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Diamine Containing VLA-4 Antagonists

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Abstract—Design and synthesis of a library as potential VLA-4 antagonists has been accomplished, based around a proposed pharmacophoric model. Compounds possessing submicromolar potency were identified and structure–activity relationships were seen across the library. Further derivatisation produced compounds with IC_{50}'s <10 nmol for inhibiting the VLA-4 mediated binding of fibronectin to RAMOS cells, providing an ideal starting point for a lead optimisation Programme.

Introduction

The cell adhesion molecule VLA-4 ($\alpha_4\beta_1$) is a member of the integrin super family of heterodimeric cell surface receptors and is found on all haemopoetic cells with the exception of neutrophils. Its primary ligands are the endothelial cell surface protein, vascular cell adhesion molecule-1 (VCAM-1) and the alternatively spliced CS-1 isoform of fibronectin, which is localised in the extracellular matrix. Recently, it has been demonstrated that besides mediating cell trafficking, VLA-4 also regulates the activation and survival of a number of cell types. Monoclonal antibodies directed against VCAM-1 and the α4 subunit have been shown to be useful modulators in animal models of chronic inflammatory diseases, including asthma and rheumatoid arthritis.[1–4] More recently, small peptide antagonists have been shown to be efficacious in preclinical models of asthma.[5–7] Subsequently, the identification of non peptide antagonists has been reported in the literature.[8,9] This has led to the belief that non-peptidic VLA-4 antagonists could be identified and used to treat diseases of this type.

At the commencement of this research a small number of peptidic antagonists had already been described in the literature.[10–12] Most interesting was a series of LDV peptidomimetics reported by Biogen.[13,14] From the reported data, it was rationalised that an aromatic ring present in the terminal amide cap and the β-carboxylate of the aspartic acid (or related residue) were essential for biological activity. De novo design was undertaken based around these moieties, with a view to constructing non-peptidic libraries which incorporated these pharmacophores.

Chemistry

The generic library **1** employing the inputs depicted in Table 1 was ideal for preparation on solid phase, employing the terminal acid pharmacophore as a point of attachment to the resin. By synthesising compounds this way, it was found that the construction, isolation and purification processes could be greatly accelerated and simplified.

The proposed synthetic sequence for the preparation of **1** was to alkylate resin bound bromoacetate, **2** and acrylate, **3**, with a series of diverse diamines (**4**–**14**) to furnish the core diamine-acid templates. Acylation of the resulting primary or secondary amine of these templates with a series of aromatic carboxylic acids caps (**15**–**28**) would provide the immobilised library members which could be cleaved from the resin using standard procedures.

A representative synthetic example for the preparation of the library is depicted in Scheme 1, using 4-(aminomethyl)piperidine, **6**, as the diamine component. Esters **2** and **3** were prepared on Wang (*p*-benzyloxybenzylalcohol) resin using standard symmetrical anhydride conditions.[15] Initial attempts to achieve high regioselectivity for the alkylation with **6** (preferential

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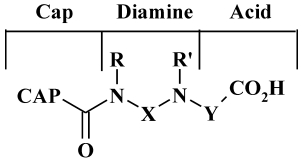
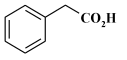
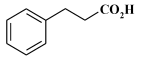
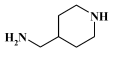
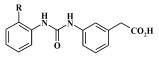
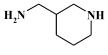
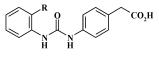
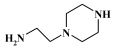
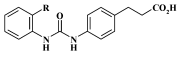
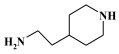
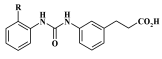
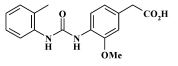
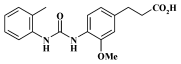
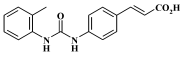
reaction of secondary amine with **2** or **3**) was not successful. Therefore, protection of the primary amino group of **6** and related non-symmetrical diamines was deemed necessary. Finding a suitable group that could be introduced easily and which was also compatible with solid phase chemistry was not trivial. The solution was to protect the primary amine as an imine.[16] Treatment of **6** with one equivalent of 3,4-dimethoxybenzaldehyde in toluene with azeotropic removal of water afforded the crude imine **29** which showed >95% purity by NMR and HPLC without the need for purification. Reaction of 5–10 equiv of **29** with resins **2** or **3** was achieved in a DMSO/diisopropylethylamine (DIPEA) mixture for 24 h. After washing of the resin, cleaved samples could be analysed by HPLC using evaporative light scattering (ELS) detection. Loadings were determined by NMR analysis employing *p*-tolylsulphone as an internal standard. Cleavage of a known quantity of resin was undertaken in 50% deuterated TFA in $CDCl_3$ for 0.5 h. The resulting spectrum were of good quality, allowing accurate integration measurement to be achieved, with purity generally >95% (typical loading values being in the region of 0.4–0.6 mmol/g).

