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Potent and Selective Inhibitors of Histone Deacetylase-3 Containing Chiral Oxazoline Capping Groups and a *N*-(2-Aminophenyl)-benzamide Binding Unit

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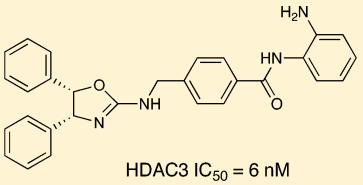
ABSTRACT: A novel series of potent chiral inhibitors of histone deacetylase (HDAC) is described that contains an oxazoline capping group and a *N*-(2-aminophenyl)-benzamide unit. Among several new inhibitors of this type exhibiting Class I selectivity and potent inhibition of HDAC3-NCoR2, *in vitro* assays for the inhibition of HDAC1, HDAC2, and HDAC3-NCoR2 by *N*-(2-aminophenyl)-benzamide **15k** gave respective IC_{50} values of 80, 110, and 6 nM. Weak inhibition of all other HDAC isoforms (HDAC4, 5, 6, 7, and 9: IC_{50} > 100 000 nM; HDAC8: IC_{50} = 25 000 nM; HDAC10: IC_{50} > 4000 nM; HDAC11: IC_{50} > 2000 nM) confirmed the Class I selectivity of **15k**. 2-Aminoimidazolinyl, 2-thioimidazolinyl, and 2-aminooxazolinyl units were shown to be effective replacements for the pyrimidine ring present in many other 2-(aminophenyl)-benzamides previously reported, but the 2-aminooxazolinyl unit was the most potent in inhibiting HDAC3-NCoR2. Many of the new HDAC inhibitors showed higher solubilities and lower binding to human serum albumin than that of Mocetinostat. Increases in histone H3K9 acetylation in the human cell lines U937 and PC-3 was observed for all three oxazolinyl inhibitors evaluated; those HDAC inhibitors also lowered cyclin E expression in U937 cells but not in PC-3 cells, indicating underlying differences in the mechanisms of action of the inhibitors on those two cell lines.



INTRODUCTION

Epigenetic mechanisms[1] are relevant to a wide range of diseases including cancer, diabetes, heart disease, and neurological disorders. At physiological pH, the alkylammonium units that form upon deacetylation of the ε-amino group of lysine residues in histone proteins bind closely to the negatively charged DNA phosphate groups. Hypoacetylation arising from increased histone deacetylase (HDAC) activity results in chromatin compaction and therefore transcriptional repression and aberrant gene regulation associated with precancerous or malignant states.[2−4] Inhibitors of histone deacetylase (HDAC)[5,6] have been much studied as a means of epigenetic cancer therapy[5,6] and can be effective in the treatment of certain leukemias, partly through relief of transcriptional repression.[7,8] HDAC inhibitors also reduce cancer cell proliferation by induction of cell cycle arrest, differentiation, and/or apoptosis.[9−12] Class I HDAC inhibitors, especially HDAC1, HDAC2, and HDAC3, are considered to be key targets for cancer treatment.

Although Romidepsin, a macrocyclic peptide containing a disulfide bridge, is a potent HDAC inhibitor used in the treatment of cutaneous T-cell lymphoma,[13] most Class I HDAC inhibitor anticancer agents[5,6] are of two structural types: (1) hydroxamic acids, including Vorinostat (suberoylanilide hydroxamic acid) used in the treatment of cutaneous T-cell lymphoma,[14] Belinostat used to treat peripheral T-cell lymphoma,[15] and Panobinostat for the treatment of multiple myeloma;[16] and (2) aminoanilides (Figure 1) such as Chidamide,[17] approved in China for the treatment of pancreatic cancer, Mocetinostat, in clinical trials for the treatment of myelogenous leukemia,[18,19] and Entinostat (MS-275), which was in Phase I clinical trials for the treatment of metastatic melanoma.[20] Additionally, the HDAC3-selective inhibitor RGFP966 has been studied for its effect on cutaneous T-cell lymphoma.[21] Given the limitations of *in vivo* efficacy and toxicity observed with many hydroxamic acids, new aminoanilide inhibitors could be expected to show prolonged inhibitory effects and longer intervals between dosing:[22,23] for example, the slow, tight binding[22] of Mocetinostat to HDACs and its slow decomplexation may be significant factors for its effects, and the observation of a lack of acquired resistance to Mocetinostat may be a potential advantage.[24] Among aminoanilide Class I HDAC inhibitors, the factors that govern isoform selectivity have been little investigated, particularly the effect of chirality on the binding of inhibitors to various HDAC isoforms. Herein are described the synthesis and preliminary evaluation of a new series of aminoanilides that contain a chiral

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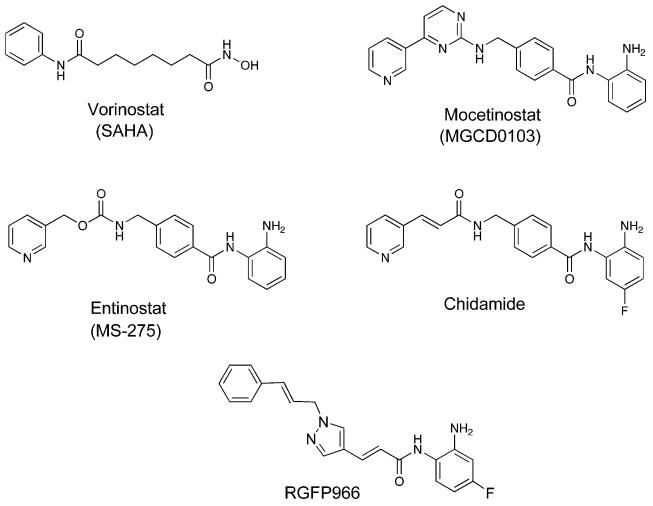


Figure 1. Examples of clinical HDAC inhibitors.

oxazoline unit and that possess specificity for HDAC3. HDAC3 is a requirement for hematopoietic stem/progenitor cells to pass through S phase of the cell cycle and also for stem cell function and lymphopoiesis.[25] In various cancers, including acute myelogenous leukemia[25,26] and lymphoma,[25,27] recruitment of SMRT/N-CoR-HDAC3 complexes that induce hypoacetylation leads to significant transcriptional repression. Additionally, HDAC3 is the target isoform associated with the progressive neurodegenerative disease, Friedrich's ataxia.[28−30]

CHEMISTRY

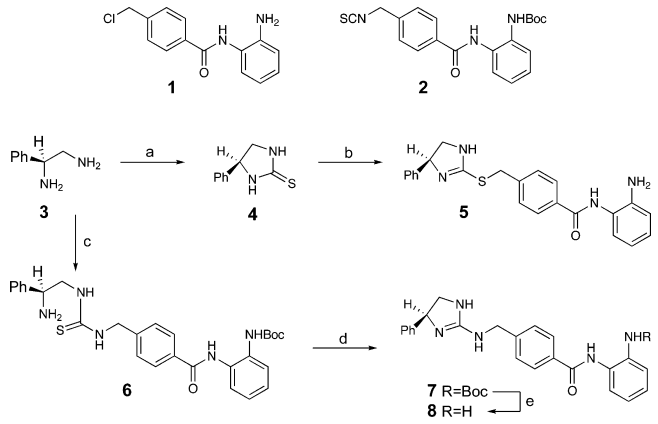
A series of analogues of Mocetinostat was sought that possessed the same linker and zinc-binding group but a five-membered heterocyclic replacement for the pyrimidine ring. Chirality would be installed within the five-membered heterocycles that also contain a heteroatom linker at the 2-position. A 2-aminooxazolinyl unit was selected as a preferred potential replacement in view of its low clogP (−0.53 cf. −0.22 for 2-aminopyrimidine), high solubility, and established use in drug design[31] as well as the availability of a variety of enantiopure 2-amino alcohols.

For comparison against the new oxazolinyl HDAC inhibitors, selected five-membered rings with either 2-thiomethyl or 2-aminomethyl linkages were prepared. *S*-Alkylation of (*S*)-4-phenylimidazolidine-2-thione (**4**)[32] with *N*-(2-aminophenyl)-4-(chloromethyl)benzamide (**1**)[33] in acetone at reflux afforded thioimidazoline **5** in 66% yield (Scheme 1), a succinct route that did not require protection of the arylamino group.

Aminoimidazoline **8** was also prepared for purpose of comparison. Since methods for the preparation of 2-(alkylamino)imidazolines[33] are limited, especially enantiopure examples, a two-step approach was developed (Scheme 1). Heating isothiocyanate **2**[34] with diamine **3** in THF at reflux afforded thiourea **6** (57%), which was quantitatively cyclized in acetone in the presence of iodomethane to give aminoimidazoline **7**, which was deprotected using TFA in dichloromethane to give the required aminoimidazoline **8** (47%).

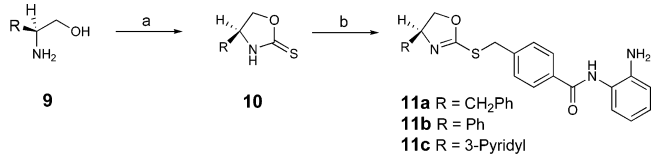
A succinct route to the 2-(thiomethyl)oxazolines **11** was developed, also without the need for protection of the arylamino group (Scheme 2). Oxazolidine-2-thiones **10**, prepared by reaction of the 2-aminoalcohols **9** with carbon disulfide in ethanol in the presence of K_2CO_3 at 50 °C followed by treatment with hydrogen peroxide,[35] underwent *S*-alkylation

Scheme 1. [a]



[a]Reagents and conditions: (a) CS_2, NEt_3, EtOH, H_2O, 60 °C, 5 h, then conc. HCl, reflux, 14 h, 31%; (b) **1**, K_2CO_3, acetone, reflux, 17 h, 66%; (c) **2**, THF, rt, 18 h, 57%; (d) iodomethane, acetone, rt, 18 h, 99% (crude); (e) TFA, DCM, rt, 2 h, 47%.

Scheme 2. [a]

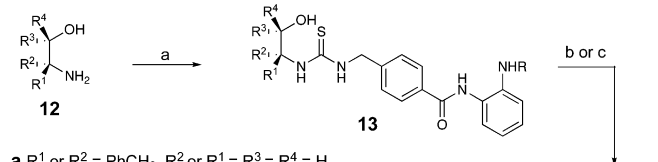


[a]Reagents and conditions: (a) CS_2, K_2CO_3, EtOH, 50 °C, then H_2O_2, 5 min, **10a**, 98%; **10b**, 80%; **10c**, 14%; (b) **1**, K_2CO_3, acetone, reflux, 16−18 h, **11a**, 70%; **11b**, 47%; **11c**, 45%.

with *N*-(2-aminophenyl)-4-(chloromethyl)benzamide (**1**)[34] in acetone at reflux to give the corresponding 2-(thiomethyl)-oxazolines **11** (Scheme 2). With suitable ring systems now in hand for comparison, attention was turned to the synthesis of 2-alkylaminooxazolines, the primary replacement motif.

Reaction of (2*S*)-2-amino-2-phenylethan-1-ol (**12b**)[36] with *tert*-butyl *N*-{2-[4-(isothiocyanatomethyl)benzamido]phenyl}-carbamate (**2**) in THF afforded thiourea (*S*)-**13b** (89%) (Scheme 3).[34] Yellow mercuric oxide has been used to cyclize

Scheme 3. [a]



a R^1 or R^2 = $PhCH_2$, R^2 or R^1 = R^3 = R^4 = H
b R^1 or R^2 = Ph, R^2 or R^1 = R^3 = R^4 = H
c R^1 or R^2= 3-pyridyl, R^2 or R^1 = R^3 = R^4 = H
d R^1= *p*-FC_6H_4, R^2 = R^3 = R^4 = H (±)
e R^1= *m*-FC_6H_4, R^2 = R^3 = R^4 = H (±)
f R^1= *p*-$CF_3C_6H_4$, R^2 = R^3 = R^4 = H (±)
g R^1= (*R*)-*p*-HOC_6H_4, R^2 = R^3 = R^4 = H
h R^3 or R^4 = Ph, R^4 or R^3 = R^1 = R^2 = H
i R^1 = R^2 = Ph, R^2 = R^3 = H
j R^1 = R^3 = Ph, R^2 = R^4 = H
k R^2 = R^3 = Ph, R^1 = R^4 = H
l R^1 or R^2 = 3-imidazolylmethyl, R^2 or R^1 =R^3 = R^4 = H

[a]Reagents and conditions: (a) **2**, THF, 20 °C, 16 h; (b) iodomethane (2 equiv), acetone, 20 °C, 4−18 h; (c) yellow HgO (2.5−5 equiv) 1:2 ethanol/toluene, reflux, 0.5−4 h (**13a**, **13b**); (d) TFA, CH_2Cl_2, 20 °C, 1.5−5.5 h.

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

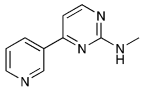
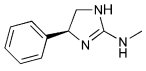
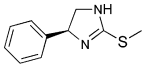
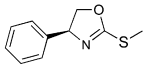
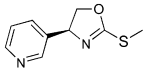
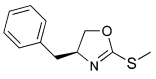
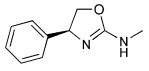
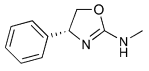
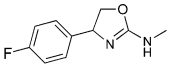
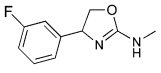
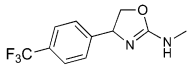
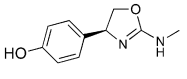
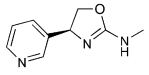
Entry	Compound	Structure	HDAC inhibitory activity[a] IC_{50} (μM)				
			HDAC1	HDAC2	HDAC3 --CoR2	HDAC6[b]	HDAC8
1	**Mocetinostat**		0.098±0.001	0.022±0.001	0.045±0.011	68%	35±2.2
2	**8**		0.24±0.08	0.27±03	0.015±0.001	78%	5.5±0.3
3	**5**		0.38±0.005	0.18±006	0.038±0.002	64%	14.3±1.8
4	**11b**		1.2±0.6	0.36±0.05	0.066±0.008	54%	9.5±2.2
5	**11c**		13%[c]	0.61±0.13	0.056±0.003	62%	112±14
6	**11a**		0.70±0.04	0.25±0.07	0.071±0.006	60%	88.0±11.0
7	**(*S*)-15b**		0.53±0.03	0.18±0.03	0.040±0.002	66%	14.7±3.2
8	**(*R*)-15b**		0.082±0.002	0.18±0.03	0.033±0.002	69%	80.7±19
9	**15d**		0.19±0.01	0.15±0.008	0.034±0.004	68%	33.9±6.1
10	**15e**		0.24±0.003	0.24±0.02	0.024±0.001	70%	52.2±8.6
11	**15f**		20%[c]	0.34±0.05	0.034±0.002	67%	82.1±13
12	**15g**		0.29±0.01	0.23±0.02	0.018±0.001	13%	83.3±12
13	**15c**		0.26±0.01	0.43±0.07	0.031±0.002	62%	111±15

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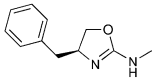
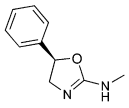
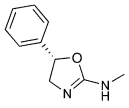
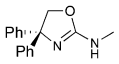
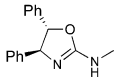
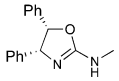
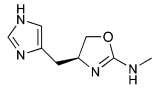
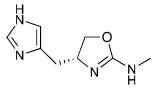
Table 1. continued

Entry	Compound	Structure	HDAC inhibitory activity[a] IC_{50} (μM)				
			HDAC1	HDAC2	HDAC3 --CoR2	HDAC6[b]	HDAC8
14	**15a**		0.20±0.02	0.13±0.04	0.041±0.001	72%	63.0±5.4
15	**(*R*)-15h**		0.076±0.005	0.192±0.007	0.011±0.003	74%	173±38
16	**(*S*)-15h**		0.39±0.01	0.094±0.038	0.035±0.002	72%	8.1±0.9
17	**15i**		21%[c]	0.40±0.08	0.055±0.02	61%	53.5±6.4
18	**15j**		28%[c]	0.28±0.03	0.12±0.02	67%	29.2±2.9
19	**15k**		0.080±0.04	0.11±0.03	0.006±0.005	71%	25.2±3.2
20	**(*S*)-15l**		0.078±0.002	0.16±0.01	0.021±0.001	67%	179±29
21	**(*R*)-15l**		0.13±0.003	0.29±0.01	0.018±0.001	61%	113±13

[a]All IC_{50} values were obtained from experiments in triplicate. [b]Percentage inhibition at 20 μM (from duplicate experiments). [c]Percentage inhibition at 0.2 μM (from duplicate experiments).

