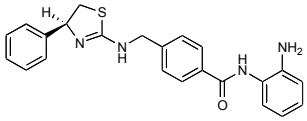
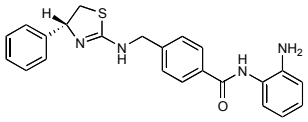
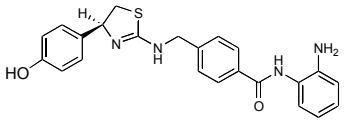
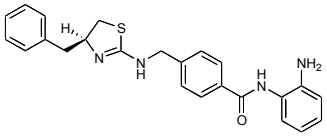
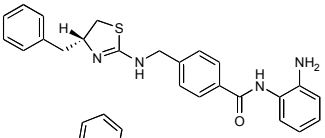
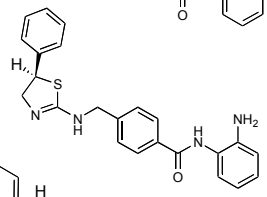
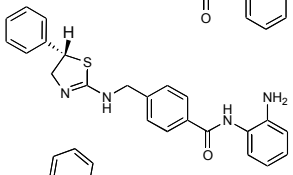
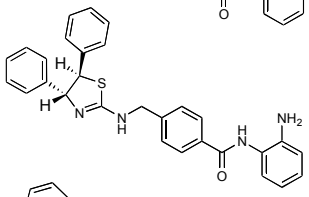
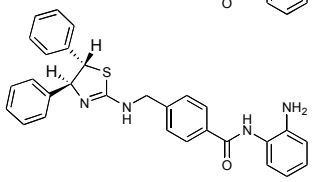
Entry	Compound	Structure	HDAC inhibitory activity IC_{50} (μM)			
			HDAC2	N-CoR-HDAC3	HDAC6	HDAC8
1	**8a**		>20	0.63±0.011	>20	>20
2	**8b**		16.7±7.2	>20	>20	>20
3	**8c**		>20	4.72±0.125	>20	>20
4	**8d**		0.248±0.023	0.111±0.029	>20	>20
5	**8e**		1.43±0.20	0.719±0.065	>20	>20
6	**8f**		>20	>20	>20	>20
7	**8g**		>20	>20	>20	>20
8	**8h**		>20	>20	>20	>20
9	**8i**		>20	>20	>20	>20
10	**MGCD0103**		0.022±0.003	0.022±0.001	>20	35.0±4.4
11	**31**		0.219±0.027	0.031±0.001	0.042±0.019	0.41±0.1

Table 2. *In Vitro* Inhibition of Histone Deacetylase Isoforms as a Function of Cap Group

Entry	Compound	Structure	HDAC inhibitory activity IC_{50} (μM)				
			HDAC1	HDAC2	N-CoR-HDAC3	HDAC6	HDAC8
1	**18b**		0.50±0.07	0.048±0.006	0.040±0.002	>20	27.2±1.0
2	**13b**		ND	0.116±0.016	0.070±0.012	>20	24.3±2.0
3	**19b**		0.19±0.06	0.078±0.007	0.104±0.016	>20	>20
4	**13a**		ND	0.26±0.02	0.083±0.010	>20	22.7±2.7
5	**19a**		ND	0.066±0.003	0.030±0.006	>20	13.0±2.8
6	**19c**		0.35±0.2	0.056±0.012	0.014±0.007	>20	8.0±1.3
7	**30b**		ND	0.92±0.45	0.89±0.24	>20	>20
8	**30a**		ND	0.58±0.12	0.509±0.016	>20	>20
9	**30c**		ND	0.44±0.06	0.063±0.026	>20	5.16±0.59
10	**MGCD0103**		0.098±0.055	0.022±0.003	0.022±0.001	>10	35.0±4.4

ND = not determined

Table 3. *In Vitro* Inhibition of Histone Deacetylase Isoforms by 2-Aminothiazolines