Table 1. Components of diamine library



1

No.	Diamine (RHN-X-NHR′)	No.	Terminal cap
4		**15**	
5		**16**	
6		**17**	
7		**18** (R=H) **19** (R=Me)	
8		**20** (R=H) **21** (R=Me)	
9		**22** (R=H) **23** (R=Me)	
10		**24** (R=H) **25** (R=Me)	
11		**26**	
12		**27**	
13		**28**	
14			

Not all combinations synthesised.

The imine protecting group of **30** was easily removed by treating the resin with a known volume of a mixture of $MeCN/H_2O/TFA$ (80:20:2). By employing HPLC, the progression of the reaction could be monitored, measuring the concentration of the liberated 3,4-dimethoxybenzaldehyde. Reactions were generally complete within an hour.

Acylation of the resulting amines, **31**, with caps **15**–**28** was achieved using *o*-(7-azabenzotriazol-1-yl)1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) mediated coupling[17] to afford the resin bound products, **32**. These could be cleaved from the resin using a 1:1 TFA/CH_2Cl_2 mixture for 1 h.

Using the protocol depicted in Scheme 1, a library of approximately 200 compounds was synthesised. Products were analysed for purity by reverse phase LC/MS (>85% in all cases) and the presence of the requisite mass ion confirmed by electrospray mass spectrometry.

Subsequent work focused on using **33** as a pivotal intermediate for the synthesis of additional VLA-4 inhibitors. It is available from commercial 4-(aminomethyl)piperidine as outlined in Scheme 2. Selective protection of the secondary amine was achieved following the method of Prugh[16] to afford **34**. Acylation, using standard conditions, gave **35** and removal of the BOC protecting group, with trifluoroacetic acid, provided **33** in quantitative yield.

The solution synthesis of **36** is depicted in Scheme 3. Reaction of ethyl crotonate and **33** proceeds smoothly at room temperature and saponification of the resulting ester provided **36** in acceptable yield.

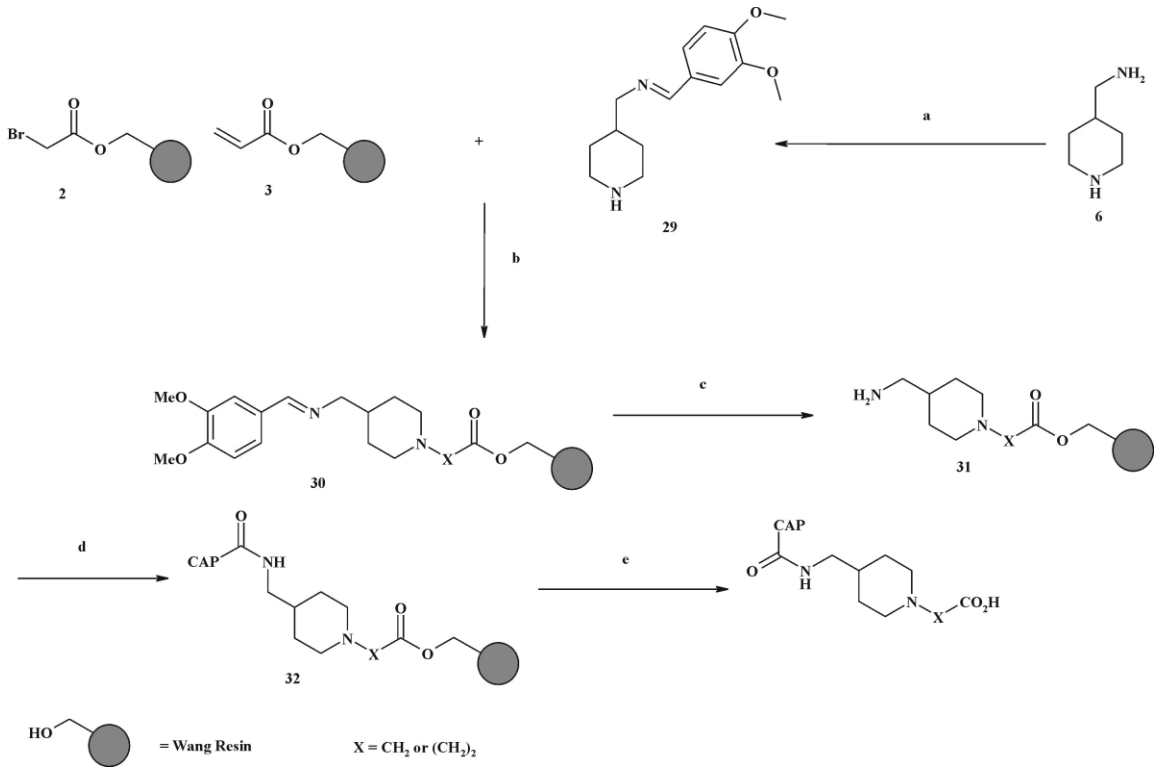
The preparation of **39** is outlined in Scheme 4. The key Cbz-aziridine derivative, **37**, was accessed according to literature precedent[18,19] from commercial ethyl-2,3-dibromopropionate in modest yields. Ring opening of **37** by **33** was highly regioselective, but slow. Hydrolysis of the desired regioisomer, **38**, provided the target molecule, **39** in low overall yield.