N-(β-hydroxyethyl)thioureas to the corresponding 2-amino-4,5-dihydrooxazoles.[37,38] However, (*S*)-**13b** did not cyclize to (*S*)-**14b** using the conditions of Uchida.[37] Accordingly, Hirashima's conditions[38] were investigated, and modification of those using additional yellow mercuric oxide and a longer reaction time proved to be efficient, affording (*S*)-**14b** in 76% yield. The benzyl derivative (*S*)-**14a** was similarly prepared (92%). In those reactions, it was found to be essential to use freshly prepared yellow mercuric oxide in order to obtain satisfactory yields; an aged commercial sample gave only low yields of dihydrooxazoles (*S*)-**14a** and (*S*)-**14b** even after extended reaction periods. Boc deprotection was achieved using TFA in dichloromethane to give dihydrooxazoles (*S*)-**15a** and (*S*)-**15b** in 47 and 74% yields, respectively (Scheme 3).

Although the above cyclizations of *N*-(β-hydroxyethyl)-thioureas (*S*)-**13a** and (*S*)-**13b** using yellow mercuric oxide were effective, the need for several equivalents of this toxic reagent led to a search for a more benign synthetic method. One example of such a cyclization using iodomethane has been described,[39] and this method was found to be applicable to the preparation of a wide variety of *N*-(β-hydroxyethyl)thioureas, **14c–l**. Boc deprotection using TFA in dichloromethane afforded the corresponding dihydrooxazoles **15c–l**.

RESULTS AND DISCUSSION

Of the few reports of alternative ring systems to the pyrimidine ring in Mocetinostat, replacement has involved the use of only planar, fused rings that decrease the solubility, albeit affording compounds with therapeutic potential.[40] Several analogues with planar rings in the capping group other than pyrimidine were less potent against HDAC1 and several cell lines than was Mocetinostat.[41] SAR detail regarding the cap region of Mocetinostat congeners has been limited, especially in regard the potential influence of chirality; to our knowledge, there is only one publication describing chiral heterocyclic cap analogues of such aminoanilides.[34]

Our previous study[34] showed that five-membered semi-saturated ring systems such as imidazolin-4-one can be effective as a replacement for the pyrimidine ring in Mocetinostat and, promisingly, even in the absence of the terminal 3-pyridyl ring present in Mocetinostat. In the absence of a 3-pyridyl group, few analogues of Mocetinostat (in which the pyrimidine ring

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was retained) are potent HDAC inhibitors.[41] Since the carbonyl group of substituted imidazolin-4-ones rendered an adjacent chiral center prone to racemization,[34] 1,2-dihydroazole units, as pyrimidine replacements, became the focus of the present work. The greater saturation of most 1,2-dihydroazoles and the greater solubility of compounds with unsaturated oxazoline and imidazoline rings compared to that of their pyrimidine counterparts were additional potential advantages in improving physicochemical properties.[42]

Comparison of the (4*S*)-4-phenyl-4,5-dihydro-1*H*-imidazole derivative **8** with Mocetinostat (Table 1, entries 1 and 2) shows that a smaller and partly saturated imidazoline ring system is consistent with HDAC inhibition: its IC_{50} for HDAC3 inhibition showing 3-fold greater potency than Mocetinostat; moreover, whereas inhibition of HDAC1, HDAC2, and HDAC3-CoR2 by Mocetinostat is similar (and less than 5-fold different for isoforms 1 and 3), inhibition of HDAC3CoR2 by **8** is 16 times greater than HDAC1 and 18 times greater than HDAC2. Comparison of entry 2 with entries 3−5 shows that the 2-aminoalkyl substituent is important for potent HDAC3 inhibition, with the 2-thioalkyl compounds being appreciably less potent.

For the 2-(arylmethylamino)-4-aryl-substituted dihydrooxazole series, little variation in the potent inhibition of HDAC3-CoR2 (IC_{50} = 24−40 nM) was found. However, the pair of enantiomers **15b** (entries 7 and 8) show that binding to HDAC1 is significantly influenced by the absolute configuration, with the (4*R*)-enantiomer being 6-fold more potent, whereas inhibition of HDAC2 and HDAC3-CoR2 is little affected by the stereochemistry of the inhibitor. A similar pattern is observed for the enantiomeric pair of 2-(arylmethylamino)-5-aryl dihydrooxazoles **15h**, with the (5*R*)-enantiomer being 5-fold more potent at inhibiting HDAC1. The simplest explanation for these results is that rotation about the exocyclic C−N bonds permits the phenyl group of (*R*)-**15b** to adopt the same orientation as that adopted by the phenyl group in (*R*)-**15h**; the bonding of HDAC1 to the respective N and O atoms of the heterocyclic rings does not appear to be the determining factor. The enantiomeric pair **15h** exhibit some preferential inhibition: (*S*)-**15h** at HDAC2 and (*R*)-**15h** at HDAC1.

The relatively bulky and diastereoisomeric 4,5-diphenyl-4,5-dihydrooxazoles **15j** and **15k** are potent inhibitors of HDAC3-CoR2, with **15k** being the more potent and also with 13- and 18-fold selectivity over HDAC1 and HDAC2, respectively. Compared to **15b**, the additional phenyl ring present in **15k** increases by 5-fold the inhibition of HDAC3-CoR2. These results are consistent with an extended lipophilic region that can accommodate the *cis*-1,2-diphenyl unit.

Other features are also of note. Compared to (*R*)-**15b**, the *p*- and *m*-fluoro substituents in **15d** and **15e** have little effect on the inhibition of HDAC2 and HDAC3-CoR2. The terminal 3-pyridyl capping group, present in Mocetinostat, is of little or no advantage, as compared with phenyl, in these five-membered heterocyclic systems (compare entries 4 and 5 and entries 7 and 13). The more flexible benzyl substituent may confer somewhat greater inhibition of HDAC1 and HDAC2 (entries 4 and 6 and entries 7 and 14) as compared with phenyl, but it showed no advantage over phenyl in regard to inhibition of HDAC3-CoR2. The same trends were observed for the 4-(1*H*-imidazolylmethyl)-4,5-dihydrooxazole pair of enantiomers **15l** (entries 20 and 21).

The appreciable selectivity (up to 18-fold) for HDAC3 over HDAC2 for several of the compounds in this work is significant, especially in view of the close homology of those two isoforms. All compounds showed feeble inhibition of HDAC6, consistent with the expected preference within Class I HDAC isoform selectivity observed for other amino anilides,[18,27] and very low or negligible inhibition of HDAC8, also as expected given previous studies.[18] The Class I selectivity of **15k**, the most potent HDAC3 inhibitor (IC_{50} = 0.006 μM) of this study, was further shown by evaluation against the Class 2 isoforms HDAC4, 5, 6, 7, and 9, with IC_{50} values for each enzyme being >100 μM. Weak inhibition was observed for HDAC8 (IC_{50} = 25 μM, Class 1) as well as little activity against HDAC10 (IC_{50} > 2 μM, Class 2) and HDAC 11 (IC_{50} > 4 μM, Class 4).

Physicochemical data (Table 2) for the five-membered heterocyclic HDAC inhibitors are generally favorable, especially with similar and in many cases higher solubilities and lower binding to human serum albumin as compared to that of Mocetinostat, with the exception of the relatively lipophilic inhibitors **11a**, **15d**, and **15i−k**. 3-Pyridyl compound **15c**, the oxazolinyl analogue of Mocetinostat, showed equipotent inhibition of HDAC3-CoR2 but approximately 10-fold selectivity over HDAC1 and HDAC2. HDAC inhibitors with one aryl substituent at the 4- or 5-position of the oxazoline ring showed suitable physicochemical properties (Table 2) and, depending on the stereochemistry, high potency against HDAC3-CoR2, usually with significant isoform selectivity.

Table 2. Physicochemical Data of HDAC Inhibitors

compound	Chrom log P	Chrom log $D_{7.4}$	solubility CLND (μg/mL)	permeability (pH 7.4, nm/s)	%HSA binding
Mocetinostat	2.96	2.96	107	350	92.5
5	4.31	3.49	116	300	91.0
8	3.46	1.92	70[a]	17	72.2
11a	5.67	5.41	31	440	96.5
11c	3.32	3.19	108	270	87.5
15a	4.15	2.86	148[a]	230	85.9
(*R*)-**15b**	3.76	3.09	151[a]	310	84.7
(*S*)-**15b**	3.74	3.10	153[a]	330	85.3
15c	1.78	1.71	124[a]	33	68.7
15d	3.97	3.39	147	270	88.1
15e	3.84	3.42	131[a]	340	88.5
15f	4.72	4.54	8	360	94.3
15g	2.13	1.74	130[a]	13	74.4
(*R*)-**15h**	3.58	2.79	98	180	84.9
(*S*)-**15h**	3.84	2.99	77	162	89.9
15i	5.59	5.29	7	390	96.6
15j	5.41	5.01	6	410	96.9
15k	5.01	4.54	73	410	94.9
(*R*)-**15l**	0.93	0.89	158[a]	<3	46.5
(*S*)-**15l**	0.95	0.90	142[a]	<3	46.8

[a]Greater than or equal to the number shown. Chrom log D and Chrom log P values[43] and permeability (artificial membrane permeability)[44] were measured using procedures previously described. Solubility was measured using chemiluminescent nitrogen detection (CLND).[45] Human serum albumin (HSA) binding was measured using fast gradient HPLC with a chemically bonded protein stationary phase.[46]

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BIOLOGY

Changes in histone acetylation status were assayed by analyzing histone H3 acetylated on lysine 9 (H3K9Ac) upon addition of *N*-(2-aminophenyl)-benzamides. To determine whether compounds **15e**, (*S*)-**15h**, (*R*)-**15h**, and Mocetinostat increase histone H3 acetylation levels, the human cell lines U937 and PC-3 were cultured in the presence of each compound (10 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 at 10 μM each of the above compounds caused an increase in histone H3K9Ac with respect to histone H3 expression in U937 and PC-3 cells. (Figure 1). These data are consistent with an inhibition of endogenous cellular HDAC activity that causes 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.[47] 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 compounds **15e**, (*R*)-**15h**, and (*S*)-**15h** also cause apoptosis. The data obtained (Figures 2–4) show that, for each of the compounds tested at

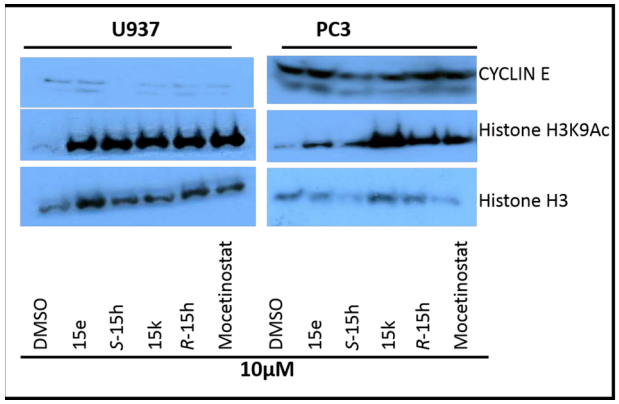

Figure 2. Analysis of histone H3 acetylation by western blotting. U937 and PC-3 cells were cultured for 24 h with DMSO control or **15e**, (*S*)-**15h**, (*R*)-**15h**, and Mocetinostat (each at 10 μM), and the expression of histone H3 and cyclin E as well as the acetylation of histone H3-lysine 9 (H3K9) was analyzed by western blotting.

10 μM in both U937 and PC-3 cells, there is an increase in the percentage of cells with sub-G_1 DNA content, consistent with induction of apoptosis. However, PC-3 cells cultured with each of the compounds, **15e**, (*R*)-**15h**, and (*S*)-**15h**, at 10 μM showed accumulation in G_0/G_1, which was not observed for U937 cells treated with those compounds, and indicates a difference in underlying mechanisms in these two cell types. In contrast, culturing PC-3 cells with Mocetinostat caused an accumulation of cells in G2/M. When cultured with U937 cells, Mocetinostat also resulted in the most significant increase in sub-G_1 DNA content. Cyclin E expression levels in U937 cells were lowered upon treatment with all compounds, most significantly with Mocetinostat and (*R*)-**15h**; however, significant changes in cyclin E expression levels were not observed in PC-3 cells using any of the above HDAC inhibitors. Inhibition of HDAC activity by different compounds can cause changes in cyclin E expression,[48,49] but this does not occur in all cases.[50] Differences in the biological effects among these compounds could be due to greater isoform selectivity of **15e**, (*R*)-**15h**, and (*S*)-**15h** compared to that of Mocetinostat, although that would need to be investigated in a further study.

SUMMARY

A new capping group, a chiral oxazoline unit bearing one or more aryl substituents, has been shown to be effective as a substructure of *N*-(2-aminophenyl)-benzamide HDAC inhibitors. Inhibition of HDAC1, HDAC2, and HDAC3-NCoR2 (Class I isoforms) was generally potent, with some compounds in this novel class of inhibitors possessing low nanomolar potency against HDAC3-NCoR2 that was greater than that of Mocetinostat, one of the most potent benzamide HDAC inhibitors previously reported. Highly convergent routes were established to HDAC inhibitors containing a variety of enantiopure heterocyclic capping groups by use of a preassembled *N*-(2-aminophenyl)-benzamide unit, which is a new methodology that has been shown to be applicable to the synthesis of several five-membered heterocyclic systems linked by a 2-amino or 2-thio substituent.

To our knowledge, this is the first extensive study of the effect of stereochemistry on HDAC inhibition. These results show that stereochemistry, both absolute and relative, can be used to distinguish the affinity of inhibitors to various HDAC isoforms and that, in some cases, preferential inhibition of an HDAC isoform can be exhibited by one enantiomer, even when only one chiral center is present. Further optimization of HDAC isoform potency and selectivity is likely to be achievable, and physicochemical data show promise for the development of new drug-like benzamide HDAC inhibitors. Such HDAC inhibitors may benefit from the absence of the commonly required 3-pyridyl terminal capping group and its metabolism to the *N*-oxide. This study demonstrates that, at least in terms of HDAC inhibition, inhibitor solubility, and low HSA binding, an oxazoline ring can beneficially replace the pyrimidine ring present in Mocetinostat and, furthermore, that its pyridine ring can also be replaced, with such inhibitors leading to restoration of acetylation status in the two cell lines studied. Thus, each of the compounds, **15e**, (*S*)-**15h**, (*R*)-**15h**, and Mocetinostat (at 10 μM), increased histone H3K9 acetylation in U937 and PC-3 cell lines. The results of this study illustrate some advantages of developing new Class I-selective HDAC inhibitors that incorporate as part of the capping region a saturated or partly saturated heterocyclic ring with substituents contributing to a center, or centers, of defined absolute configuration.

