Journal of Medicinal Chemistry

Article

Selective Arylsulfonamide Inhibitors of ADAM-17: Hit Optimization and Activity in Ovarian Cancer Cell Models

Elisa Nuti, Francesca Casalini, salvatore Santamaria, Marina Fabbi, Grazia Carbotti, Silvano Ferrini, Luciana Marinelli, Valeria La Pietra, Ettore Novellino, Caterina Camodeca, Elisabetta Orlandini, Susanna Nencetti, and Armando Rossello

J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/jm4011753 • Publication Date (Web): 17 Sep 2013

Journal of Medicinal Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Selective Arylsulfonamide Inhibitors of ADAM-17: Hit Optimization and Activity in Ovarian Cancer Cell Models.

Elisa Nuti,[1] Francesca Casalini,[1] Salvatore Santamaria,[1†] Marina Fabbi,[2] Grazia Carbotti,[2] Silvano Ferrini,[2] Luciana Marinelli,[3] Valeria La Pietra,[3] Ettore Novellino,[3] Caterina Camodeca,[1] Elisabetta Orlandini,[1] Susanna Nencetti,[1] and Armando Rossello[1]*

[1] Dipartimento di Farmacia, Università di Pisa, via Bonanno 6, 56126 Pisa, Italy.

[2] Dipartimento di Terapie Oncologiche Integrate, IRCCS AOU San Martino-IST Istituto Nazionale per la Ricerca sul Cancro, Largo R. Benzi 10, 16132 Genova, Italy

[3] Dipartimento di Chimica Farmaceutica e Tossicologica, Università di Napoli "Federico II", Via Domenico Montesano 49, 80131 Napoli, Italy

* To whom correspondence should be addressed. phone: +39 050 2219562; fax: +39 050 2219605; e-mail: aros@farm.unipi.it.

† Present address: Kennedy Institute of Rheumatology, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, Roosevelt Drive, Oxford OX3 7FY, United Kingdom

Abstract

Activated Leukocyte Cell Adhesion Molecule (ALCAM) is expressed at the surface of epithelial ovarian cancer (EOC) cells and is released in a soluble form (sALCAM) by ADAM-17-mediated shedding. This process is relevant to EOC cell motility and invasiveness, which is reduced by inhibitors of ADAM-17. In addition, ADAM-17 plays a key role in EGFR signalling and thus may represent a useful target in anticancer therapy. Herein we report our hit optimization effort to

identify potent and selective ADAM-17 inhibitors, starting with previously identified inhibitor **1**. A new series of secondary sulfonamido-based hydroxamates was designed and synthesized. The biological activity of the newly synthesized compounds was tested *in vitro* on isolated enzymes and human EOC cell lines. The optimization process led to compound **21,** which showed an IC_{50} of 1.9 nM on ADAM-17 with greatly increased selectivity. This compound maintained good inhibitory properties on sALCAM shedding in several *in vitro* assays.

1. Introduction

ADAM-17 or Tumor Necrosis Factor-α Converting Enzyme (TACE) is an integral plasma membrane zinc-dependent metalloprotease and member of the A Disintegrin And Metalloprotease (ADAM) family of molecules. ADAM-17 is produced as an inactive proenzyme of 120 kDa, which is physiologically activated via stimulation of G protein–coupled receptors and furin-like enzyme cleavage of the ADAM-17 prodomain leading to generation of the 100 kDa active enzyme. ADAM-17 is a sheddase and/or activator of several biologically important membrane-anchored proteins, including TNF, epithelial cell growth factors, and cell adhesion molecules.[1] In recent years, we focused our attention on studying interaction between ADAM-17 and cell adhesion molecules (CAMs) of the immunoglobulin superfamily. In particular, we have shown that Activated Leukocyte Cell Adhesion Molecule (ALCAM or CD166) is expressed at the surface of epithelial ovarian cancer (EOC) cells, can be internalized following soluble ligand engagement[2] and is released in a soluble form (sALCAM) by ADAM-17-mediated shedding.[3] This process is relevant to EOC cell motility, which is reduced by inhibitors of ADAM-17. In addition, ADAM-17 plays a key role in Epidermal Growth Factor Receptor (EGFR) signalling[4] and thus may represent a useful target in anticancer therapy in EGF-dependent tumours.[5] Recently, we have reported the synthesis and biological evaluation of a series of arylsulfonamide hydroxamates as potent ADAM-17 inhibitors.[6] Among these, compound **1** (Figure 1) showed a nanomolar activity for ADAM-17 isolated enzyme. This compound proved to be also potent in inhibiting soluble ALCAM release in