Results and Discussion

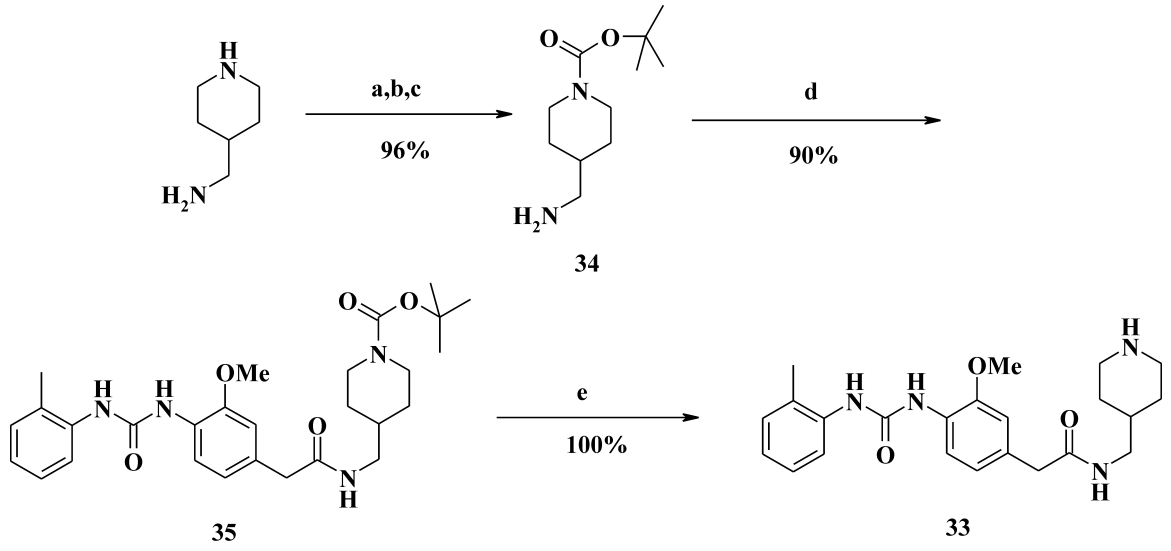
The target library **1**, was chosen with a view to linking the two proposed VLA-4 pharmacophores (aromatic ring and carboxylic acid) through a suitable template. Spatial variation of these moieties was achieved by employing diverse diamines/linkers as the central

spacer. The library was tested for its ability to inhibit VLA-4 mediated binding of H^3-RAMOS cells to fibronectin and VCAM-1 at doses of 30, 10 and 3 μmol. A total of 44 compounds showed >50% inhibition at 30 μmol in the fibronectin assay (inhibitions >100 and <0% are artefacts of the assay conditions). Data for selected compounds is shown in Table 2.