EXPERIMENTAL SECTION

Chemistry. All chemicals were used as supplied, except *o*-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 PerkinElmer 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

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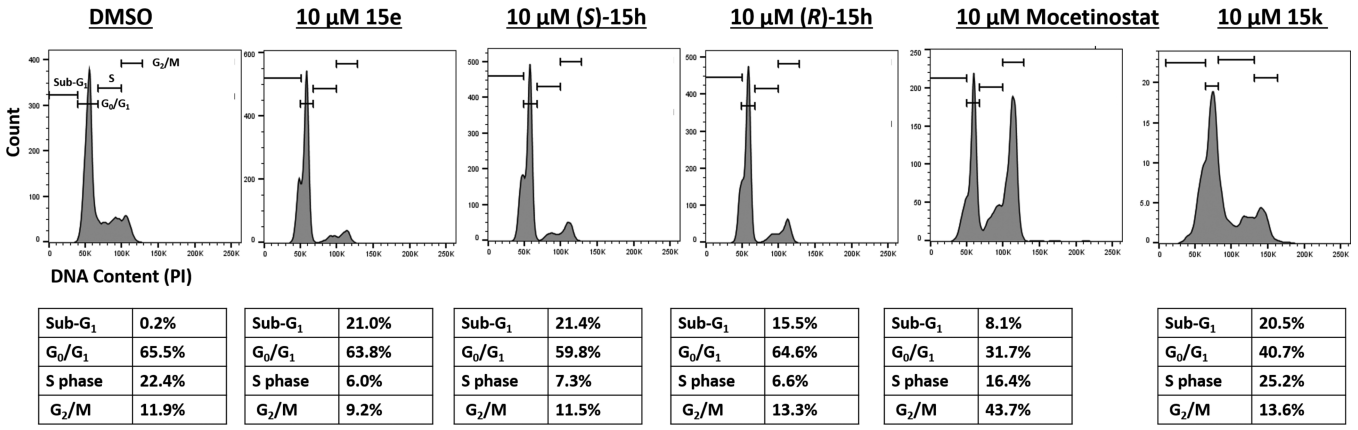


Sub-G_1	0.2%
G_0/G_1	65.5%
S phase	22.4%
G_2/M	11.9%

Sub-G_1	21.0%
G_0/G_1	63.8%
S phase	6.0%
G_2/M	9.2%

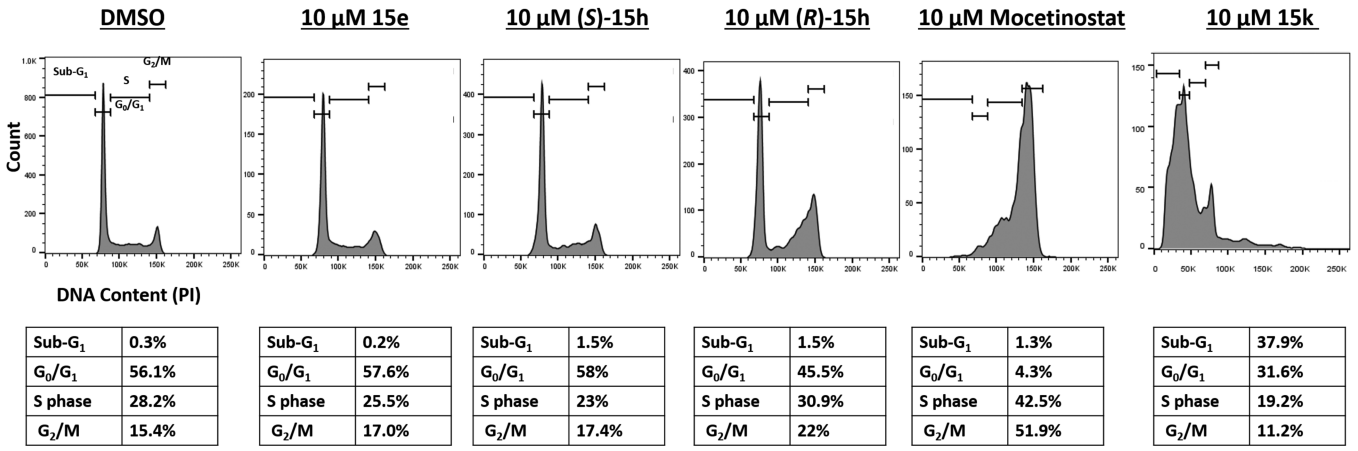
Sub-G_1	21.4%
G_0/G_1	59.8%
S phase	7.3%
G_2/M	11.5%

Sub-G_1	15.5%
G_0/G_1	64.6%
S phase	6.6%
G_2/M	13.3%

Sub-G_1	8.1%
G_0/G_1	31.7%
S phase	16.4%
G_2/M	43.7%

Sub-G_1	20.5%
G_0/G_1	40.7%
S phase	25.2%
G_2/M	13.6%

Figure 3. Analysis of DNA and protein content in PC-3 cells using compounds at 10 μM. Samples were stained with dyes to identify protein and DNA, followed by flow cytometry. 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 is shown.



Sub-G_1	0.3%
G_0/G_1	56.1%
S phase	28.2%
G_2/M	15.4%

Sub-G_1	0.2%
G_0/G_1	57.6%
S phase	25.5%
G_2/M	17.0%

Sub-G_1	1.5%
G_0/G_1	58%
S phase	23%
G_2/M	17.4%

Sub-G_1	1.5%
G_0/G_1	45.5%
S phase	30.9%
G_2/M	22%

Sub-G_1	1.3%
G_0/G_1	4.3%
S phase	42.5%
G_2/M	51.9%

Sub-G_1	37.9%
G_0/G_1	31.6%
S phase	19.2%
G_2/M	11.2%

Figure 4. Analysis of DNA and protein content in U937 cells using compounds at 10 μM. Samples were stained with dyes to identify protein and DNA, followed by flow cytometry. 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 is shown.

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 LC-MS analysis unless otherwise indicated. For all samples, 0.1% TFA was added to both eluents.

The following compounds were prepared according to the literature: 2-amino-2,2-diphenylethanol;[51] (*S*)-2-amino-2-phenylacetamide;[52] methyl (*S*)-2-amino-2-phenylacetate hydrochloride;[53] *N*-(2-aminophenyl)-4-(chloromethyl)benzamide (**1**);[34] *N*-Boc-*o*-phenylenediamine;[34] (*S*)-*N*-((*S*)-2-((*tert*-butyldimethylsilyl)oxy)-1-(pyridin-3-yl)ethyl)-2-methylpropane-2-sulfinamide;[54] (*S*)-1-phenylethane-1,2-diamine (**3**);[55] (*S*)-4-phenylimidazolidine-2-thione (**4**);[32] (*S*)-2-amino-2-(pyridin-3-yl)ethanol (**9c**);[54] (*S*)-4-benzyloxazolidine-2-thione (**10a**);[56] (*S*)-4-phenyloxazolidine-2-thione (**10b**);[56] (*S*,*E*)-*N*-(2-((*tert*-butyldimethylsilyl)oxy)ethylidene)-2-methylpropane-2-sulfinamide;[56] (2*S*)-2-amino-2-phenylethan-1-ol (**12b**);[36] 2-amino-2-(3-fluorophenyl)ethanol (**12c**);[57] *tert*-butyl (*R*)-(2-(4-((3-(2-hydroxy-1-phenylethyl)thioureido)methyl)benzamido)phenyl)carbamate (*S*)-**13a**;[34] *tert*-butyl (*S*)-(2-(4-((3-(2-hydroxy-1-phenylethyl)thioureido)-methyl)benzamido)phenyl)carbamate (*S*)-**13b**;[34] and *N*-(2-aminophenyl)-4-({[4-(pyridin-3-yl)pyrimidin-2-yl]amino}methyl)benzamide (Mocetinostat).[58]

***tert*-Butyl *N*-{2-[4-(Isothiocyanatomethyl)benzamido]-phenyl}carbamate (2).** A literature procedure[34] was modified as follows. Carbon disulfide (3.48 mL, 57.5 mmol) was added to a mixture of 4-(aminomethyl)benzoic acid (3.0 g, 19.8 mmol) and triethylamine (6.62 mL, 47.5 mmol) in THF (13 mL) and water (13 mL). The mixture was stirred vigorously for 22 h and then cooled to 0 °C, and a solution of iodine (5.38 g, 21.2 mmol) in THF (13 mL) was added dropwise over 10 min. The mixture was stirred at 0 °C for 2 h; then, hydrochloric acid (20 mL, 1M) and sodium sulfite (0.49 g, 3.9 mmol) were added, and the mixture ws stirred. The mixture was then extracted with ethyl acetate (100 mL, then 50 mL), and the combined organic layers were washed with hydrochloric acid (2 × 50 mL, 1M) and then with brine (50 mL), dried ($MgSO_4$), and evaporated to give 4-(isothiocyanatomethyl)benzoic acid as a cream solid. To a suspension of the cream solid in dichloromethane (35 mL) was added oxalyl chloride (2.28 mL, 27.0 mmol) dropwise, followed by a few drops of DMF. The resulting mixture was stirred at 20 °C for 1 h (until evolution of gas had ceased), and the resulting solution was stirred at reflux for 1 h. Evaporated gave a residue that was coevaporated with toluene (20 mL) to ensure complete removal of oxalyl chloride. The residue was dissolved in dichloromethane (25 mL), and the solution was added dropwise over 10 min to a solution of *N*-Boc-*o*-phenylenediamine[34] (3.75 g, 18 mmol) in dichloro-

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methane (25 mL) and pyridine (25 mL) at 0 °C. The mixture was stirred at 0 °C for 1 h, warmed to 20 °C, and stirred for a further 16.5 h. Then aqueous saturated sodium hydrogen carbonate (150 mL) was added, and the mixture was extracted with chloroform (2 × 100 mL). The combined organic layers were washed with hydrochloric acid (2 × 150 mL, 1 M) and then with brine (150 mL), dried over $MgSO_4$, and evaporated to give an orange oil that was purified by column chromatography (3% ethyl acetate/dichloromethane) to give **2** (5.11 g, 74%) as a cream solid. Spectroscopic data were as previously reported.[34]

(*S*)-*N*-(2-Aminophenyl)-4-(((4-phenyl-4,5-dihydro-1*H*-imidazol-2-yl)thio)methyl)benzamide (5). To a solution of thione **4** (80 mg, 0.45 mmol) and benzyl chloride **1** (117 mg, 0.45 mmol) in acetone (5 mL) was added potassium carbonate (93 mg, 0.673 mmol). The mixture was then stirred at reflux for 17 h. After allowing the mixture to cool, the solvent was evaporated, and the residue was partitioned between water (15 mL) and ethyl acetate (30 mL). The aqueous layer was extracted with ethyl acetate (2 × 30 mL), and the combined organic layers were washed with brine (15 mL), dried (Na_2SO_4), and evaporated to give a beige solid (200 mg) that was purified by column chromatography on silica gel (5:95 methanol/ethyl acetate) to give a white solid (120 mg, 66%). A small sample was further purified by recrystallization from chloroform to give **5** as a white solid, mp 150−151 °C; $[\alpha]_D^{25}$ −2.1 (*c* 1.0, methanol); ν_{max} (cm^{-1}) 3239, 1688, 1642; ^1H NMR (600 MHz, chloroform-*d*) δ ppm 3.58 (1H, br s), 3.86 (2H, br s), 4.12 (1H, br s), 4.39 (1H, d, *J* = 13.6 Hz), 4.44 (1H, d, *J* = 13.6 Hz), 4.97 (1H, br s), 6.82−6.89 (2H, m), 7.10 (1H, m), 7.22−7.29 (2H, m), 7.30−7.36 (3H, m), 7.53 (2H, d, *J* = 7.9 Hz), 7.81−7.92 (3H, m); ^{13}C NMR (150 MHz, chloroform-*d*) δ ppm 35.2, 118.6, 120.0, 124.7, 125.2, 126.4, 127.4, 127.7, 128.8, 129.6, 133.3, 140.7, 141.9, 143.4, 162.8, 165.5; *m*/*z* (ESI^+) 403 ($[M + H]^+$, 100%); HRMS calcd for $C_{23}H_{23}N_4OS^+$, 403.1587; found, 403.1577.

(*S*)-*tert*-Butyl (2-(4-((3-(2-Amino-2-phenylethyl)thioureido)-methyl)benzamido)phenyl)carbamate (6). To a solution of (*S*)-1-phenylethane-1,2-diamine[55] (213 mg, 1.56 mmol) in THF (4 mL) was added a solution of **2** (200 mg, 0.522 mmol) in THF (4 mL). The resulting solution was stirred at 20 °C for 17.5 h and then evaporated, and the residue was purified by flash column chromatography on silica gel (gradient elution from 1 to 8% of methanolic 2 M ammonia in dichloromethane) to give **6** (154 mg, 57%) as a white solid, mp 105−106 °C; ν_{max} (cm^{-1}) 3276, 1689, 1660; ^1H NMR (500 MHz, chloroform-*d*) δ ppm 1.47−1.54 (9H, m), 2.32 (4H, br s), 3.50 (1H, m), 3.76 (1H, br s), 4.12 (1H, dd, *J* = 8.4, 4.3 Hz), 4.74 (2H, br s), 7.03 (1H, br s), 7.10−7.20 (3H, m), 7.20−7.44 (7H, m), 7.65 (1H, m), 7.78 (2H, d, *J* = 7.7 Hz), 9.35 (1H, br s); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 28.3, 48.0, 51.8, 55.3, 81.5, 124.7, 125.8, 126.2, 126.3, 127.7, 127.7, 127, 128.8, 130.4, 130.5, 132.9, 142.2, 154.7, 165.9, 183.4; *m*/*z* (ESI^+) 520 ($[M + H]^+$, 100%); HRMS calcd for $C_{28}H_{34}N_5O_3S^+$, 520.2377; found, 520.2365.

(*S*)-*tert*-Butyl (2-(4-(((4-Phenyl-4,5-dihydro-1*H*-imidazol-2-yl)amino)methyl)benzamido)phenyl)carbamate (7). To a solution of thiourea **6** (154 mg, 0.296 mmol) in acetone (3 mL) was added iodomethane (0.037 mL, 0.593 mmol), and the solution was stirred at 20 °C for 18 h. Saturated aqueous sodium hydrogen carbonate (1.5 mL) was added, and the mixture was stirred for 10 min. Saturated aqueous sodium hydrogen carbonate (20 mL) and water (5 mL) were added, and the mixture was then extracted with ethyl acetate (2 × 20 mL). The combined organic layers were dried (Na_2SO_4) and evaporated to give **7** (143 mg, 99%) as a cream solid, mp 130−132 °C; ν_{max} (cm^{-1}) 3171 (NH), 1667 (C═O); ^1H NMR (400 MHz, chloroform-*d*) δ ppm 1.42 (9H, s, $C(CH_3)_3$), 3.35 (1H, t, *J* = 7.0 Hz), 3.84 (1H, t, *J* = 8.1 Hz), 4.53 (2H, br s), 4.86 (1H, br s), 7.04 (1H, t, *J* = 7.3 Hz), 7.10−7.22 (3H, m), 7.22−7.37 (6H, m), 7.46 (1H, d, *J* = 7.8 Hz), 7.50 (1H, d, *J* = 7.6 Hz), 7.69 (1H, br s), 7.86 (2H, d, *J* = 6.6 Hz), 8.77 (1H, br s), 9.83 (1H, br s); ^{13}C NMR (126 MHz, chloroform-*d*) δ ppm 28.3, 46.3, 51.2, 58.7, 81.1, 124.8, 125.1, 126.1, 126.1, 126.4, 127.4, 128.3, 128.7, 129.1, 129.8, 131.5, 133.2, 139.0, 140.0, 154.5, 159.3, 166.2; *m*/*z* (ESI^+) 486 ($[M + H]^+$, 100%); HRMS calcd for $C_{28}H_{32}N_5O_3^+$, 486.2500; found, 486.2496. (90% purity by LC-MS and NMR).

(*S*)-*N*-(2-Aminophenyl)-4-(((4-phenyl-4,5-dihydro-1*H*-imidazol-2-yl)amino)methyl)benzamide (8). To a solution of **7** (140 mg, 0.288 mmol) in dichloromethane (3 mL) was added trifluoroacetic acid (0.44 mL, 5.77 mmol) dropwise. The resulting solution was stirred at 20 °C for 2 h and then evaporated, and the resulting white solid purified by MDAP to give **8** (52 mg, 47%) as a white solid, mp 130−133 °C; $[\alpha]_D^{25}$ + 11.1 (*c* 1.0, methanol); ν_{max} (cm^{-1}) 3217, 1672, 1613; ^1H NMR (400 MHz, methanol-d_4) δ ppm 3.41 (1H, dd, *J* = 9.9, 7.2 Hz), 4.02 (1H, t, *J* = 9.8 Hz), 4.47−4.59 (2H, m), 5.01 (1H, dd, *J* = 9.5, 7.3 Hz), 6.79 (1H, t, *J* = 7.3 Hz), 6.93 (1H, dd, *J* = 8.1, 0.7 Hz), 7.10 (1H, td, *J* = 7.6, 1.2 Hz), 7.21 (1H, d, *J* = 7.6 Hz), 7.26−7.41 (5H, m), 7.53 (2H, d, *J* = 8.2 Hz), 8.01 (2H, d, *J* = 8.2 Hz); ^{13}C NMR (100 MHz, methanol-d_4) δ ppm 45.7, 54.0, 61.3, 117.4, 118.3, 123.9, 125.9, 126.3, 127.0, 127.2, 127.5, 127.8, 128.4, 133.2, 142.4, 142.6, 161.3, 167.2; *m*/*z* (ESI^+) 386 ($[M + H]^+$, 100%); HRMS calcd for $C_{23}H_{24}N_5O^+$, 386.1975; found, 386.1974.