Entry	Compound	Structure	HDAC inhibitory activity IC_{50} (μM)				
			HDAC1	HDAC2	N-CoR-HDAC3	HDAC6	HDAC8
1	**24a**		0.93±0.30	0.085±0.015	0.012±0.008	>20	4.1±0.85
2	**24b**		0.37±0.06	0.056±0.007	0.079±0.01	>20	10.7±1.1
3	**24c**		0.18±0.06	0.044±0.005	0.016±0.005	ND	15.0±1.8
4	**24d**		0.92±0.32	0.104±0.017	0.052±0.003	>20	34.9±7.1
5	**24e**		0.41±0.14	0.082±0.026	0.036±0.006	>20	12.7±2.5
6	**24f**		ND	0.111±0.008	0.023±0.009	ND	1.4±0.1
7	**24g**		0.55±0.23	0.081±0.003	0.012±0.01	ND	2.3±0.2
8	**24h**		ND	0.425±0.074	0.287±0.123	ND	14.9±0.5
9	**24i**		ND	0.538±0.101	0.388±0.216	ND	12.6±2.1

ND = not determined

Table 4. Cell growth inhibition data (IC_{50})[a]

Compound	A549	DU145	HCT116	MCF-7
19a	>50	>50	16.6±0.22	>50
19c	>50	>50	18.2±0.15	30.1±0.43
24a	5.78±0.03	6.40±0.02	2.17±0.03	5.43±0.09
24d	6.37±0.03	5.45±0.06	1.95±0.05	3.09±0.03
30b	9.53±0.38	2.80±0.10	3.64±0.21	9.91±0.20
Mocetinostat	1.73±0.03	2.06±0.03	0.70±0.04	1.26±0.03
Vorinostat	2.27±0.04	2.58±0.02	1.39±0.03	2.29±0.06

[a] Average of triplicate runs, μM.

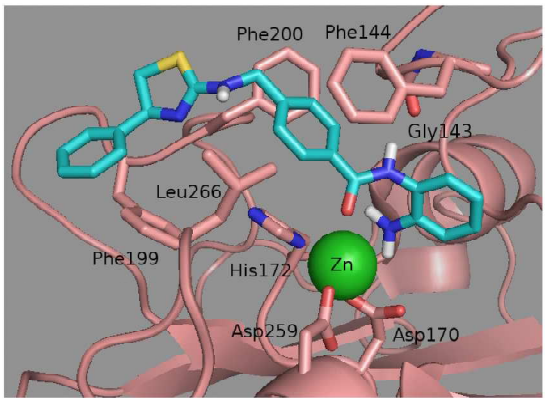


Fig. 2

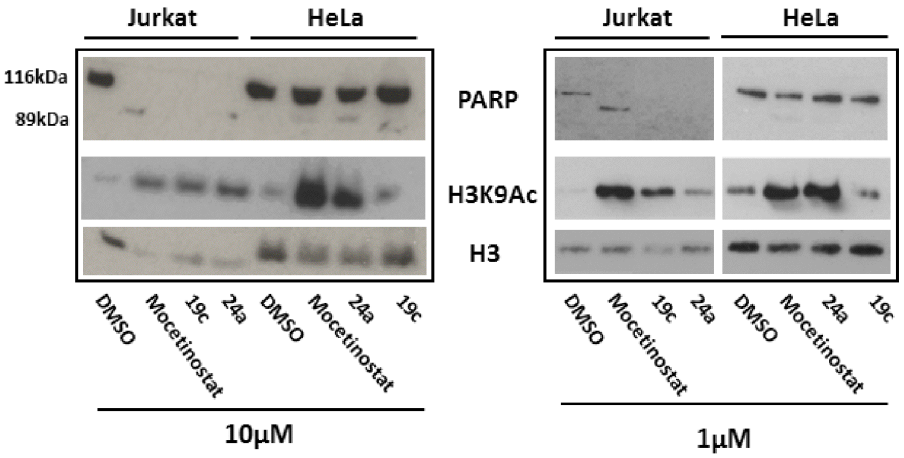

Jurkat
HeLa
Jurkat
HeLa
116kDa
89kDa
PARP
H3K9Ac
H3
DMSO
Mocetinostat
19c
24a
DMSO
Mocetinostat
24a
19c
DMSO
Mocetinostat
19c
24a
DMSO
Mocetinostat
24a
19c
10μM
1μM