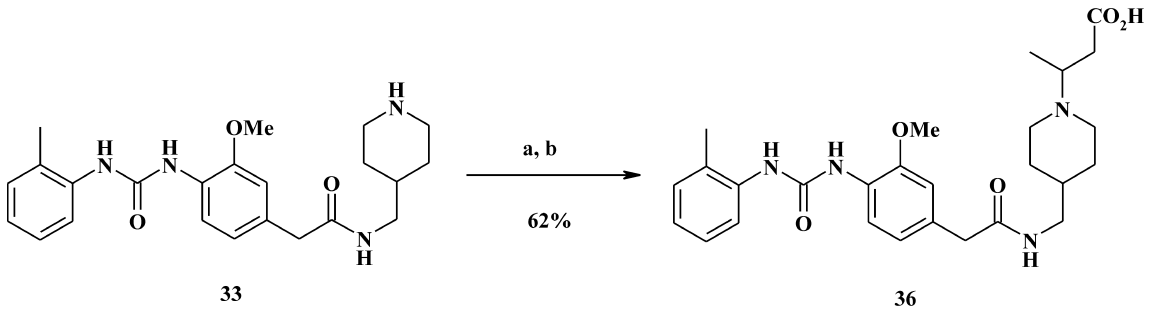
Clear structure–activity relationships (SAR) can be seen across the library. The most interesting results generally being derived from the 3-(4-aminomethyl-piperidin-1-yl)propionic acid template. The diphenyl urea is essential for VLA-4 antagonism in these systems, with the *para* ureas always showing superior potency over their corresponding *meta* analogues (**41** vs **42**). Improvements in activity are seen with the incorporation of the *ortho*-methyl substituent in the terminal phenyl ring and more strikingly, with the introduction of the 3-methoxy moiety into the phenylacetyl portion (**40**, **41** and **43**). The requirement of a specific distance between the acid and urea is confirmed by the fact that both **43** and **44** possess similar potency. This would seem to indicate that the



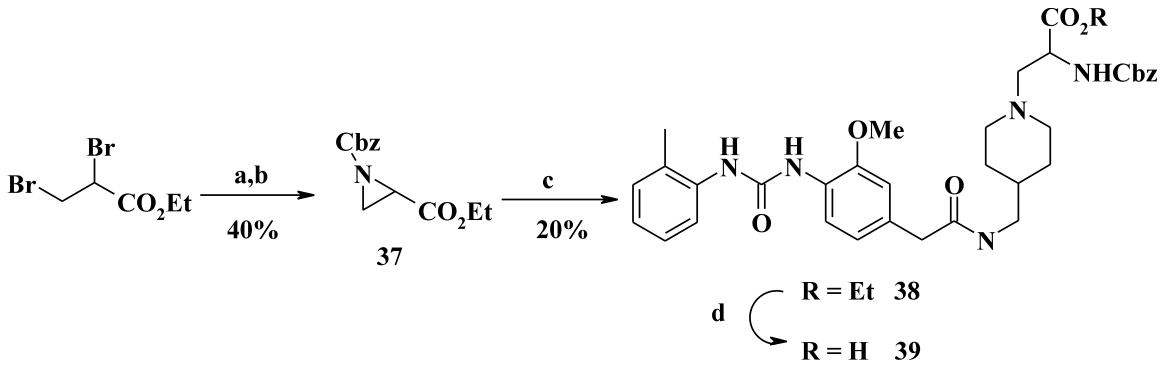
Scheme 1. Reagents: (a) 3,4-dimethoxybenzaldehyde, PhMe reflux; (b) DIPEA, DMSO; (c) MeCN/H_2O/TFA (80:20:2) 1 h; (d) **15**–**28**, HATU, DIPEA, DMF; (e) 50% TFA in DCM 1 h.



Scheme 2. Reagents: (a) benzaldehyde, PhMe reflux; (b) di-*tert*-butylcarbonate; (c) 1 M $KHSO_4$; (d) **26**, HATU, DIPEA, DMF; (e) 25% TFA in DCM.



Scheme 3. Reagents: (a) ethyl crotonate, DIPEA, MeOH; (b) 1 M NaOH/THF.



Scheme 4. Reagents: (a) ammonia, MeCN; (b) Cbz-succinimide, DIPEA, THF; (c) **33**, THF reflux; (d) 1 M NaOH/MeOH.

core template is acting only as a spacer in positioning the proposed pharmacophores in a suitable orientation, and not contributing to the mode of binding. However, varying the positioning of the central amide bond relative to the diaryl urea dramatically effects potency (cf. **43** with **45**), suggesting the amide to be either a key pharmacophore or exerting a particular conformation on the molecules which is essential for VLA-4 activity. Finally, comparisons between **46** and lactam **47**,[20] highlights either the necessity of the carbonyl group or the preference for an sp^2 nitrogen for good biological activity with this specific substitution pattern.

Encouraged that novel inhibitors of fibronectin and VCAM-1 binding to the α4β1 integrin had been identified from de novo design, the next step was to try and improve the potency. As limited variations around the terminal cap and spacer had already been incorporated into the scaffold, the 4-(aminomethyl)piperidine and diphenyl urea portions were initially kept constant. Intermediate **33** was therefore prepared and used to probe the SAR around the carboxylic acid portion of the scaffold.