cancer cells, showing a nanomolar activity on A2774 and SKOV3 cell lines. No toxicity for this compound was detected on parallel cultures by MTT assay. In addition, it inhibited the EGF-triggered invasive properties of ovarian cancer cells in vitro.[7] These preliminary results allowed us to validate ADAM-17/ALCAM pathway as new target in anticancer therapy and prompted us to undertake a structural optimization of **1** in order to find out more selective ADAM-17 inhibitors. In fact, compound **1** also inhibited other metalloproteases such as MMP-2, MMP-9 and MMP-14 and it is widely believed that some side effects that have been clinically observed with the use of broad spectrum MMP inhibitors, such as musculoskeletal syndrome (MSS), could be related to MMP-1/MMP-14 inhibition.[8] Moreover, a more target-directed inhibition could be helpful to discern the actual ADAM-17 biological activity from overlapping effects due to other metalloproteases and to reduce toxicity.

FIGURE 1 HERE

In the present paper, based on recent SAR data from our lab and others,[9] we designed and synthesized a series of secondary sulfonamido-based inhibitors, compounds **2-13**[10] (Table 1), with bended P1' substituents different from the highly exploited 4-(but-2-ynyloxy)benzene group. In fact, as revealed by X-ray crystal structure of catalytic domain[11], ADAM-17 is characterized by an "L-shaped" pocket with a polar access between S1' and S3', and this singular shape of the specificity pocket has been largely used to design specific ADAM-17 inhibitors by insertion of bended substituents. Two examples of these selective inhibitors reported in literature for the treatment of inflammatory diseases are DPC-333[12] from Bristol-Myers Squibb (which entered clinical trials for rheumatoid arthritis) and WAY-281418,[13] developed by Wyeth researchers (Figure 2).

FIGURE 2 HERE

Hydroxamates **2-13** were screened against recombinant human ADAM-17 in order to select the P1' substituent able to give the best results of selectivity for ADAM-17 over MMPs. After the most suitable and innovative P1' substituent has been found, we sought to improve activity against ADAM-17 by introducing an amidic chain in the α position relative to the hydroxamate (P1), compounds **19-21**[10] (Table 3). In fact, as previously shown,[6] an α substituent in this arylsulfonamidic scaffold could highly increase potency against ADAM-17. The compounds which gave the best results on isolated enzymes were finally tested on human EOC cell lines (A2774, SKOV3-luc and A2780), which express ADAM-17 and its substrate ALCAM.

2. Chemistry.

Compound **2** (Table 1) has been synthesized as previously described.[14] Benzyloxy-benzene derivatives **3-13** (Table 1) and **16** (Table 2) have been prepared as reported in Scheme 1.

SCHEME 1 HERE

Sulfonyl chlorides **33-43** were synthesized starting from sodium 4-hydroxybenzenesulfonate dihydrate, which was alkylated with the appropriate aryl bromides using sodium hydroxide as base. Reaction of sodium salts **22-32** with oxalyl chloride in the presence of DMF in dichloromethane afforded sulfonyl chlorides **33-43,** which were coupled with glycine to give carboxylic acids **44-53** and **16**. Carboxylates **44-46** and **49-53** were condensed with *O*-(*tert*-butyldimethylsilyl)hydroxylamine in presence of 1-[3-(Dimethylamino)propyl]-3-ethyl carbodiimide hydrochloride (EDC) to give silyl intermediates **54-61,** which afforded hydroxamates **3-5** and **8-12** after acid cleavage with TFA. Alternatively, carboxylates **47**, **48** and **16** were first converted into protected hydroxamates **62-64** by condensation with *O*-(tetrahydro-2H-pyran-2-

yl)hydroxylamine (THP-hydroxylamine) in the presence of EDC and then hydrolyzed with 4 N HCl to hydroxamates **6**, **7** and **13**, respectively.