Fig. 3

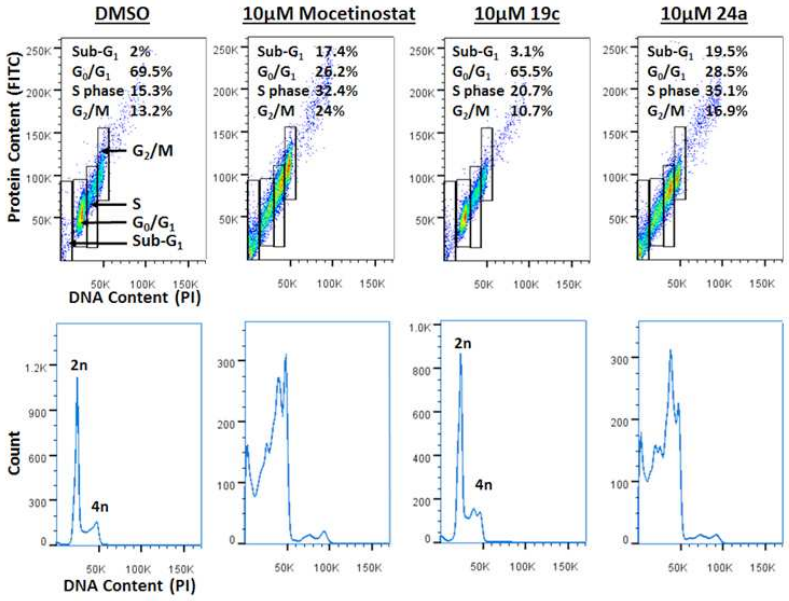

DMSO
10μM Mocetinostat
10μM 19c
10μM 24a
Sub-G1 2%
G0/G1 69.5%
S phase 15.3%
G2/M 13.2%
Sub-G1 17.4%
G0/G1 26.2%
S phase 32.4%
G2/M 24%
Sub-G1 3.1%
G0/G1 65.5%
S phase 20.7%
G2/M 10.7%
Sub-G1 19.5%
G0/G1 28.5%
S phase 35.1%
G2/M 16.9%
Protein Content (FITC)
G2/M
S
G0/G1
Sub-G1
DNA Content (PI)
Count
2n
4n
DNA Content (PI)

Fig. 4

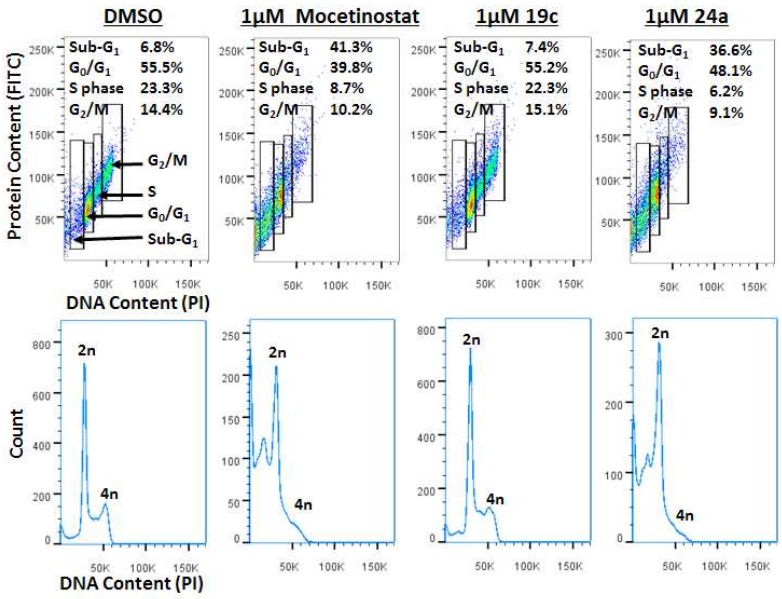

DMSO
1μM Mocetinostat
1μM 19c
1μM 24a
Sub-G1 6.8%
G0/G1 55.5%
S phase 23.3%
G2/M 14.4%
Sub-G1 41.3%
G0/G1 39.8%
S phase 8.7%
G2/M 10.2%
Sub-G1 7.4%
G0/G1 55.2%
S phase 22.3%
G2/M 15.1%
Sub-G1 36.6%
G0/G1 48.1%
S phase 6.2%
G2/M 9.1%
Protein Content (FITC)
DNA Content (PI)
Count
2n
4n