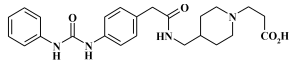
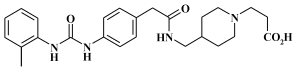
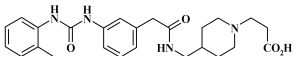
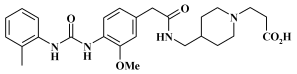
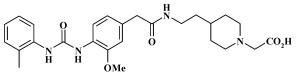
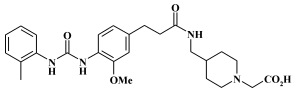
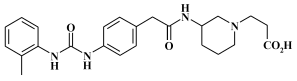
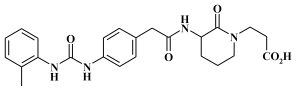
Our previous work, based on the lactam templates, had shown that increases in biological activity could be obtained by the introduction of substituents β to the carboxylic acid.[20] Compound **36** was therefore chosen as a suitable target. Contrary to results experienced in the lactam series, no increase in activity was observed compared to the unsubstituted analogue **43** (Table 3). This again highlights the differences in conformations and subsequent biological activities between the two series. An alternative strategy was therefore necessary, if increases in biological activity were to be achieved. As variations in the potency of other series had been identified by modifying substituents around the carboxylic acid,[20] attention turned to other potential pharmacophores that could be incorporated around this part of the molecule. Substitution α to the acid had not yet been investigated and it was decided to introduce an amino group in this position. If successful, this moiety could also be used as a 'handle' to incorporate a variety of potential pharmacophores. Compound **39** was therefore chosen as an initial target to evaluate this hypothesis.

Interestingly, this modification to the general structure gave a 10-fold increase in potency for the inhibition of binding of fibronectin to the integrin (Table 3). The Cbz protecting group was the first amino derivative that had been incorporated at this position and was unlikely to be optimal. Thus, **39** provides a useful starting point for derivatisation in a bid to further optimise potency and evaluate SAR.

Conclusion

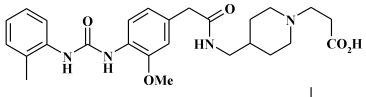
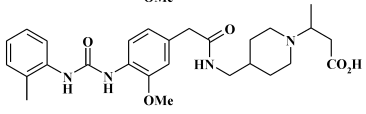
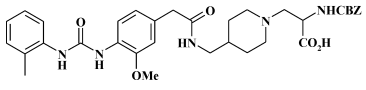
A de novo library has been designed, based on a putative pharmacophoric model. This led to the identification of novel, non peptidic inhibitors of binding of fibronectin to the $\alpha_4\beta_1$ integrin, with well defined SAR being established. Further derivatisation around the lead template has enabled compounds with low nanomolar potency to be identified, providing a useful starting point for a lead optimisation process. Additional work in this area will be reported elsewhere.

Table 2. Activities of selected library compounds for their inhibition of binding of ^{3}H-RAMOS cells to Fibronectin and VCAM-1

No.	Structure	Inhibition of VLA-4 binding to Fibronectin		Inhibition of VLA-4 binding to VCAM-1		Retention time[a]	MH + [a]	Purity[a] (%)
		30 μM (%)	IC_{50} (μM)	30 μM (%)	IC_{50} (μM)			
40		28		9		2.51	439	91%
41		78	7	10	>30	2.69	453	92%
42		19		6		2.46	453	>99%
43		115	0.1	88	1.7	2.58	483	>99%
44		124	0.1	100	4	3.92	483	95%
45		18		6		2.78	483	95%
46		−13		−1		2.49	439	87%
47		85	4		13	—	—	—

[a]For conditions, see Experimental.

Table 3. Activities of selected compounds for their inhibition of binding of RAMOS cells to fibronectin and VCAM-1

No.	Structure	Inhibition of VLA-4 binding to Fibronectin	Inhibition of VLA-4 binding to VCAM-1
		IC_{50} (nM)	IC_{50} (nM)
43		100	1700
36		150	1080
39		7	282

Experimental

Chemical methods

Reagents, starting materials and solvents were purchased from common commercial suppliers and used as received or purified by standard methods. All organic solutions were dried over magnesium sulphate and concentrated at reduced pressure under aspirator vacuum using a Buchi rotary evaporator. Reaction products were purified, when necessary, by flash chromatography on silica gel (40–63 μm) eluting with the solvent system indicated. Yields are not optimised. Melting points were determined on a Gallenkamp 595 apparatus and are uncorrected. The structure and purity of all compounds were confirmed by microanalytical and/or spectroscopic methods. ^{1}H NMR spectra were acquired on a

Varian VXR-400 or 500 MHz spectrometers; peak positions are reported in parts per million relative to internal tetramethylsilane on the δ scale. Elemental analyses were performed by the Physical Chemistry Department at Aventis. Satisfactory microanalyses (±0.4%) were obtained for C, H, N unless otherwise stated. LC/MS: Samples were run on a Micromass Platform II mass spectrometer by +ve/−ve ion Electrospray under the following LC conditions.