(*S*)-4-(Pyridin-3-yl)oxazolidine-2-thione (10c). To a flask located behind a blast shield that contained a stirred mixture of amino alcohol **9c** (160 mg, 1.19 mmol), potassium carbonate (80 mg, 0.58 mmol), and carbon disulfide (0.14 mL, 2.32 mmol) in ethanol (1 mL) at 50 °C was added dropwise hydrogen peroxide (0.15 mL, 1.72 mmol) (**Caution**: *Exothermic!*). After completing the addition, the mixture was cooled to 20 °C and filtered. The filtrate was diluted with ethyl acetate (30 mL), washed with water (2 × 15 mL), aqueous sodium sulfite (10%, 15 mL), and brine, dried (Na_2SO_4), and evaporated. The yellow solid was purified by flash column chromatography on silica gel (0−5% of 2 M ammonia/methanol in dichloromethane) to give **10c** (29 mg, 14%) as a white solid, mp 157−159 °C; ν_{max} (cm^{-1}) 3083; ^1H NMR (400 MHz, methanol-d_4) δ ppm 4.52 (1H, dd, *J* = 9.2, 6.2 Hz), 5.04 (1H, t, *J* = 9.2 Hz), 5.28 (1H, dd, *J* = 9.2, 6.2 Hz), 7.53 (1H, dd, *J* = 7.8, 4.9 Hz), 7.87 (1H, d, *J* = 7.8 Hz), 8.49−8.63 (2H, m); ^{13}C NMR (100 MHz, methanol-d_4) δ ppm 57.6, 76.8, 124.4, 134.8, 136.0, 147.2, 149.1, 190.2; *m*/*z* (ESI^+) 181 ($[M + H]^+$, 100%); HRMS calcd for $C_8H_9N_2S^+$, 181.0436; found, 181.0430.

(*S*)-*N*-(2-Aminophenyl)-4-(((4-benzyl-4,5-dihydrooxazol-2-yl)thio)methyl)benzamide (11a). To a solution of thione **10a** (84 mg, 0.44 mmol) and benzyl chloride **1** (95 mg, 0.36 mmol) in acetone (4 mL) was added potassium carbonate (75 mg, 0.543 mmol). The mixture was then stirred at reflux for 16.5 h and then evaporated. The residue was partitioned between water (15 mL) and ethyl acetate (20 mL), and the layers were then separated. The aqueous layer was extracted with ethyl acetate (15 mL), and the combined organic layers were washed with brine, dried ($MgSO_4$), and evaporated to give a solid that was purified by column chromatography on silica gel (1:1 ethyl acetate/hexane) to give **11a** (106 mg, 70%) as a cream solid, mp 137−140 °C; $[\alpha]_D^{25}$ −32.0 (*c* 0.5, methanol); ν_{max} (cm^{-1}) 3274, 1649; ^1H NMR (300 MHz, chloroform-*d*) δ ppm 2.66 (1H, dd, *J* = 13.8, 8.3 Hz), 3.08 (1H, dd, *J* = 13.8, 5.5 Hz), 3.86 (2H, br s), 4.03−4.12 (1H, m), 4.19−4.35 (3H, m), 4.43 (1H, m), 6.75−6.89 (2H, m), 7.07 (1H, m), 7.14−7.35 (6H, m,), 7.48 (2H, d, *J* = 8.0 Hz, H(11 and 13)), 7.83 (2H, d, *J* = 8.0 Hz), 7.96 (1H, br s); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 36.2, 42.0, 68.2, 74.0, 118.8, 120.2, 125.0, 125.6, 127.0, 127.7, 128.0, 129.0, 129.6, 129.8, 133.7, 138.0, 141.1, 141.7, 165.3, 165.8; *m*/*z* (ESI^-) 416 ($[M - H]^-$, 100%); HRMS calcd for $C_{24}H_{22}N_3O_2S^-$, 416.1438; found, 416.1433.

(*S*)-*N*-(2-Aminophenyl)-4-(((4-phenyl-4,5-dihydrooxazol-2-yl)thio)methyl)benzamide (11b). To a solution of thione **10b** (185 mg, 1.03 mmol) and benzyl chloride **1** (269 mg, 1.03 mmol) in acetone (10 mL) was added potassium carbonate (213 mg, 1.56 mmol). The mixture was stirred at reflux for 18 h and then evaporated. The residue was partitioned between water (40 mL) and ethyl acetate (40 mL). The organic layer was washed with brine, dried ($MgSO_4$), and evaporated to give a solid that was purified by column chromatography on silica gel (ethyl acetate/hexane, 1:1) and was then purified again by column chromatography on silica gel (dichloromethane/ethyl acetate, 4:1 to 3:1) to give **11b** as a cream solid (66 mg, 16%), mp 64−65 °C; $[\alpha]_D^{25}$ −14.0 (*c* 0.5, methanol); ν_{max} (cm^{-1}) 3292, 1653; ^1H NMR (400 MHz, chloroform-*d*) δ ppm 3.91 (2H, br s), 4.22 (1H, t, *J* = 8.0 Hz), 4.31−4.46 (2H, m), 4.75 (1H, t, *J* = 8.9 Hz), 5.25 (6H, dd, *J* = 9.5, 8.0 Hz), 6.83−6.93 (2H, m, H), 7.13

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(1H, t, J = 7.3 Hz), 7.21 (2H, d, J = 7.0 Hz), 7.26−7.41 (6H, m), 7.56 (2H, d, J = 8.0 Hz), 7.87 (3H, d, J = 7.5 Hz); ^{13}C NMR (125 MHz, chloroform-d) δ ppm 35.9, 69.9, 76.6, 118.5, 119.8, 124.6, 125.4, 126.6, 127.4, 127.7, 127.9, 128.8, 129.5, 133.4, 140.8, 141.4, 141.9, 165.6, 166.0; m/z (CI) 404 ([M + H]$^+$, 100%); HRMS calcd for $C_{23}H_{22}N_3O_2S^+$, 404.1427; found, 404.1426; 66% purity by LC-MS.

(*S*)-*N*-(2-Aminophenyl)-4-(((4-(pyridin-3-yl)-4,5-dihydrooxazol-2-yl)thio)methyl)benzamide (11c). To a solution of amino alcohol **10c** (25 mg, 0.139 mmol) and the arylmethyl chloride **1** (36.2 mg, 0.139 mmol) in acetone (2 mL) was added potassium carbonate (28.8 mg, 0.208 mmol), and the mixture was stirred at reflux for 17 h. Since the volume had decreased, acetone (3 mL) was added, and then mixture was then stirred at reflux for 6 h. After allowing the mixture to cool, the mixture was partitioned between water (15 mL) and ethyl acetate (20 mL), and the layers then separated. The aqueous layer was extracted with ethyl acetate (15 mL), and the combined organic layers were dried (Na_2SO_4) and evaporated to give a solid that was purified by MDAP to give **11c** (25 mg, 45%) as a white solid, mp 107−110 °C; $[\alpha]_D^{25}$ + 22.0 (c 0.25, methanol); ν_{max} (cm^{-1}) 3212, 1645; ^{1}H NMR (500 MHz, methanol-d_4) δ ppm 4.23 (1H, t, J = 8.0 Hz), 4.38 (1H, d, J = 13.7 Hz), 4.44 (1H, d, J = 13.7 Hz), 4.83 (1H, t, J = 9.3 Hz), 5.35 (1H, dd, J = 9.3, 7.7 Hz), 6.80 (1H, t, J = 7.4 Hz), 6.93 (1H, d, J = 8.0 Hz), 7.11 (1H, t, J = 7.4 Hz), 7.22 (1H, d, J = 7.7 Hz), 7.43 (1H, dd, J = 7.7, 4.9 Hz), 7.60 (3H, d, J = 8.0 Hz), 7.96 (2H, d, J = 8.0 Hz), 8.40 (1H, s), 8.49 (1H, d, J = 4.7 Hz); ^{13}C NMR (125 MHz, methanol-d_4) δ ppm 34.8, 67.1, 75.9, 117.3, 118.3, 123.9, 124.1, 126.3, 127.2, 127.7, 128.4, 133.4, 135.1, 138.3, 141.5, 142.4, 147.2, 148.0, 167.1, 167.7; m/z (ESI$^+$) 405 ([M + H]$^+$, 100%); HRMS calcd for $C_{22}H_{21}N_4O_2S^+$, 405.1380; found, 405.1384; 93% purity by LC-MS.

2-Amino-2-(4-fluorophenyl)ethan-1-ol (12d). A three-necked flask was fitted with a magnetic stirrer and a reflux condenser and then flame-dried. Under an atmosphere of nitrogen, sodium borohydride (0.224 g, 5.92 mmol) was added, followed by anhydrous THF (10 mL). Then, a dropping funnel was fitted, and the mixture was cooled in an ice bath to 0 °C. A solution of iodine (1.05 g, 2.96 mmol) in THF (10 mL) was added dropwise over 30 min. After the vigorous evolution of gas had ceased, 4-fluoro-DL-phenylglycine (0.50 g, 2.96 mmol) was added, and the mixture was heated at reflux for 17 h. After allowing the mixture to cool, methanol was added slowly until the solution became clear. The mixture was stirred for 30 min, and then the solvent was evaporated. The resulting white paste was dissolved in aqueous potassium hydroxide (20%, 30 mL). After stirring it for 6 h, the mixture was extracted with dichloromethane (3 × 30 mL). The combined organic layers were washed with brine (20 mL then 75 mL), dried (Na_2SO_4), and evaporated to give crude **12d** as a white solid that was used without further purification (0.22 g, 48%); ^{1}H NMR (400 MHz, chloroform-d) δ ppm 2.51 (3H, br s), 3.54 (1H, dd, J = 10.8, 8.3 Hz), 3.72 (1H, dd, J = 10.8, 4.3 Hz), 4.07 (1H, dd, J = 8.3, 4.3 Hz), 7.05 (2H, t, J = 8.8 Hz), 7.32 (2H, dd, J = 8.5, 5.5 Hz).

2-Amino-2-(4-(trifluoromethyl)phenyl)ethanol (12f). To a suspension of 2-amino-2-(4-(trifluoromethyl)phenyl)acetic acid (1.50 g, 6.84 mmol) in anhydrous THF (15 mL) at 20 °C was added borane−THF complex (1 M in THF, 17.1 mL) dropwise over 5 min; then, the solution was stirred at 70 °C for 16.5 h. After cooling the mixture to 20 °C, methanol (10 mL) was added cautiously, and the mixture was then stirred for 30 min. The solvent was evaporated, and the resulting yellow paste was stirred with potassium hydroxide (20% aq w/w, 30 mL) for 4 h at 20 °C. The mixture was then extracted with ethyl acetate (3 × 30 mL), and the combined organic layers were washed with brine, dried (Na_2SO_4), and evaporated to give a yellow semisolid (1.3 g). Purification by flash column chromatography on silica gel (2−10% of 2 M ammonia/methanol in dichloromethane) gave **12f** (0.646 g, 46%) as a white solid, mp 110−116 °C; ^{1}H NMR (500 MHz, chloroform-d) δ ppm 2.14 (3H, br s), 3.58 (1H, dd, J = 10.7, 8.0 Hz), 3.78 (1H, dd, J = 10.7, 4.1 Hz), 4.16 (1H, dd, J = 8.0, 4.1 Hz), 7.49 (2H, d, J = 8.1 Hz), 7.63 (2H, d, J = 8.1 Hz); ^{13}C NMR (125 MHz, chloroform-d) δ ppm 57.0 (s), 67.8 (s), 124.1 (q, J = 271.9 Hz), 125.5 (q, J = 3.7 Hz), 126.9 (s), 129.8 (q, J = 32.5 Hz), 146.6 (s); m/z (ESI$^+$) 206 ([M + H]$^+$, 100%); HRMS calcd for $C_9H_{11}NOF_3^+$, 206.0787; found, 206.0790.

(*R*)-4-(1-Amino-2-hydroxyethyl)phenol (12g). To a suspension of (*R*)-2-amino-2-(4-hydroxyphenyl)acetic acid (1.0 g, 5.98 mmol) in anhydrous THF (10 mL) at 20 °C was added borane−THF complex in THF (20 mL, 1M) dropwise over 5 min; then, the solution was stirred at 70 °C for 44 h. After cooling the mixture to 20 °C, methanol (10 mL) was added cautiously, and the mixture was stirred for 30 min. The solvent was then evaporated, and the resulting white paste was stirred with potassium hydroxide (20% aqueous w/w, 20 mL) for 4 h at 20 °C. The solution was neutralized to pH 7 with hydrochloric acid (2M) and then washed with ethyl acetate (20 mL). The aqueous layer was evaporated, and the resulting white solid was stirred for 1 h with 1:4 ethanol/chloroform (50 mL), filtered, and evaporated to give **12g** (300 mg, 33%) as a colorless oil that was used without further purification.

Preparation of Yellow Mercuric Oxide. To a solution of mercury(II) chloride (1.0 g, **Caution**: *TOXIC*!) in water (22 mL) was added a sirred solution of sodium hydroxide (0.60 g) in water (8 mL). The mixture was stirred at 20 °C for 5 min. The precipitate was filtered and dried under vacuum overnight over phosphorus pentoxide to give mercuric oxide as a yellow solid (0.58 g).

***tert*-Butyl (*S*)-(2-(4-(((4-Benzyl-4,5-dihydrooxazol-2-yl)-amino)methyl)benzamido)phenyl)carbamate (14a).** To a solution of *tert*-butyl (*R*)-(2-(4-((3-(2-hydroxy-1-phenylethyl)-thioureido)methyl)benzamido)phenyl)carbamate (*S*)-**13a**[34] (0.524 g, 0.98 mmol) in ethanol (7 mL) and toluene (14 mL) was added freshly prepared yellow mercuric oxide (0.58 g, 2.6 mmol); the resulting mixture was stirred at reflux for 30 min. After cooling the mixture to 20 °C, it was filtered through Celite, and the filtrate was evaporated to give **14a** as a white solid (0.45 g, 92%), mp 200−201 °C; ν_{max} (cm^{-1}) 3308, 1664, 1650; ^{1}H NMR (500 MHz, chloroform-d) δ ppm 1.51 (9H, s), 2.66 (1H, dd, J = 13.5, 8.5 Hz), 3.06 (1H, dd, J = 13.5, 4.7 Hz), 4.03 (1H, t, J = 7.1 Hz), 4.22 (1H, t, J = 8.2 Hz), 4.32 (1H, m), 4.43 (1H, d, J = 15.3 Hz), 4.47 (1H, d, J = 15.3 Hz), 6.93 (1H, br s), 7.13−7.32 (5H, m), 7.38 (2H, d, J = 7.9 Hz), 7.79 (1H, d, J = 7.7 Hz), 7.92 (2H, d, J = 7.9 Hz), 9.19 (1H, br s); ^{13}C NMR (125 MHz, chloroform-d) δ ppm 28.4, 42.4, 46.7, 65.1, 72.5, 81.5, 124.6, 125.8, 126.0, 126.1, 126.5, 127.4, 127.8, 128.6, 129.3, 130.0, 131.0, 133.5, 138.0, 142.9, 154.7, 160.8, 165.4; m/z (ESI$^-$) 499 ([M − H]$^-$, 100%); HRMS calcd for $C_{29}H_{31}N_4O_4^-$, 499.2351; found, 499.2358.