Fig. 5

Captions for Figures

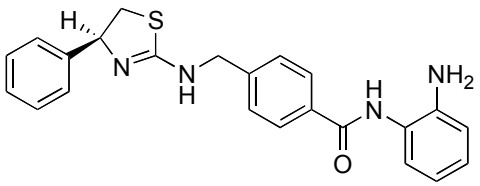
Figure 1. Examples of clinical HDAC inhibitors.

Figure 2. Molecular modeling of thiazoline **24a** (turquoise) bound to HDAC3 (derived by modification of PDB code: 4A69 using AutoDockTools 1.5.4) and docked using AutoDock Vina.[34] A side-on view of **24a** is depicted in PyMOL with residues bound to zinc and other residues near **24a** shown in pink.

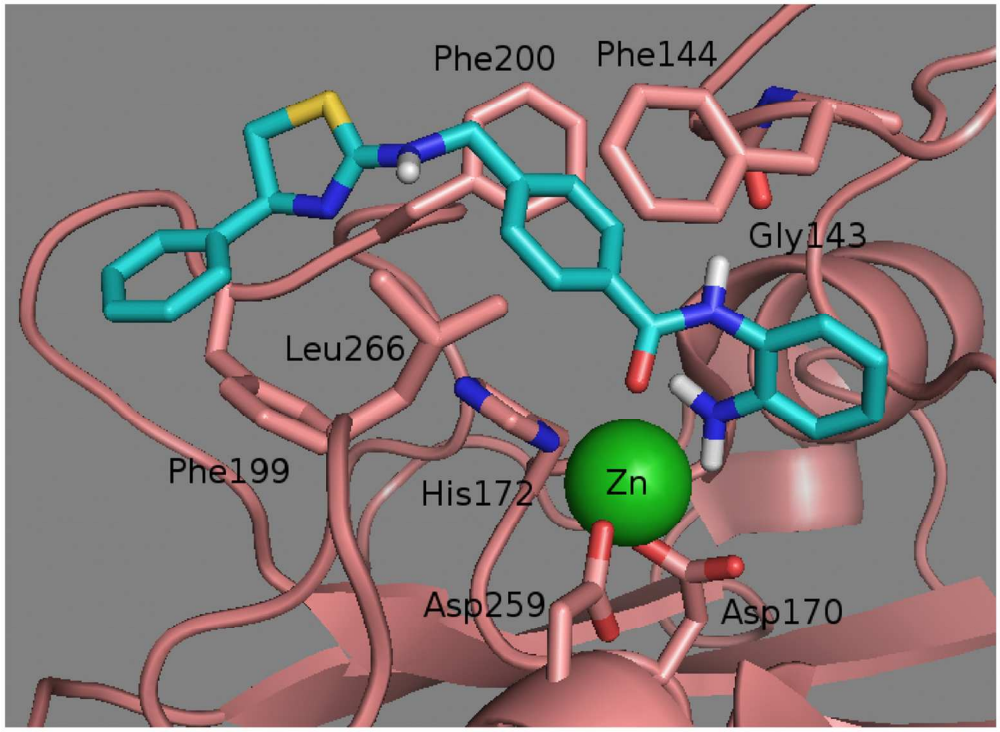
Figure 3. Analysis of histone H3 acetylation by Western blotting. Jurkat and HeLa cells cultured for 24 h with DMSO control and Mocetinostat, **19c** and **24a** (each at 10 μM and 1 μM).

Figures 4 and 5. Analysis of DNA and protein content using compounds at 10 μM (Fig. 4) and 1 μM (Fig. 5). Samples cultured as described in Fig. 3 were stained with dyes to identify protein and DNA, followed by flow cytometry. Fig. 4 shows the percentage of cells in G_0/G_1, S and G_2/M cell cycle phases and with sub-G_1 DNA content and low protein content characteristic of apoptotic cells.