Mobile phase. (A) Water 0.1% formic acid; (B) acetonitrile 0.1% formic acid.

Gradient

Time flow (mL/min)		%A	%B
0.00	2.0	95	5
0.50	2.0	95	5
4.50	2.0	5	95
5.00	2.0	5	95
5.50	2.0	95	5

Column Luna 3u C18 (2) 30×4.6. Detection: diode array, ELS detection in line ELS, temp 50 °C, Gain 6–1.8 mL/min, injection volume 10–40 μL dependant on library. Source temp 150 °C. Approx. 200 μL/min split to mass spectrometer. Mass spectra were recorded on a Micromass Platform II mass spectrometer fitted with an Electrospray source and an HP1100 liquid chromatograph; using a mixture of acetonitrile and water (1:1, v/v) as the mobile phase, a flow rate of 0.3 mL/min, an injection volume of 20 μL, a run time of 2.0 min, a scan range of 150–850 Daltons positive/negative, a scan time of 2.0 s, an ESI voltage of 3.5 Kv, an ESI pressure of 20 N/m^2 nitrogen. The ions recorded are positive ions.

Inhibitory activity in cell adhesion assays

A functional cell adhesion assay was used as the primary screen to identify and optimise inhibitors of the interaction between the cell adhesion molecule VLA-4 and its physiological ligands, VCAM-1 and fibronectin. This assay utilises the VLA-4 positive RAMOS cell line and measures adhesion to 96-well plates pre-coated with either VCAM-1 or fibronectin.

RAMOS cells were seeded at a density of 200,000 cells/mL in RPMI 1640 culture medium supplemented with 10% FCS and grown for 3 days. Cells were then centrifuged at 800 rpm for 5 min at 20 °C and re-suspended at a concentration of 500,000 cells/mL in methionine deficient RPMI 1640 supplemented with 10% FCS and 1 mM L-glutamine. These cells were then radiolabelled for 18 h with 1 μCi/mL [3H]-methionine and finally resuspended in Puck's buffer for assay.

Labelled cells (200,000 per well) were incubated in Flashplates™ (96-well plates with scintillant contained under each well) precoated with fibronectin (30 μg/mL) or VCAM-1 (10 μg/mL) in the presence of test compound or vehicle (DMSO 1.8% final concentration) at 37 °C for 1 h. Following incubation, adherent cells were quantified by counting the plates on a Topcount Microplate Scintillation counter.

Diamine library

4-(3,4-Dimethoxybenzylideneaminomethyl)piperidine **29** and related compounds were prepared by refluxing equimolar amounts of diamine and 3,4-dimethoxybenzaldehyde in toluene with azetropic removal of water.[16] These compounds were used crude without purification.

A representative example is given below for the synthesis of 3-(4-{[3-methoxy-4-(3-(2-methylphenyl)ureido)-phenyl]-acetylaminomethyl}-piperidin-1-yl)-propionic acid (**43**).

Step 1. A stirred solution of acrylic acid (0.67 g, 9.3 mmol) in dimethylformamide (10 mL) and tetrahydrofuran (5 mL) was treated with diisopropyl-carbodiimide (0.59 g, 4.67 mmol). After stirring for 5 min, the solution was treated with *N*,*N*-4-(dimethylamino)pyridine (10 mg) and then with Wang resin (1 g, 1 mmol/g, Advanced ChemTech). The mixture was allowed to stand at ambient temperature for 18 h. The resin was drained and then washed three times with dimethylformamide, then three times with tetrahydrofuran, then three times with dichloromethane, sucked dry and then dried under vacuum.

Step 2. The resin from Step 1 (0.5 g, 0.92 mmol/g loading) was treated with a mixture of 4-[(3,4-dimethoxybenzylidenamino)methyl]piperidine (1.0 g) and DIPEA (500 μL) in dimethylsulphoxide (10 mL). After standing at room temperature overnight the resin was drained, then washed three times with dimethylformamide, then three times with tetrahydrofuran, then three times with dichloromethane, sucked dry, and then dried under high vacuum.

Step 3. The resin from Step 2 (50 mg, 0.046 mmol/g loading) was suspended in a mixture of acetonitrile, water and trifluoroacetic acid (2.5 mL, 80:20:2, v/v/v) at room temperature. The mixture was kept at room temperature until HPLC analysis of the supernatant solution showed no more 3,4-dimethoxybenzaldehyde was being produced. The resin was then drained, then washed three times with acetonitrile, then three times with dimethylformamide, then twice with 5% DIPEA in dimethylformamide, then three times with dimethylformamide, then three times with tetrahydrofuran, then three times with dichloromethane, sucked dry and then dried under high vacuum.