(*S*)-(*tert*-Butyl 2-(4-(((4-Phenyl-4,5-dihydrooxazol-2-yl)-amino)methyl)benzamido)phenyl)carbamate (*S*)-(14b). To a solution of *tert*-butyl (*S*)-2-(2-(4-((3-(2-hydroxy-1-phenylethyl)-thioureido)methyl)benzamido)phenyl)carbamate (*S*)-**13b**[34] (180 mg, 0.345 mmol) in ethanol (3.5 mL) and toluene (7 mL) was added yellow mercuric oxide (375 mg, 1.73 mmol) in portions. The resulting mixture was stirred at reflux for 2 h. The cool mixture was filtered through Celite, and the filtrate was evaporated. The residue was dissolved in ethanol (3.5 mL) and toluene (7 mL); then, yellow mercuric oxide (375 mg, 1.73 mmol) was added, and the mixture stirred at reflux for 4 h. When it was cool, the mixture was filtered through Celite, yellow mercuric oxide (375 mg, 1.73 mmol) was added, and the mixture was stirred at reflux for 4 h. Filtration of the mixture through Celite and evaporation of the filtrate afforded (*S*)-**14b** as a foamy white solid (0.127 g, 76%), mp 100−103 °C; ν_{max} (cm^{-1}) 3274, 1654; ^{1}H NMR (400 MHz, chloroform-d) δ ppm 1.51 (9H, s), 4.11 (1H, t, J = 7.5 Hz), 4.47 (2H, s), 4.66 (1H, t, J = 8.5 Hz), 5.12 (1H, dd, J = 9.0, 7.3 Hz), 7.15 (1H, td, J = 7.5, 1.6 Hz), 7.19 (1H, td, J = 7.5, 1.6 Hz), 7.24−7.30 (5H, m), 7.31−7.39 (4H, m), 7.73 (1H, d, J = 7.3 Hz), 7.89 (2H, d, J = 8.0 Hz), 9.33 (1H, br s); ^{13}C NMR (125 MHz, chloroform-d) δ ppm 28.7), 47.0, 67.8, 75.8, 81.6, 124.9, 126.1, 126.2, 126.3, 126.8, 127.7, 127.9, 128.1, 129.0, 130.5, 131.2, 133.6, 143.3, 143.8, 155.0, 162.0, 165.9; m/z (ESI$^-$) 485 ([M − H]$^-$, 95%); HRMS calcd for $C_{28}H_{29}N_4O_4^-$, 485.2194; found, 485.2206.

(*R*)-*tert*-Butyl 2-(4-(((4-Phenyl-4,5-dihydrooxazol-2-yl)-amino)methyl)benzamido)phenyl)carbamate (*R*)-(14b). To a solution of (*R*)-phenylalaninol (110 mg, 0.80 mmol) in THF (12 mL) was added isothiocyanate **2** (337 mg, 0.88 mmol), and the mixture was stirred at 20 °C for 18 h. Evaporation gave a residue that was dissolved in acetone (6 mL); iodomethane (0.10 mL, 1.6 mmol) was added, and the solution was stirred at 20 °C for 9 h. Saturated aqueous sodium

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hydrogen carbonate (2 mL) was then added, and the mixture was stirred for 10 min. Saturated aqueous sodium hydrogen carbonate (20 mL) was then added, and the mixture was extracted with ethyl acetate (2 × 20 mL). The combined organic layers were washed with brine (20 mL), dried (Na_2SO_4), and evaporated to give a white solid that was purified by column chromatography on silica gel (5:95 methanol/ethyl acetate) to give (*R*)-**14b** (159 mg, 41%) as a white solid, mp 104−106 °C; ν_{max} (cm^{-1}) 3277, 1653; ^1H NMR (300 MHz, chloroform-*d*) δ ppm 1.48 (9H, s), 4.08 (1H, t, *J* = 7.5 Hz), 4.44 (2H, s), 4.63 (1H, t, *J* = 8.6 Hz), 5.09 (1H, dd, *J* = 8.9, 7.3 Hz), 7.03−7.39 (13H, m), 7.70 (1H, m), 7.85 (2H, d, *J* = 8.1 Hz), 9.30 (1H, br s); ^{13}C NMR (75 MHz, chloroform-*d*) δ ppm 28.4, 46.7, 67.6, 75.6, 81.3, 124.7, 125.8, 125.9, 126.1, 126.6, 127.4, 127.6, 127.9, 128.8, 130.3, 130.9, 133.3, 143.1, 143.6, 154.8, 161.8, 165.7; *m*/*z* (ESI^+) 487 ($[M + H]^+$, 100%); HRMS calcd for $C_{28}H_{31}N_4O_4^+$, 487.2340; found, 487.2336.

(*S*)-*tert*-Butyl (2-(4-(((4-(Pyridin-3-yl)-4,5-dihydrooxazol-2-yl)amino)methyl)benzamido)phenyl)carbamate (14c). To a solution of **9c** (155 mg, 1.12 mmol) in THF (8 mL) and methanol (3 mL) was added isothiocyanate **2** (330 mg, 0.86 mmol), and the mixture was stirred at 20 °C for 17 h. Evaporation gave a residue that was dissolved in acetone (4 mL); iodomethane (0.108 mL, 1.72 mmol) was then added, and the solution was stirred at 20 °C for 5 h. The solution was then worked up as described for (*R*)-**14b** to give a solid that was purified by flash column chromatography on silica gel (gradient elution from 2 to 10% of methanolic 2 M ammonia in dichloromethane) to give **14c** (86 mg, 21%) as a white solid, mp 107−108 °C; ν_{max} (cm^{-1}) 3265, 1651; ^1H NMR (400 MHz, chloroform-*d*) δ ppm 1.50 (9H, s), 4.06 (1H, dd, *J* = 7.8, 7.2 Hz), 4.48 (2H, s), 4.66 (1H, dd, *J* = 7.8, 7.0), 5.12 (1H, dd, *J* = 8.7, 7.2 Hz), 5.43 (1H, br s), 7.09−7.22 (2H, m), 7.26 (1H, m), 7.30−7.42 (4H, m), 7.56 (1H, d, *J* = 7.8 Hz), 7.73 (1H, d, *J* = 6.8 Hz), 7.88 (2H, d, *J* = 7.8 Hz), 8.42 (1H, s), 8.49 (1H, d, *J* = 3.7 Hz), 9.39 (1H, br s); ^{13}C NMR (100 MHz, chloroform-*d*) δ ppm 28.3, 46.6, 65.5, 74.9, 81.1, 123.6, 124.5, 125.6, 125.7, 126.0, 127.4, 127.8, 130.3, 130.7, 133.5, 134.2, 139.2, 142.6, 148.2, 148.7, 154.6, 162.0, 165.7; *m*/*z* (ESI^+) 488 ($[M + H]^+$, 100%); HRMS calcd for $C_{27}H_{30}N_5O_4^+$, 488.2292; found, 488.2285.

***tert*-Butyl (2-(4-(((4-(4-Fluorophenyl)-4,5-dihydrooxazol-2-yl)amino)methyl)benzamido)phenyl)carbamate (14d).** To a solution of amino alcohol **12d** (220 mg, 1.42 mmol) in THF (10 mL) was added isothiocyanate **2** (272 mg, 0.71 mmol); the resulting solution was stirred at 20 °C for 18 h. The solvent was evaporated, and the residue was dissolved in acetone (5 mL); iodomethane (0.09 mL, 1.45 mmol) was then added, and the solution stirred at 20 °C for 16 h. The solution was then worked up as described for (*R*)-**14b** to give a solid that was purified by column chromatography on silica gel (4:1 ethyl acetate/hexane) to give **14d** (238 mg, 67%) as a white solid, mp 105−108 °C; ν_{max} (cm^{-1}) 3258, 1650; ^1H NMR (400 MHz, chloroform-*d*) δ ppm 1.51 (9H, s), 4.06 (1H, t, *J* = 7.7 Hz), 4.50 (2H, s), 4.65 (1H, t, *J* = 8.5 Hz), 5.11 (1H, dd, *J* = 8.9, 7.4 Hz), 7.02 (2H, t, *J* = 8.7 Hz), 7.12−7.30 (6H, m), 7.39 (2H, d, *J* = 8.0 Hz), 7.77 (1H, d, *J* = 7.5 Hz), 7.91 (2H, d, *J* = 8.0 Hz), 9.32 (1H, br s); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 28.3, 46.6, 66.8, 75.4, 81.3, 115.4 (d, *J* = 22.1 Hz), 124.5, 125.7, 125.9, 127.3, 127.7, 128.0 (d, *J* = 8.6 Hz), 130.0, 130.8, 133.3, 139.2, 142.7, 154.6, 162.1 (d, *J* = 244.7 Hz), 161.5, 165.3; *m*/*z* (CI) 505 ($[M + H]^+$, 93%); HRMS calcd for $C_{28}H_{30}FN_4O_4^+$, 505.2246; found, 505.2254.

***tert*-Butyl (2-(4-(((4-(3-Fluorophenyl)-4,5-dihydrooxazol-2-yl)amino)methyl)benzamido)phenyl)carbamate (14e).** To a solution of **12e** (81 mg, 0.522 mmol) in THF (6 mL) was added isothiocyanate **2** (200 mg, 0.522 mmol), and the resulting solution was stirred at 20 °C for 16 h. Evaporation gave a residue that was dissolved in acetone (3 mL); iodomethane (0.10 mL, 1.56 mmol) was then added, and the solution was stirred at 20 °C for 4 h. The solution was then worked up as described for (*R*)-**14b** to give a solid that was purified by flash column chromatography on silica gel (75−100% ethyl acetate/cyclohexane) to give **14e** (200 mg, 76%) as a white solid, mp 104−107 °C; ν_{max} (cm^{-1}) 3283, 1652; ^1H NMR (400 MHz, chloroform-*d*) δ ppm 1.51 (1H, s), 3.95 (1H, br s), 4.07 (1H, dd, *J* = 8.1, 7.1 Hz), 4.44−4.54 (2H, m), 4.64 (1H, dd, *J* = 9.0, 8.1 Hz), 5.11 (1H, dd, *J* = 9.0, 7.1 Hz), 6.91−7.04 (3H, m), 7.11−7.21 (2H, m)), 7.22−7.32 (3H, m), 7.36 (2H, d, *J* = 8.3 Hz), 7.73 (1H, dd, *J* = 7.8, 1.2 Hz), 7.89 (2H, d, *J* = 8.3 Hz), 9.28 (1H, br s); ^{13}C NMR (100 MHz, chloroform-*d*) δ ppm 28.3, 46.6, 67.0, 75.2, 81.2, 113.3 (d, *J* = 22.0 Hz), 114.3 (d, *J* = 21.3 Hz), 122.0 (d, *J* = 2.2 Hz), 124.5, 125.7, 125.8, 125.9, 127.3, 127.8, 130.1 (d, *J* = 8.1 Hz), 130.2, 130.8, 133.3, 142.7, 146.3 (d, *J* = 6.6 Hz), 154.6, 161.8, 163.1 (d, *J* = 245.8 Hz), 165.5; *m*/*z* (ESI^+) 505 ($[M + H]^+$, 100%); HRMS calcd for $C_{28}H_{30}FN_4O_4^+$, 505.2246; found, 505.2240.

***tert*-Butyl (2-(4-(((4-(4-(Trifluoromethyl)phenyl)-4,5-dihydrooxazol-2-yl)amino)methyl)benzamido)phenyl)carbamate (14f).** To a solution of **12f** (107 mg, 0.522 mmol) in THF (6 mL) was added **2** (200 mg, 0.522 mmol). The mixture was stirred at 20 °C for 17.5 h. Evaporation gave a residue that was dissolved in acetone (3 mL); iodomethane (0.065 mL, 1.04 mmol) was added, and the solution was stirred at 20 °C for 6 h. A further 65 μL of iodomethane was then added, and the mixture stirred for another 2.5 h at 20 °C. The mixture was worked up as described for (*R*)-**14b** to give a solid that was purified by flash column chromatography on silica gel (gradient elution from 1 to 5% of methanolic 2 M ammonia in dichloromethane) to give **14f** (200 mg, 69%) as a white solid, mp 114−116 °C; ν_{max} (cm^{-1}) 3282, 1658; ^1H NMR (400 MHz, chloroform-*d*) δ ppm 1.49 (9H, s), 4.04 (1H, t, *J* = 7.6 Hz), 4.44 (2H, s), 4.65 (1H, t, *J* = 8.7 Hz), 4.94 (1H, br s), 5.14 (1H, t, *J* = 8.1 Hz), 7.05−7.18 (2H, m), 7.24 (1H, dd, *J* = 7.6, 1.2 Hz), 7.31 (2H, d, *J* = 8.1 Hz), 7.36 (2H, d, *J* = 8.1 Hz), 7.47 (1H, br s), 7.57 (2H, d, *J* = 8.1 Hz), 7.70 (1H, d, *J* = 7.1 Hz), 7.85 (2H, d, *J* = 8.1 Hz), 9.39 (1H, br s); ^{13}C NMR (100 MHz, chloroform-*d*) δ ppm 28.3, 46.5, 67.0, 75.0, 81.1, 124.1 (q, *J* = 272.9 Hz), 124.5, 125.5 (q, *J* = 3.7 Hz), 125.6, 125.9, 126.8, 127.2, 127.7, 129.6 (q, *J* = 32.3 Hz), 130.3, 130.7, 133.2, 142.8, 147.6, 154.7, 162.1, 165.6; *m*/*z* (ESI^+) 387 ($[M + H]^+$, 100%); HRMS calcd for $C_{29}H_{30}N_4O_4F_3^+$, 555.2214; found, 555.2214.

(*S*)-*tert*-Butyl (2-(4-(((4-(4-Hydroxyphenyl)-4,5-dihydrooxazol-2-yl)amino)methyl)benzamido)phenyl)carbamate (14g). To a solution of thiourea **13g**[29] (270 mg, 0.503 mmol) in acetone (4 mL) was added iodomethane (0.045 mL, 0.75 mmol); the solution was then stirred at 20 °C for 4 h. More iodomethane (0.045 mL, 0.75 mmol) was then added, followed by stirring at 20 °C for an additional 17.5 h. The mixture was worked up as described for (*R*)-**14b** to give **14g** (250 mg, 99%) as a white solid, mp 133−139 °C; ν_{max} (cm^{-1}) 3282, 1650; ^1H NMR (400 MHz, chloroform-*d*) δ ppm 1.49 (9H, s), 4.09 (1H, t, *J* = 7.5 Hz), 4.33−4.50 (2H, m), 4.48 (1H, br s), (4.58 (1H, t, *J* = 8.6 Hz), 4.98 (1H, dd, *J* = 8.8, 7.1 Hz), 6.58 (2H, d, *J* = 8.5 Hz), 6.91 (2H, d, *J* = 8.5 Hz), 7.11−7.21 (2H, m), 7.27−7.35 (4H, m), 7.68 (1H, m), 7.83 (2H, d, *J* = 8.3 Hz), 9.37 (1H, br s); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 28.3, 46.3, 66.2, 75.7, 81.3, 115.9, 124.6, 125.6, 125.7, 126.1, 127.2, 127.5, 127.4, 130.4, 130.6, 132.9, 133.6, 142.7, 154.6, 156.3, 161.5, 165.8; *m*/*z* (ESI^+) 503 ($[M + H]^+$, 100%); HRMS calcd for $C_{28}H_{31}N_4O_5^+$, 503.2289; found, 503.2281.