TOC Graphic



HDAC3 IC_{50} = 12 nM



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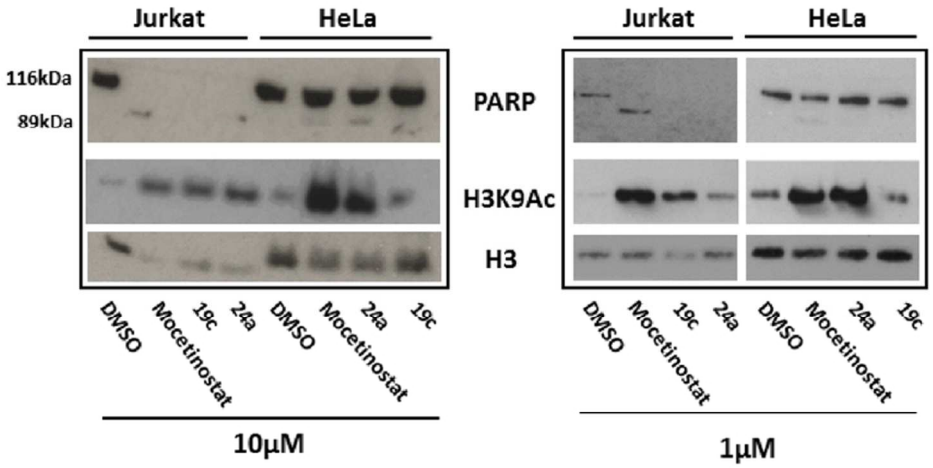

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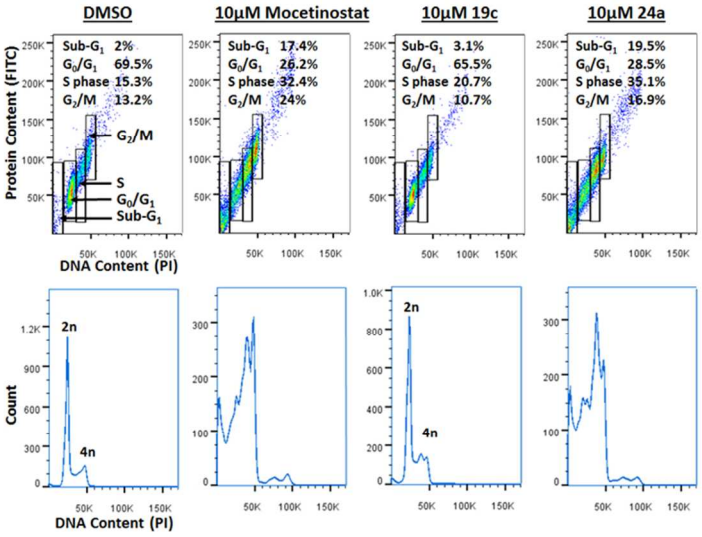

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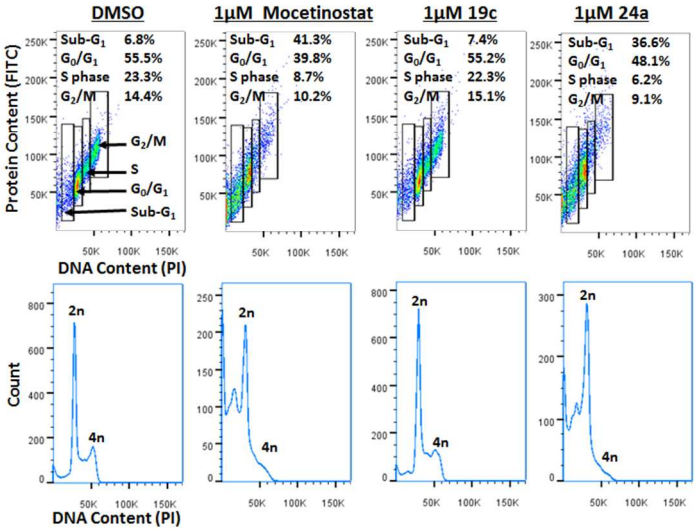

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