Step 4. The resin from Step 3 (50 mg, nominal 0.046 mmol/g loading) was treated successively at room temperature with a solution of 3-methoxy-4-[3-(2-methylphenyl)ureido]phenylacetic acid[21] (0.092 mmol) in dimethylformamide (0.75 mL), then with diisopropylethylamine (50 μL) and then with a solution of *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (0.092 mmol) in dimethylformamide (0.75 mL). The mixture was kept at room temperature

for 1–2 h, then the resin drained, and then washed three times with dimethylformamide, three times with tetrahydrofuran, three times with dichloromethane, and dried under high vacuum.

Step 5. The resin from step 4 was treated with a mixture of trifluoroacetic acid and dichloromethane (2 mL, 1:1, v/v). After 1–2 h at room temperature, the resin was drained and then washed with a mixture of trifluoroacetic acid and dichloromethane (2 mL). The combined filtrate and washings were evaporated to give the 3-(4-{[3-methoxy-4-(3-(2-methylphenyl)ureido)-phenyl]-acetylaminomethyl}-piperidin-1-yl)-propionic acid (**43**). MS: MH^+ 483. HPLC retention time = 2.58 min.

Analytical data for this and other selected members of the library are shown in Table 2 using standard conditions.

Preparation of 4-({2-[3-methoxy-4-(3-*o*-tolyl-ureido)-phenyl]acetylamino}-methyl)-piperidine-1-carboxylic acid *tert*-butyl ester (35). A solution of 3-methoxy-4-[3-(2-methylphenyl)ureido]phenylacetic acid (5 g, 16.12 mmol) in dimethylformamide (150 mL) was treated with *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (PerSeptive Biosystems, 6.06 g, 15.9 mmol) and diisopropylethylamine (6.45 mL). 4-Aminomethylpiperidine-1-carboxylic acid *tert*-butyl ester[16] (3.4 g, 15.9 mmol) was added and the mixture stirred at ambient temperature for 3 h. The mixture was poured into 10% (w/w) sodium carbonate solution (500 mL) and extracted with tetrahydrofuran (4×100 mL). The organic extracts were washed with saturated brine (250 mL) and dried over magnesium sulphate, filtered and evaporated. The resulting oil was subjected to flash chromatography on silica gel eluting with dichloromethane and methanol (95:5, v/v) to afford **35** (8.0 g, 98%) as a white solid: mp 211–212 °C MS: MH^+ 511. ^{1}H NMR ($CDCl_3$) 8.22 (d, J = 10 Hz, 1H), 8.02 (s, 1H), 7.71 (m, 2H), 7.19 (m, 2H), 7.04 (m, 1H), 6.79 (m, 2H), 5.85 (m, 1H), 4.04 (m, 2H), 3.83 (s, 3H), 3.51 (s, 2H), 3.06 (m, 2H), 2.62 (m, 2H), 2.24 (s, 3H), 1.59 (m, 3H), 1.04 (m, 2H). Anal. ($C_{28}H_{38}N_4O_5$: $0.2CH_2Cl_2$) calcd C, 65.0; H, 7.35; N, 10.8; found: C, 65.3; H, 7.45; N, 10.75.

Preparation of 2-[3-Methoxy-4-(3-*o*-tolyl-ureido)-phenyl]-*N*-piperidine-4-ylmethyl-acetamide (33). 4-({2-[3-Methoxy-4-(3-*o*-tolyl-ureido)-phenyl]acetylamino}-methyl)-piperidine-1-carboxylic acid *tert*-butyl ester (7.9 g, 15.5 mmol) was dissolved in a mixture of dichloromethane and trifluoroacetic acid (50 mL, 4:1, v/v) and stirred at ambient temperature for 1 h. The solution was concentrated under vacuum. The resulting oil was rapidly stirred with 4 M sodium hydroxide for 5 min and the resulting white precipitate filtered off and washed with water (20×50 mL) and dried on high vacuum to afford **33** (5.9 g, 92%) as a white solid: mp 154–160 °C MS: MH^+ 411. ^{1}H NMR ($CDCl_3$) 8.26 (s, 1H), 8.22 (d, J = 10 Hz, 1H), 8.07 (s, 1H), 7.79 (m, 1H), 7.19 (m, 2H), 6.95 (m, 1H), 6.81 (m, 2H), 6.41 (m, 1H), 3.86 (s, 3H), 3.48 (s, 2H), 3.07 (m, 4H), 2.57 (m, 2H), 2.25 (s, 3H), 1.61 (m, 3H), 1.06 (m, 2H). Anal. ($C_{23}H_{30}N_4O_3$: $1.5H_2O$) calcd C, 63.2; H, 7.5; N, 12.8; found: C, 63.3; H, 7.5; N, 12.55.