(*R*)-*tert*-Butyl (2-(4-(((5-Phenyl-4,5-dihydrooxazol-2-yl)-amino)methyl)benzamido)phenyl)carbamate (*R*)-(14h). To a solution of (*R*)-2-amino-1-phenylethanol (110 mg, 0.80 mmol) in THF (8 mL) was added isothiocyanate **2** (307 mg, 0.8 mmol); the mixture was stirred at 20 °C for 18 h. Evaporation gave a residue that was dissolved in acetone (4 mL); iodomethane (0.10 mL, 1.60 mmol) was then added, and the mixture was stirred at 20 °C for 6 h. Saturated aqueous sodium hydrogen carbonate (2 mL) was added, and the mixture stirred for 10 min, diluted with saturated aqueous sodium hydrogen carbonate (20 mL), and extracted with ethyl acetate (2 × 20 mL). The combined organic layers were dried (Na_2SO_4) and evaporated to give a yellow oil that was purified by flash column chromatography on silica gel (gradient elution from 1 to 5% of methanolic 2 M ammonia in dichloromethane) to give (*R*)-**14h** (291 mg, 75%) as a white solid, mp 97−99 °C; ν_{max} (cm^{-1}) 3277, 1659; ^1H NMR (400 MHz, chloroform-*d*) δ ppm 1.48 (9H, s), 3.67 (1H, dd, *J* = 12.2, 7.3 Hz), 4.14 (1H, dd, *J* = 12.2, 9.2 Hz), 4.41 (2H, s), 5.02 (1H, br s), 5.49 (1H, t, *J* = 8.3 Hz), 7.04−7.19 (2H, m), 7.22−7.48 (9H, m), 7.65 (1H, m), 7.84 (2H, d, *J* = 8.3 Hz), 9.48 (1H, br s); ^{13}C NMR (100 MHz, chloroform-*d*) δ ppm 28.3, 46.4, 60.1, 80.9, 81.7, 124.5, 125.4, 125.6, 125.7, 125.9, 127.1, 127.8, 128.4, 128.8, 130.5, 130.6,

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133.1, 140.3, 142.8, 154.7, 160.8, 165.7; *m/z* (ESI$^+$) 487 ([M + H]$^+$, 100%); HRMS calcd for $C_{28}H_{31}N_4O_4^+$, 487.2340; found, 487.2342.

(*S*)-*tert*-Butyl (2-(4-(((5-Phenyl-4,5-dihydrooxazol-2-yl)-amino)methyl)benzamido)phenyl)carbamate (*S*)-(14h). To a solution of (*S*)-2-amino-1-phenylethanol (110 mg, 0.8 mmol) in THF (8 mL) was added isothiocyanate **2** (307 mg, 0.80 mmol); the mixture was stirred at 20 °C for 18 h. Evaporation gave a residue that was dissolved in acetone (4 mL); iodomethane (0.10 mL, 1.6 mmol) was then added, and the solution was stirred at 20 °C for 4 h. The mixture was worked up as described for (*S*)-**14h** to give a solid that was purified by flash column chromatography on silica gel (gradient elution from 1 to 5% of methanolic 2 M ammonia in dichloromethane) to give (*S*)-**14h** (275 mg, 71%) as a white solid, mp 100−102 °C; ν_{max} (cm^{-1}) 3274 (NH), 1655 (C═O); ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 1.51 (9H, s), 3.73 (1H, dd, *J* = 12.2, 7.3 Hz), 4.20 (1H, dd, *J* = 12.3, 9.2 Hz), 4.49 (2H, s), 5.52 (1H, dd, *J* = 9.2, 7.3 Hz), 7.12−7.23 (2H, m), 7.27−7.43 (9H, m), 7.75 (1H, d, *J* = 7.6 Hz), 7.91 (2H, d, *J* = 8.1 Hz), 9.31 (1H, br s); ^{13}C NMR (100 MHz, chloroform-*d*) δ ppm 28.3, 46.6, 60.7, 81.2, 81.7, 124.5, 125.6, 125.7, 125.8, 125.9, 127.3, 127.8, 128.3, 128.8, 130.2, 130.8, 133.3, 140.5, 142.9, 154.6, 160.5, 165.5; *m/z* (ESI$^+$) 487 ([M + H]$^+$, 100%); HRMS calcd for $C_{28}H_{31}N_4O_4^+$, 487.2340; found, 487.2327.

***tert*-Butyl (2-(4-(((4,4-Diphenyl-4,5-dihydrooxazol-2-yl)-amino)methyl)benzamido)phenyl)carbamate (14i).** To a solution of 2-amino-2,2-diphenylethanol (122 mg, 0.574 mmol) in THF (6 mL) was added **2** (200 mg, 0.522 mmol), and the resulting solution was stirred at 20 °C for 24 h and then at reflux for 18 h. Evaporation of the solvent gave a residue that was dissolved in acetone (3 mL); iodomethane (0.098 mL, 1.565 mmol) was then added, and the solution was stirred at 20 °C for 4 h. The mixture was worked up as described for (*R*)-**14h** to give a solid that was purified by flash column chromatography on silica gel (gradient elution from 1 to 5% of methanolic 2 M ammonia in dichloromethane) to give **14i** (290 mg, 99%) as a white solid, mp 104−106 °C; ν_{max} (cm^{-1}) 3276, 1658; ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 1.50 (9H, s), 4.49 (2H, s), 4.80 (2H, s), 7.08−7.39 (16H, m), 7.72 (1H, d, *J* = 6.8 Hz), 7.87 (2H, d, *J* = 8.3 Hz), 9.26 (1H, br s); ^{13}C NMR (100 MHz, chloroform-*d*) δ ppm 28.3, 46.5, 80.0, 81.2, 124.6, 125.7, 125.8, 126.0, 126.6, 126.9, 127.4, 127.7, 128.3, 130.2, 130.8, 133.1, 143.3, 146.9, 154.7, 160.1, 165.6; *m/z* (ESI$^+$) 563 ([M + H]$^+$, 100%); HRMS calcd for $C_{34}H_{35}N_4O_4^+$, 563.2653; found, 563.2639.

***tert*-Butyl (2-(4-((((4*S*,5*S*)-4,5-Diphenyl-4,5-dihydrooxazol-2-yl)amino)methyl)benzamido)phenyl)carbamate (14j).** To a solution of (1*S*,2*S*)-2-amino-1,2-diphenylethanol (122 mg, 0.574 mmol) in THF (6 mL) was added isothiocyanate **2** (200 mg, 0.522 mmol); the mixture was then stirred at 20 °C for 16 h. Evaporation gave a residue that was dissolved in acetone (3 mL); iodomethane (0.01 mL, 1.56 mmol) was then added, and the solution was stirred at 20 °C for 5.5 h. The mixture was worked up as described for (*R*)-**14h** to give a solid that was purified by flash column chromatography on silica gel (gradient elution from 1 to 5% of methanolic 2 M ammonia in dichloromethane) to give **14j** (165 mg, 56%) as a white solid, mp 137−139 °C; ν_{max} (cm^{-1}) 3271, 1656; ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 1.51 (9H, s), 4.15 (1H, br s), 4.46−4.60 (2H, m), 4.98 (1H, d, *J* = 6.8 Hz), 5.23 (1H, d, *J* = 6.8 Hz), 7.08−7.47 (16H, m), 7.75 (1H, dd, *J* = 7.8, 1.0 Hz), 7.92 (2H, d, *J* = 8.1 Hz), 9.32 (1H, br s); ^{13}C NMR (100 MHz, chloroform-*d*) δ ppm 28.3, 46.5, 76.1, 81.2, 89.6, 124.5, 125.7, 125.8, 125.9, 126.5, 127.3, 127.6, 127.8, 128.6, 128.7, 128.9, 130.2, 130.8, 133.3, 139.7, 142.7, 142.9, 154.7, 160.8, 165.5; *m/z* (ESI$^+$) 563 ([M + H]$^+$, 100%); HRMS calcd for $C_{34}H_{35}N_4O_4^+$, 563.2653; found, 563.2639.

***tert*-Butyl (2-(4-((((4*R*,5*S*)-4,5-Diphenyl-4,5-dihydrooxazol-2-yl)amino)methyl)benzamido)phenyl)carbamate (14k).** To a solution of (1*S*,2*R*)-2-amino-1,2-diphenylethanol (171 mg, 0.8 mmol) in THF (8 mL) was added isothiocyanate **2** (307 mg, 0.80 mmol); the mixture was then stirred at 20 °C for 18 h. Evaporation gave a residue that was dissolved in acetone (4 mL); iodomethane (0.10 mL, 1.60 mmol) was then added, and the solution was stirred at 20 °C for 6 h. The mixture was worked up as described for (*R*)-**14h** to give an oil that was purified by flash column chromatography on silica gel (gradient elution from 1 to 5% of methanolic 2 M ammonia in dichloromethane) and then with a SCX-2 cartridge (washed with methanol and then eluted with 2 M ammonia in methanol) to give **14k** (421 mg, 85%) as a white solid, mp 121−123 °C; ν_{max} (cm^{-1}) 3277, 1653; ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 1.51 (9H, s), 4.48−4.59 (2H, m), 5.40 (1H, d, *J* = 8.9 Hz), 5.84 (1H, d, *J* = 8.9 Hz), 6.84−6.92 (4H, m), 6.96−7.09 (6H, m), 7.10−7.22 (2H, m), 7.28 (1H, dd, *J* = 7.2, 2.1 Hz), 7.35−7.46 (3H, m), 7.74 (1H, d, *J* = 7.6 Hz), 7.90 (2H, d, *J* = 8.1 Hz), 9.36 (1H, br s); ^{13}C NMR (100 MHz, chloroform-*d*) δ ppm 28.3, 46.5, 71.9, 81.1, 85.8, 124.6, 125.7, 126.0, 126.2, 126.8, 127.4, 127.5, 127.6, 127.6, 127.8, 130.3, 130.7, 133.2, 136.4, 139.1, 143.1, 154.7, 161.6, 165.6; *m/z* (ESI$^+$) 563 ([M + H]$^+$, 100%); HRMS calcd for $C_{34}H_{35}N_4O_4^+$, 563.2653; found, 563.2641.

(*R*)-*tert*-Butyl (2-(4-(((4-((1*H*-Imidazol-4-yl)methyl)-4,5-dihydrooxazol-2-yl)amino)methyl)benzamido)phenyl)carbamate (*R*)-(14l). To a mixture of (*R*)-2-amino-3-(1*H*-imidazol-4-yl)propan-1-ol dihydrochloride (123 mg, 0.574 mmol) in THF (10 mL) were added potassium carbonate (0.16 mg, 1.15 mmol) and **2** (200 mg, 0.522 mmol). The mixture was stirred at 72 °C for 72 h. The mixture was filtered, and evaporation of the filtrate gave a residue that was dissolved in acetone (6 mL); iodomethane (0.065 mL, 1.04 mmol) was then added, and the solution was stirred at 20 °C for 18 h. The mixture was worked up as described for (*R*)-**14h** to give a solid that was purified by flash column chromatography on silica gel (gradient elution from 2 to 20% of methanolic 2 M ammonia in dichloromethane) to give (*R*)-**14l** (110 mg, 43%) as a white solid, mp 159−163 °C; ν_{max} (cm^{-1}) 3664 (NH), 3382 (NH), 1679, 1641; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.46 (9H, s), 2.62 (1H, dd, *J* = 15.4, 5.8 Hz), 3.09 (1H, dd, *J* = 15.4, 7.4 Hz), 3.75 (1H, dd, *J* = 11.0, 5.5 Hz), 4.24 (1H, dd, *J* = 11.0, 7.0 Hz), 4.31 (2H, d, *J* = 6.0 Hz), 4.75 (1H, m), 6.53 (1H, t, *J* = 6.0 Hz), 6.62 (1H, s), 6.66 (1H, d, *J* = 7.4 Hz), 7.12−7.26 (2H, m), 7.41 (2H, d, *J* = 8.2 Hz), 7.49−7.61 (3H, m), 7.91 (2H, d, *J* = 8.0 Hz), 8.69 (1H, br s), 9.81 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 28.5, 29.6, 43.1, 50.7, 55.0, 80.1, 119.8, 124.4, 124.6, 126.0, 126.4, 127.4, 128.0, 130.3, 131.4, 132.1, 133.0, 134.7, 145.5, 153.9, 157.9, 165.6; *m/z* (ESI$^+$) 491 ([M + H]$^+$, 100%); HRMS calcd for $C_{26}H_{31}N_6O_4^+$, 491.2401; found, 491.2390.

(*S*)-*tert*-Butyl (2-(4-(((4-((1*H*-Imidazol-4-yl)methyl)-4,5-dihydrooxazol-2-yl)amino)methyl)benzamido)phenyl)carbamate (*S*)-(14l). To a mixture of (*S*)-2-amino-3-(1*H*-imidazol-4-yl)propan-1-ol dihydrochloride (178 mg, 0.83 mmol) in THF (6 mL) was added potassium carbonate (230 mg, 1.67 mmol) followed by a solution of **2** (319 mg, 0.833 mmol) in THF (6 mL). The resulting mixture was stirred at 20 °C for 17 h and was then heated at reflux for 72 h. The mixture was filtered, and evaporation of the filtrate gave a residue that was dissolved in acetone (6 mL); iodomethane (0.104 mL, 1.67 mmol) was then added, and the solution was stirred at 20 °C for 5 h. The mixture was worked up as described for (*R*)-**14h** to give a solid that was purified by flash column chromatography on silica gel (gradient elution from 2 to 20% of methanolic 2 M ammonia in dichloromethane) to give (*S*)-**14l** (135 mg, 33%) as a white solid, mp 155−157 °C; ν_{max} (cm^{-1}) 3310, 1635; ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 1.51 (9H, s), 2.74 (1H, ddd, *J* = 15.6, 5.5, 1.0 Hz), 3.23 (1H, ddd, *J* = 15.6, 7.3, 1.0 Hz), 3.87 (1H, dd, *J* = 11.4, 5.3 Hz), 4.35 (1H, dd, *J* = 11.4, 6.7 Hz), 4.43 (2H, s), 4.95 (1H, m), 6.69 (1H, s), 7.19−7.29 (2H, m), 7.40−7.49 (3H, m), 7.57 (1H, s), 7.63 (1H, m), 7.94 (2H, d, *J* = 8.1 Hz); ^{13}C NMR (100 MHz, methanol-d_4) δ ppm 27.2, 28.8, 42.9, 50.6, 54.8, 80.3, 118.8, 124.3, 124.9, 125.7, 126.0, 126.9, 127.4, 130.3, 130.9, 131.6, 132.7, 134.6, 144.6, 154.9, 158.8, 166.7; *m/z* (ESI$^+$) 491 ([M + H]$^+$, 100%); HRMS calcd for $C_{26}H_{31}N_6O_4^+$, 491.2401; found, 491.2382.

(*S*)-*N*-(2-Aminophenyl)-4-(((4-benzyl-4,5-dihydrooxazol-2-yl)amino)methyl)benzamide (15a). To a solution of **14a** (0.44 g, 0.88 mmol) in dichloromethane (3.4 mL) was added trifluoroacetic acid (0.6 mL). The solution was stirred at 20 °C for 5 h; then, trifluoroacetic acid (0.20 mL) was added, and the solution was stirred at 20 °C for a further 30 min. To the mixture were then added dichloromethane (50 mL) and saturated aqueous sodium hydrogen carbonate (30 mL), and the mixture was then stirred vigorously for 5 min. The organic layer was washed with brine (30 mL), dried

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(Na_2SO_4), and evaporated. The residue was purified by column chromatography on silica gel (1:1:98 ammonia (30% aqueous)/methanol/ethyl acetate) to give **15a** as a white solid (0.167 g, 47%), mp 146−147 °C; $[\alpha]_D^{25}$ + 8.8 (*c* 0.5, methanol); ν_{max} (cm^{-1}) 3202, 1674, 1647; ^1H NMR (500 MHz, chloroform-*d*) δ ppm 2.62 (1H, dd, *J* = 13.5, 8.3 Hz), 3.00 (1H, dd, *J* = 13.5, 5.0 Hz), 3.87 (2H, br s), 3.98 (1H, t, *J* = 7.7 Hz), 4.18 (1H, t, *J* = 8.1 Hz), 4.26 (1H, m), 4.32−4.44 (2H, m), 6.74−6.85 (2H, m), 7.07 (1H, t, *J* = 7.3 Hz), 7.15 (2H, d, *J* = 7.1 Hz), 7.20 (1H, t, *J* = 6.9 Hz), 7.23−7.36 (5H, m), 7.81 (2H, d, *J* = 7.4 Hz), 8.22 (1H, br s); *m/z* (CI) 401 ([M + H]$^+$, 100%); HRMS calcd for $C_{24}H_{25}N_4O_2^+$, 401.1972; found, 401.1963.

(*S*)-*N*-(2-Aminophenyl)-4-(((4-phenyl-4,5-dihydrooxazol-2-yl)amino)methyl)benzamide (*S*)-(15b). To a solution of (*S*)-**14b** (103 mg, 0.21 mmol) in dichloromethane (0.90 mL) was added trifluoroacetic acid (0.10 mL). The solution was stirred at 20 °C for 1.5 h; then, trifluoroacetic acid (0.05 mL) was added, and the solution was stirred at 20 °C for a further 2.5 h. The reaction was diluted with dichloromethane (10 mL) and saturated aqueous sodium hydrogen carbonate (10 mL) and then stirred vigorously for 5 min. The organic layer washed with saturated aqueous sodium hydrogen carbonate (10 mL), dried (Na_2SO_4), and evaporated. Purification by column chromatography on silica gel (1:1:98 ammonia (30% aqueous)/methanol/ethyl acetate) gave (*S*)-**15b** as a white solid (60 mg, 74%), mp 85−86 °C; $[\alpha]_D^{25}$ + 12.2 (*c* 0.5, methanol); ν_{max} (cm^{-1}) 3254, 1649; ^1H NMR (500 MHz, chloroform-*d*) δ ppm 3.91 (2H, br s), 4.09 (1H, t, *J* = 7.6 Hz), 4.42 (2H, br s), 4.62 (1H, t, *J* = 8.5 Hz), 5.09 (1H, t, *J* = 7.9 Hz), 6.75−6.88 (2H, m), 7.09 (1H, t, *J* = 7.6 Hz), 7.20−7.38 (8H, m), 7.80 (2H, d, *J* = 6.3 Hz), 8.32 (1H, br s); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 46.9, 67.9, 75.7, 118.7, 120.0, 125.0, 125.8, 126.8, 127.6, 127.7, 127.8, 128.1, 129.0, 133.5, 141.3, 143.6, 144.0, 162.1, 166.2; *m/z* (ESI$^-$) 385 ([M − H]$^-$, 100%); HRMS calcd for $C_{23}H_{21}N_4O_2^-$, 385.1670; found, 385.1673.

(*R*)-*N*-(2-Aminophenyl)-4-(((4-phenyl-4,5-dihydrooxazol-2-yl)amino)methyl)benzamide (*R*)-(15b). To a solution of (*R*)-**14b** (150 mg, 0.308 mmol) in dichloromethane (5 mL) was added trifluoroacetic acid (0.50 mL, 6.2 mmol) dropwise; then, the mixture was stirred at 20 °C for 3 h. Saturated aqueous sodium hydrogen carbonate (20 mL) was then added, and the mixture was extracted with ethyl acetate (2 × 30 mL). The combined organic layers were washed with brine (30 mL), dried (Na_2SO_4), and evaporated to give a white solid that was purified by column chromatography on silica gel (1:1:98 ammonia (30% aqueous)/methanol/ethyl acetate) to give (*R*)-**15b** as a white solid (38 mg, 32%), mp 115−118 °C; $[\alpha]_D^{25}$ −14.0 (*c* 1.0, methanol); ν_{max} (cm^{-1}) 3287, 1649; ^1H NMR (300 MHz, chloroform-*d*) δ ppm 4.04 (1H, t, *J* = 7.5 Hz), 4.11 (1H, br s), 4.33 (2H, s), 4.56 (1H, t, *J* = 8.6 Hz), 5.02 (1H, dd, *J* = 8.8, 7.3 Hz), 6.68−6.61 (2H, m), 7.04 (1H, td, *J* = 7.6, 1.0 Hz), 7.12−7.36 (8H, m), 7.73 (2H, d, *J* = 7.9 Hz), 8.52 (1H, br s); ^{13}C NMR (75 MHz, chloroform-*d*) δ ppm 46.5, 67.4, 75.5, 118.3, 119.6, 124.6, 125.8, 126.6, 127.4, 127.6, 127.9, 128.8, 133.1, 141.2, 143.2, 143.6, 161.9, 166.1; *m/z* (ESI$^+$) 387 ([M + H]$^+$, 100%); HRMS calcd for $C_{23}H_{23}N_4O_2^+$, 387.1816; found, 387.1808.

(*S*)-*N*-(2-Aminophenyl)-4-(((4-(pyridin-3-yl)-4,5-dihydrooxazol-2-yl)/amino)methyl)benzamide (15c). To a solution of **14c** (80 mg, 0.164 mmol) in dichloromethane (1.0 mL) was added trifluoroacetic acid (0.25 mL, 3.25 mmol). The resulting solution was stirred at 20 °C for 1.5 h. The mixture was worked up as for (*R*)-**15b** to give a solid that was purified by flash column chromatography on silica gel (gradient elution from 2 to 10% of methanolic 2 M ammonia in dichloromethane) to give **15c** (38 mg, 60%) as a white solid, mp 89−90 °C (dec.); $[\alpha]_D^{25}$ −20.0 (*c* 1.0, methanol); ν_{max} (cm^{-1}) 3214, 1652; ^1H NMR (400 MHz, methanol-d_4) δ ppm 4.12 (1H, dd, *J* = 8.3, 6.6 Hz), 4.42−4.58 (2H, m), 4.73 (1H, dd, *J* = 9.1, 8.3 Hz), 5.16 (1H, dd, *J* = 9.1, 6.6 Hz), 6.79 (1H, td, *J* = 7.6, 1.0 Hz), 6.93 (1H, dd, *J* = 8.1, 1.0 Hz), 7.10 (1H, td, *J* = 7.6, 1.3 Hz), 7.22 (1H, d, *J* = 7.6 Hz), 7.41 (1H, dd, *J* = 7.8, 5.1 Hz), 7.53 (2H, d, *J* = 8.1 Hz), 7.70 (1H, dt, *J* = 7.8, 1.8 Hz), 8.00 (2H, d, *J* = 8.1 Hz), 8.41−8.49 (2H, m); ^{13}C NMR (100 MHz, methanol-d_4) δ ppm 45.5, 64.5, 74.6, 117.3, 118.3, 123.9, 126.3, 126.9, 127.1, 127.7, 127.7, 133.1, 135.0, 140.1, 142.4, 143.3, 147.2, 147.7, 163.3, 167.3; *m/z* (ESI$^+$) 388 ([M + H]$^+$, 100%); HRMS calcd for $C_{22}H_{22}N_5O_2^+$, 388.1768; found, 388.1774.

***N*-(2-Aminophenyl)-4-(((4-(4-fluorophenyl)-4,5-dihydrooxazol-2-yl)amino)methyl)benzamide (15d).** To a solution of **14d** (220 mg, 0.436 mmol) in dichloromethane (6 mL) was added trifluoroacetic acid (0.67 mL, 8.7 mmol) dropwise; then, the mixture was stirred at 20 °C for 3 h. The mixture was then worked up as for (*R*)-**15b** to give a solid that was purified by column chromatography on silica gel (1:1:98 ammonia (30% aqueous)/methanol/ethyl acetate) and again (1:6:93 ammonia (30% aqueous)/methanol/ethyl acetate) to give **15d** as a white solid (108 mg, 61%), mp 104−108 °C; ν_{max} (cm^{-1}) 3245, 1649; ^1H NMR (500 MHz, chloroform-*d*) δ ppm 4.02 (1H, m), 4.09 (2H, br s), 4.39 (2H, s), 4.60 (1H, m), 5.08 (1H, m), 6.71−6.86 (2H, m), 6.93−7.03 (2H, m), 7.07 (1H, m), 7.12−7.22 (2H, m), 7.22−7.36 (3H, m), 7.70−7.87 (2H, m), 8.44 (1H, br s); ^{13}C NMR (125 MHz, chloroform-*d*) δ ppm 46.4, 66.7, 75.3, 115.3 (d, *J* = 22.1 Hz), 118.1, 119.5, 124.4, 125.5, 127.2, 127.6, 127.9 (d, *J* = 8.6 Hz), 133.0, 139.2, 140.9, 142.9, 162.0 (d, *J* = 245.7 Hz), 161.7), 165.7; *m/z* (ESI$^+$) 405 ([M + H]$^+$, 100%); HRMS calcd for $C_{23}H_{22}FN_4O_2^+$, 405.1721; found, 405.1710.

***N*-(2-Aminophenyl)-4-(((4-(3-fluorophenyl)-4,5-dihydrooxazol-2-yl)amino)methyl)benzamide (15e).** To a solution of **14e** (190 mg, 0.377 mmol) in dichloromethane (3 mL) was added trifluoroacetic acid (0.60 mL, 7.5 mmol) dropwise. The resulting solution was stirred at 20 °C for 2 h. The mixture was then worked up as for (*R*)-**15b** to give a solid that was purified by flash column chromatography on silica gel (gradient elution from 1 to 6% of methanolic 2 M ammonia in dichloromethane) to give **15e** (131 mg, 86%) as a white solid, mp 84−86 °C; ν_{max} (cm^{-1}) 3276, 1652; ^1H NMR (400 MHz, methanol-d_4) δ ppm 4.05 (1H, dd, *J* = 8.2, 6.4 Hz), 4.41−4.56 (2H, m), 4.68 (1H, dd, *J* = 9.1, 8.3 Hz), 5.09 (1H, dd, *J* = 9.0, 6.4 Hz), 6.79 (1H, td, J = 7.6, 1.0 Hz), 6.88−7.02 (3H, m), 7.03−7.14 (2H, m), 7.21 (1H, d, *J* = 7.8 Hz), 7.33 (1H, td, *J* = 7.9, 5.9 Hz), 7.53 (2H, d, *J* = 8.1 Hz), 8.00 (2H, d, *J* = 8.1 Hz); ^{13}C NMR (100 MHz, methanol-d_4) δ ppm 45.6, 66.2, 74.9, 112.7 (d, *J* = 22.4 Hz), 113.7 (d, *J* = 20.8 Hz), 117.3, 118.3, 121.9 (d, *J* = 3.2 Hz), 124.0, 126.3, 127.0, 127.2, 127.7, 130.0 (d, *J* = 8.0 Hz), 133.0, 142.4, 143.4, 146.8 (d, *J* = 7.2 Hz), 163.0 (d, *J* = 245.3 Hz), 163.0, 167.2; *m/z* (ESI$^+$) 405 ([M + H]$^+$, 100%); HRMS calcd for $C_{23}H_{22}FN_4O_2^+$, 405.1721; found, 405.1723; 94% purity by LC-MS.

***N*-(2-Aminophenyl)-4-(((4-(4-(trifluoromethyl)phenyl)-4,5-dihydrooxazol-2-yl)amino)methyl)benzamide (15f).** To a solution of **14f** (190 mg, 0.343 mmol) in dichloromethane (4 mL) was added trifluoroacetic acid (0.53 mL, 6.85 mmol). The resulting solution was stirred at 20 °C for 2.5 h. The mixture was then worked up as for (*R*)-**15b** to give a solid that was purified by flash column chromatography on silica gel (gradient elution from 1 to 6% of methanolic 2 M ammonia in dichloromethane) to give **15f** (110 mg, 71%) as a white solid, mp 198−199 °C; ν_{max} (cm^{-1}) 3265, 1653; ^1H NMR (500 MHz, methanol-d_4) δ ppm 4.08 (1H, dd, *J* = 8.2, 6.6 Hz), 4.44−4.58 (2H, m), 4.73 (1H, dd, *J* = 9.2, 8.2 Hz), 5.18 (1H, dd, *J* = 9.2, 6.6 Hz), 6.80 (1H, t, *J* = 7.4 Hz), 6.93 (1H, d, *J* = 8.0 Hz), 7.11 (1H, t, *J* = 7.1 Hz), 7.22 (1H, d, *J* = 7.7 Hz), 7.43 (2H, d, *J* = 8.0 Hz), 7.54 (2H, d, *J* = 8.0 Hz), 7.64 (2H, d, *J* = 8.0 Hz), 8.01 (2H, d, *J* = 8.0 Hz); ^{13}C NMR (125 MHz, methanol-d_4) δ ppm 45.6, 66.3, 74.8, 117.4, 118.3, 124.3 (q, *J* = 271.0 Hz), 124.0, 125.1 (q, *J* = 3.9 Hz), 126.3, 126.7, 127.0, 127.2, 127.7, 129.2 (q, *J* = 32.4 Hz), 133.0, 142.4, 143.4, 148.3, 163.2, 167.2; *m/z* (ESI$^+$) 455 ([M + H]$^+$, 100%); HRMS calcd for $C_{24}H_{22}F_3N_4O_2^+$, 455.1689; found, 455.1686; 92% purity by LC-MS.

(*S*)-*N*-(2-Aminophenyl)-4-(((4-(4-hydroxyphenyl)-4,5-dihydrooxazol-2-yl)amino)methyl)benzamide (15g). To a solution of **14g** (250 mg, 0.497 mmol) in dichloromethane (6 mL) was added trifluoroacetic acid (0.77 mL, 10 mmol). The resulting solution was stirred at 20 °C for 2.5 h. The mixture was then worked up as for (*R*)-**15b** to give a solid that was purified by flash column chromatography on silica gel (gradient elution from 1 to 10% of methanolic 2 M ammonia in dichloromethane) to give **15g** (141 mg, 70%) as a white solid, mp 158−160 °C; $[\alpha]_D^{25}$ + 17.6 (*c* 0.5, methanol); ν_{max} (cm^{-1}) 3262, 1646; ^1H NMR (400 MHz, methanol-d_4) δ ppm 4.03 (1H, dd, *J* = 8.0, 6.8 Hz), 4.39−4.54 (2H, m), 4.62 (1H, dd, *J* = 9.0, 8.0 Hz), 4.99

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(1H, dd, J = 9.0, 6.8 Hz), 6.74 (2H, d, J = 8.6 Hz), 6.79 (1H, td, J = 7.6, 1.0 Hz), 6.92 (1H, dd, J = 7.8, 1.2 Hz), 7.02−7.14 (3H, m), 7.22 (1H, d, J = 7.8 Hz), 7.52 (2H, d, J = 8.1 Hz), 7.98 (2H, d, J = 8.3 Hz); ^{13}C NMR (100 MHz, methanol-d_4) δ ppm 45.6, 66.3, 75.3, 114.9, 117.4, 118.3, 124.0, 126.3, 127.0, 127.1, 127.2, 127.6, 132.9, 134.4, 142.3, 143.6, 156.5, 162.5, 167.3; m/z (ESI$^+$) 403 ([M + H]$^+$, 100%); HRMS calcd for $C_{23}H_{23}N_4O_3^+$, 403.1765; found, 403.1765.

(*R*)-*N*-(2-Aminophenyl)-4-(((5-phenyl-4,5-dihydrooxazol-2-yl)amino)methyl)benzamide (*R*)-(15h). To a solution of (*R*)-**14h** (280 mg, 0.575 mmol) in dichloromethane (4 mL) was added trifluoroacetic acid (0.60 mL, 7.8 mmol) dropwise. The resulting solution was stirred at 20 °C for 3.5 h. The mixture was then worked up as for (*R*)-**15b** to give a solid that was purified by flash column chromatography on silica gel (gradient elution from 1.6 to 8% of methanolic 2 M ammonia in dichloromethane) to give a white solid (140 mg). Further purification by MDAP gave (*R*)-**15h** (75 mg, 34%) as a white solid, mp 196−197 °C; $[\alpha]_D^{25}$ −23.0 (c 1.0, methanol); ν_{max} (cm^{-1}) 3398, 3238, 3340, 1668, 1641; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.44 (1H, dd, J = 12.2, 6.6 Hz), 4.04 (1H, dd, J = 12.2, 9.2 Hz), 4.38 (2H, s), 4.90 (2H, br s, NH_2), 5.51 (1H, dd, J = 9.2, 6.6 Hz), 6.62 (1H, t, J = 7.3 Hz,), 6.80 (1H, d, J = 7.8 Hz), 6.98 (1H, t, J = 7.3 Hz), 7.10−7.24 (2H, m), 7.26−7.36 (3H, m), 7.40 (2H, t, J = 7.1 Hz), 7.46 (2H, d, J = 8.0 Hz), 7.96 (2H, d, J = 8.0 Hz), 9.64 (1H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 46.1, 61.2, 80.3, 116.6, 116.8, 123.9, 125.9, 126.9, 127.1, 127.3, 128.2, 128.3, 129.1, 133.5, 142.2, 143.6, 144.2, 160.5, 165.7; m/z (ESI$^+$) 387 ([M + H]$^+$, 100%); HRMS calcd for $C_{23}H_{23}N_4O_2^+$, 387.1816; found, 387.1813.