Preparation of 3-[4-({2-[3-Methoxy-4-(3-*o*-tolyl-ureido)-phenyl]acetylamino}-methyl)-piperidin-1-yl]butyric acid (36). A solution of **33** (250 mg, 0.61 mmol) in methanol (5 mL) was added ethyl crotonate (138 mg, 1.2 mmol) and triethylamine (1 mL). The mixture was refluxed for 24 h and concentrated under vacuum. The resulting oil was dissolved in methanol (2 mL) and treated with 1 M sodium hydroxide (1.8 mL). The mixture was refluxed for 2 h and concentrated under vacuum. The residue was taken up in the minimum volume of tetrahydrofuran, the pH adjusted to 4 with trifluoroacetic acid and purified by HPLC [HPLC column Dynamax 60 Å C18 column operated under gradient elution conditions with a mixture of acetonitrile and water plus 0.1% trifluoroacetic acid as the mobile phase (0–20 min 0% acetonitrile, ramped up to 100% acetonitrile after 20 min, then maintained at 100% acetonitrile) and UV detection at 220 nm] to afford **36** as a light tan solid. Mp 141–142 °C MS: MH^+ 497. ^{1}H NMR (DMSO) 9.05 (s, 1H), 8.59 (s, 1H), 8.43 (s, 1H), 8.05 (m, 1H), 8.00 (d, J = 10 Hz, 1H), 7.76 (m, 1H), 7.14 (m, 2H), 6.94 (m, 2H), 6.77 (m, 1H), 3.85 (s, 3H), 3.6 (m, 2H), 3.38 (m, 4H), 2.86 (m, 4H), 2.22 (s, 3H), 1.77 (m, 2H), 1.62 (m, 2H), 1.36 (m, 2H), 1.23 (d, J = 8 Hz, 3H).

Preparation of 2-benzyloxycarbonylamino-3-[4-({2-[3-methoxy-4-(3-*o*-tolyl-ureido)-phenyl]acetylamino}-methyl)-piperidin-1-yl]propionic acid (39). A mixture aziridine-2-carboxylic acid ethyl ester[18] (1.15 g, 10 mmol), *N*-(benzyloxycarbonyl)succinimide (2.5 g, 10 mmol) and diisopropylethylamine (1.95 mL, 10.2 mmol) in acetonitrile and tetrahydrofuran (100 mL 1:1 v/v) were stirred at ambient temperature for 4 h. The mixture was concentrated under vacuum and ethyl acetate (100 mL) added. The solution washed with water (3×100 mL) and brine (100 mL). The organic layer was dried over magnesium sulphate, filtered and concentrated under vacuum. The residue was subjected to flash chromatography on silica gel eluting with ethyl acetate and cyclohexane (1:6, v/v) to afford **37** (1.4 g) as a pale yellow oil. This oil was dissolved in dimethylformamide (5 mL) and triethylamine (1 mL) and **33** (542 mg, 1.32 mmol) added. The mixture was refluxed for 48 h. This was then concentrated under vacuum and the residue was subjected to flash chromatography on silica gel eluting with ethyl acetate and methanol (95:5, v/v) to afford **38** (180 mg) as a pale yellow solid which was dissolved in methanol (2 mL) and 1 M sodium hydroxide (1 mL) added to the mixture. This was heated at 40 °C for 8 h and concentrated under vacuum. The residue was taken up in the minimum volume of tetrahydrofuran, the pH adjusted to 4 with trifluoroacetic acid and purified by HPLC, as described above, to afford **39** (50 mg) as a tan solid: mp 128–129 °C MS: MH^+ 632. ^{1}H NMR (DMSO): 8.58 (s, 1H), 8.41 (s, 1H), 8.03 (m, 1H), 8.01 (d, J = 10 Hz, 1H), 7.77 (m, 1H), 7.60 (m, 1H), 7.31 (m, 5H), 7.12 (m, 2H), 6.92 (m, 2H), 6.77 (m, 1H), 5.02 (s, 2H), 4.00 (m, 1H), 3.87 (s, 3H), 3.61 (m, 2H), 3.35 (m, 4H), 2.96 (m, 4H), 2.22 (s, 3H), 1.76 (m, 2H), 1.61 (m, 1H), 1.41 (m, 2H).

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