(*S*)-*N*-(2-Aminophenyl)-4-(((5-phenyl-4,5-dihydrooxazol-2-yl)amino)methyl)benzamide (*S*)-(15h). To a solution of (*S*)-**14h** (0.275 g, 0.565 mmol) in dichloromethane (6 mL) was added trifluoroacetic acid (0.90 mL, 11.5 mmol). The resulting solution was stirred at 20 °C for 3 h. The mixture was then worked up as for (*R*)-**15b** to give a solid that was purified by MDAP to give (*S*)-**15h** (75 mg, 34%) as a cream solid, mp 214−215 °C; $[\alpha]_D^{25}$ + 22.0 (c 0.5, methanol); ν_{max} (cm^{-1}) 3338, 3237, 1668, 1641; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 3.49 (1H, dd, J = 11.8, 7.0 Hz), 4.08 (1H, dd, J = 11.8, 9.1 Hz), 4.41 (2H, s), 4.91 (2H, br s), 5.61 (1H, t, J = 8.0 Hz), 6.62 (1H, t, J = 7.3 Hz), 6.80 (1H, d, J = 7.7 Hz), 6.99 (1H, t, J = 7.0 Hz), 7.19 (1H, d, J = 7.4 Hz), 7.31−7.38 (3H, m), 7.39−7.44 (2H, m), 7.46 (2H, d, J = 8.0 Hz), 7.97 (2H, d, J = 7.7 Hz), 9.67 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 46.0, 59.5, 80.9, 116.6, 116.7, 123.8, 126.1, 126.9, 127.2, 127.3, 128.3, 128.6, 129.1, 133.7, 141.3, 143.6, 143.6, 160.7, 165.6; m/z (ESI$^+$) 387 ([M + H]$^+$, 100%); HRMS calcd for $C_{23}H_{23}N_4O_2^+$, 387.1816; found, 387.1822.

***N*-(2-Aminophenyl)-4-(((4,4-diphenyl-4,5-dihydrooxazol-2-yl)amino)methyl)benzamide (15i).** To a solution of **14i** (280 mg, 0.498 mmol) in dichloromethane (6 mL) was added trifluoroacetic acid (0.80 mL, 10 mmol). The resulting solution was stirred at 20 °C for 2.5 h. The mixture was then worked up as for (*R*)-**15b** to give a solid that was purified by flash column chromatography on silica gel (gradient elution from 1 to 6% of methanolic 2 M ammonia in dichloromethane) to give **15i** (135 mg, 59%) as a white solid, mp 176−177 °C; ν_{max} (cm^{-1}) 3194, 1670, 1649; ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 4.54 (2H, s), 4.79 (2H, s), 6.79 (1H, td, J = 7.6, 0.8 Hz), 6.92 (1H, dd, J = 8.1, 1.2 Hz), 7.10 (1H, td, J = 7.7, 1.3 Hz), 7.17−7.37 (11H, m), 7.49 (2H, d, J = 8.2 Hz), 7.96 (2H, d, J = 8.2 Hz); ^{13}C NMR (100 MHz, methanol-d_4) δ ppm 45.4, 76.4, 80.0, 117.4, 118.3, 124.0, 126.3, 126.6, 126.6, 127.0, 127.1, 127.6, 127.8, 132.9, 142.4, 143.6, 146.4, 161.4, 167.2; m/z (ESI$^+$) 463 ([M + H]$^+$, 100%); HRMS calcd for $C_{29}H_{27}N_4O_2^+$, 463.2129; found, 463.2118.

***N*-(2-Aminophenyl)-4-((((4*S*,5*S*)-4,5-diphenyl-4,5-dihydrooxazol-2-yl)amino)methyl)benzamide (15j).** To a solution of **14j** (155 mg, 0.275 mmol) in dichloromethane (2 mL) was added trifluoroacetic acid (0.42 mL, 5.5 mmol) dropwise. The resulting solution was stirred at 20 °C for 2 h. The mixture was then worked up as for (*R*)-**15b** to give a solid that was purified by flash column chromatography on silica gel (gradient elution from 1 to 6% of methanolic 2 M ammonia in dichloromethane) to give **15j** (125 mg, 98%) as a white solid, mp 110−113 °C; $[\alpha]_D^{25}$ −33.2 (c 1.0, methanol); ν_{max} (cm^{-1}) 3264, 1657; m/z (ESI$^+$) 463 ([M + H]$^+$, 100%); ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 4.46−4.63 (2H, m), 4.89 (1H, m), 5.23 (1H, d, J = 6.6 Hz), 6.79 (1H, t, J = 7.5 Hz), 6.92 (1H, dd, J = 8.1, 1.3 Hz), 7.10 (1H, td, J = 7.7, 1.5 Hz), 7.19−7.44 (11H, m), 7.57 (2H, d, J = 8.3 Hz), 8.02 (2H, d, J = 8.1 Hz); ^{13}C NMR (100 MHz, methanol-d_4) δ ppm 45.6, 75.9, 89.3, 117.4, 118.3, 124.0, 125.3, 126.2, 126.3, 127.1, 127.2, 127.4, 127.7, 128.2, 128.4, 128.5, 133.0, 139.9, 142.4, 142.8, 143.4, 162.0, 167.3; HRMS calcd for $C_{29}H_{27}N_4O_2^+$, 463.2129; found, 463.2128; 94% purity by LC-MS.

***N*-(2-Aminophenyl)-4-((((4*R*,5*S*)-4,5-diphenyl-4,5-dihydrooxazol-2-yl)amino)methyl)benzamide (15k).** To a solution of **14k** (414 mg, 0.736 mmol) in dichloromethane (6 mL) was added trifluoroacetic acid (1.15 mL, 15 mmol) dropwise. The resulting solution was stirred at 20 °C for 5 h. To the mixture were added saturated aqueous sodium hydrogen carbonate (20 mL) and ethyl acetate (50 mL). After extraction, the aqueous layer was separated and extracted with another portion of ethyl acetate (20 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), and evaporated to give a white solid. Purification by flash column chromatography on silica gel (gradient elution from 3 to 6% of methanolic 2 M ammonia in dichloromethane) followed by MDAP gave **15k** (107 mg, 31%) as a white solid, mp 112−113 °C; $[\alpha]_D^{25}$ + 51.6 (c 0.5, methanol); ν_{max} (cm^{-1}) 3270, 1652; ^{1}H NMR (400 MHz, chloroform-d) δ ppm 3.90 (2H, br s), 4.39−4.63 (2H, m), 5.38 (1H, d, J = 8.8 Hz), 5.81 (1H, d, J = 8.8 Hz), 6.73−6.94 (6H, m), 6.94−7.17 (7H, m), 7.30 (1H, m), 7.40 (2H, d, J = 7.8 Hz), 7.84 (2H, d, J = 7.8 Hz), 8.30 (1H, br s); ^{13}C NMR (100 MHz, chloroform-d) δ ppm 46.5, 72.0, 85.7, 118.3, 119.7, 124.6, 125.4, 126.2, 126.8, 127.2, 127.4, 127.5, 127.5, 127.6, 127.6, 127.7, 133.2, 136.4, 139.2, 140.9, 143.3, 161.5, 165.8; m/z (ESI$^+$) 463 ([M + H]$^+$, 100%); HRMS calcd for $C_{29}H_{27}N_4O_2^+$, 463.2129; found, 463.2128.

(*R*)-4-(((4-((1*H*-Imidazol-4-yl)methyl)-4,5-dihydrooxazol-2-yl)amino)methyl)-*N*-(2-aminophenyl)benzamide (*R*)-(15l). To a suspension of (*R*)-**14l** (100 mg, 0.204 mmol) in dichloromethane (2 mL) was added trifluoroacetic acid (0.31 mL, 4.1 mmol) dropwise. The resulting solution was stirred at 20 °C for 2 h and then evaporated, and the residue was purified by MDAP to (*R*)-**15l** (43 mg, 54%) as a white solid, mp 191−193 °C; $[\alpha]_D^{25}$ + 6.3 (c 1.0, methanol); ν_{max} (cm^{-1}) 3227, 1673, 1627; ^{1}H NMR (500 MHz, methanol-d_4) δ ppm 2.74 (1H, dd, J = 15.5, 5.4 Hz), 3.24 (1H, dd, J = 15.5, 7.3 Hz), 3.88 (1H, dd, J = 11.3, 5.2 Hz), 4.36 (1H, dd, J = 11.1, 6.7 Hz), 4.43 (2H, s), 4.95 (1H, m), 6.70 (1H, s), 6.79 (1H, t, J = 7.3 Hz), 6.93 (1H, d, J = 7.7 Hz), 7.10 (1H, m), 7.21 (1H, d, J = 7.4 Hz), 7.45 (2H, d, J = 7.7 Hz), 7.59 (1H, s), 7.97 (2H, d, J = 8.0 Hz); ^{13}C NMR (125 MHz, methanol-d_4) δ ppm 28.8, 42.9, 50.6, 54.7, 117.3, 118.3, 118.8, 123.9, 126.2, 126.8, 127.1, 127.6, 130.9, 132.8, 134.6, 142.4, 144.2, 158.8, 167.3; m/z (ESI$^+$) 391 ([M + H]$^+$, 100%); HRMS calcd for $C_{21}H_{23}N_6O_2^+$, 391.1877; found, 391.1881.

(*S*)-4-(((4-((1*H*-Imidazol-4-yl)methyl)-4,5-dihydrooxazol-2-yl)amino)methyl)-*N*-(2-aminophenyl)benzamide (*S*)-(15l). To a suspension of **14k** (130 mg, 0.265 mmol) in dichloromethane (2 mL) was added trifluoroacetic acid (0.20 mL, 2.6 mmol) dropwise. The resulting solution was stirred at 20 °C for 3 h; then, additional trifluoroacetic acid (0.20 mL, 2.6 mmol) was added, and the mixture stirred at 20 °C for 2 h. Addition of saturated aqueous sodium hydrogen carbonate (15 mL) and then ethyl acetate (20 mL) gave an insoluble white precipitate. The organic layer was separated, and the aqueous layer was adjusted to pH 10 with sodium hydroxide (2M) and then extracted with 1:4 ethanol/chloroform (4 × 25 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), and evaporated to give a gray solid (40 mg). LC-MS showed the product was dissolved in the aqueous layer. The aqueous layer was passed over a 10 g SCX-2 cartridge, which was then washed with methanol (100 mL) and eluted with 2 M ammonia/methanol (100 mL), but LC-MS showed the product to be in the eluent and methanolic washing fraction. The methanol and water were evaporated to give a white solid that was stirred with 1:9 methanol/dichloromethane for 30 min and filtered, and the filtrate was evaporated to give a white solid that was purified by MDAP to give (*S*)-**15l** (23 mg, 22%) as a white solid, mp 192−194 °C; $[\alpha]_D^{25}$ −18.0 (c 0.1, methanol); ν_{max} (cm^{-1}) 3241, 1655; ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 2.74 (1H, ddd, J = 15.5, 5.5,

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1.0 Hz), 3.24 (1H, ddd, J = 15.5, 7.4, 1.0 Hz), 3.87 (1H, dd, J = 11.3, 5.1 Hz), 4.35 (1H, dd, J = 11.3, 6.8 Hz), 4.43 (2H, s), 4.95 (1H, m), 6.69 (1H, s), 6.79 (1H, t, J = 7.2 Hz), 6.92 (1H, dd, J = 8.1, 1.2 Hz), 7.09 (1H, m), 7.20 (1H, d, J = 7.6 Hz), 7.44 (2H, d, J = 8.3 Hz), 7.57 (1H, s), 7.96 (2H, d, J = 8.1 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 29.5, 43.1, 50.7, 55.0, 116.6, 116.7, 119.9, 123.8, 126.9, 127.1, 127.2, 128.2, 131.4, 133.4, 134.6, 143.6, 144.9, 157.9, 165.6; m/z (ESI$^+$) 391 ([M + H]$^+$, 100%); HRMS calcd for $C_{21}H_{23}N_6O_2^+$, 391.1877; found, 391.1878.

***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 synthesized according to the literature.[59] The *in vitro* HDAC assay used was based on a homogeneous fluorogenic HDAC assay.[60] Inhibitor solutions were prepared by serial dilution of a 5 mM DMSO stock solution with assay buffer. In a 96-well white NBS microplate was added 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 was added (for HDAC1, HDAC2, HDAC3-NCoR1, and HDAC8: MAL (20 μL) to give concentrations of 60, 80, 14, and 200 μM and for HDAC6 and Fluor-de-Lys SIRT1 to give a concentration of 13 μM), 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 μM TSA in assay buffer; for HDAC6 Fluor-de-Lys developer II and 2 μM 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 six different concentrations. The SEM is the calculated standard error in the IC_{50} value of the fitted line.

Calculation and Measurement of Physicochemical Properties. Chrom log D and Chrom log P values[43] and cell permeability (artificial membrane permeability)[44] were measured using procedures previously described. Solubility was measured using chemiluminescent nitrogen detection.[45]

Cellular Histone H3 Acetylation Assay and Analysis of Cell Cycle Phases and Apoptosis. *Cell Culture and Application of Compounds.* Human PC-3 (epithelial prostate-derived adenocarcinoma) and human U937 (human leukemic monocyte lymphoma) cell lines were cultured in a humidified incubator at 37 °C and 5% CO_2. PC-3 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% v/v fetal bovine serum (Life Technologies) and L-glutamine−penicillin−streptomycin solution (Sigma). The U937 cell line was maintained in RPMI 1640 medium supplemented with 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 × 10^6 cells in culture medium (10 mL) in 25 mL tissue culture flasks. A 10 mM stock solution of each HDAC inhibitor in DMSO was serially diluted, with the final inhibitor concentration being 10 μM in fresh culture medium (500 μL); that solution was added dropwise to the PC-3 cultures and U937 cell suspensions, followed by gentle mixing and then incubation for 24 h. An equivalent volume of DMSO was added to control cell cultures.

Cell Cycle Analysis by Flow Cytometry. The method described by Lea et al.[61] was used. Briefly, samples of 1 × 10^5 cells were taken following 24 h incubation with each compound and fixed in ethanol (400 μL, 70% v/v) at −20 °C. Following centrifugation at 400g 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 and then analyzed 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-G1 DNA content.

Immunoblotting. PC-3 and U937 cell pellets containing 1 × 10^6 cells were resuspended in 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 skim milk (Marvel, 5% w/v) diluted in PBS−Tween 20 (0.05% v/v) and kept for 1 h. After a PBS−Tween-20 rinse, the membrane was immunoblotted with primary antibodies against histone H3 (DIH2), acetylated histone H3 (Lys 9) (C5BII, Cell Signaling Technologies), and cyclin E (HE12) (SC-247). Detection was performed with anti-mouse or anti-rabbit horseradish 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).

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

A549, adenocarcinomic human alveolar cell line; Bis-tris, 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)-1,3-propanediol); DMEM, Dulbecco's modified Eagle's medium; FACS, fluorescence-activated cell sorting; FITC, fluorescein isothiocyanate; H3K9, histone H3 lysine 9; HDAC, histone deacetylase; HSA, human serum albumin; MAL, Boc-Lys(Ac)-7-amino-4-methylcoumarin; MDAP, mass-directed automatic purification; Mocetinostat, MGCD0103, *N*-(2-aminophenyl)-4-({[4-(pyridin-3-yl)pyrimidin-2-yl]amino}methyl)-benzamide; NBS, nonbinding surface; NCoR2, nuclear receptor corepressor 2; PC-3, human prostate cancer cell line; Pd/C, palladium on activated carbon; SCX-2, strong cation exchange resin; SEM, standard error of the mean; TSA, trichostatin A; U937, human leukemic monocyte lymphoma cell line; Vorinostat, suberoylanilide hydroxamic acid

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