Compounds **17** and **18** (Table 2), close analogues of **13**, were synthesized according to the route described in Scheme 2. 4-(3,5-Dibromobenzyloxy)benzene-1-sulfonyl chloride **43** was converted into the sulfonamide **65** by reaction with glycine *tert*-butyl ester hydrochloride in H_2O and dioxane in the presence of TEA. This ester was successively converted by Pd-catalyzed Suzuki coupling to mono-substituted biaryl derivatives **66** and **67**, which were hydrolyzed to carboxylic acids **68** and **69** respectively. These carboxylates were finally converted to their corresponding hydroxamates **17** and **18** by condensation with *O*-(*tert*-butyldimethylsilyl)hydroxylamine, followed by acid hydrolysis with TFA.

SCHEME 2 HERE

N-Methyl derivative **15** (Table 2) and the α-substituted analogues of **13**, compounds **19-21** (Table 3), were prepared as reported in Scheme 3. All these compounds were synthesized starting from 4-(3,5-Dibromobenzyloxy)benzene-1-sulfonyl chloride **43**, obtained as described above. **43** was converted into the corresponding Boc-sulfonamide **76** by reaction with ammonia, followed by treatment with di-(*tert*-butyl)dicarbonate. Sulfonamide **76** underwent Mitsunobu reaction in the presence of diisopropylazodicarboxylate (DIAD) and triphenylphophine with optically active alcohol **77**[6] to give ester **78** of (*R*) configuration. Acid hydrolysis of **78** yielded the *NH*-sulfonamide carboxylate **79**, which was finally converted into the corresponding hydroxamate **19** by condensation with THP-hydroxylamine in DMF and acid cleavage with 4 N HCl. The sulfonyl chloride **43** was also made reacting with non-natural aminoacids such as sarcosine *tert*-butyl ester hydrochloride or D-Cbz-ornithine or D-carbamoyl-ornithine in the presence of TEA to give sulfonamides **70**, **72** and **73**, respectively. Acid hydrolysis with TFA of *tert*-butyl ester **70** afforded carboxylate **71**. Condensation of **71** and **72** with THP-hydroxylamine in the presence of EDC

yielded tetrahydropyranyl intermediates **74** and **75** which, after mild hydrolysis with HCl 4N, gave the corresponding hydroxamates **15** and **20**. Finally, hydroxamic acid **21** was obtained from carboxylate **73** by condensation with *O*-(*tert*-butyldimethylsilyl)hydroxylamine, followed by acid hydrolysis with TFA.

SCHEME 3 HERE

3. Results and Discussion

Biological Activity on Isolated Enzymes.

The first series of hydroxamates, compounds **2-13**, were tested in vitro for their ability to inhibit human recombinant ADAM-17, MMP-1, -2, -9 and -14 by a fluorimetric assay, which uses a fluorogenic peptide[15] as the substrate (Table 1). Compound **14**[16], previously reported by Wyeth researchers as ADAM-17 inhibitor and bearing the highly exploited 4-(but-2-ynyloxy)benzene group in P1', was taken as reference compound to evaluate the effect of the different R groups on the simple glycine-like scaffold. On the basis of our previous results[6], we focused our attention on secondary sulfonamides because the substitution of sulfonamide NH was seen to be detrimental to ADAM-17 inhibitory activity.

Among the substituted benzyloxy derivatives, the best results of activity were achieved by compound **3**, which resulted as potent as the reference compound **14** on ADAM-17 (IC_{50}=16 nM). Unfortunately, this 4-bromobenzyloxy derivative was poorly selective, showing nanomolar activity also against MMP-2 and MMP-9. The replacement of the bromine atom with a fluorine or a trifluoromethoxy group in the *para*-position led to a drop of activity for ADAM-17, with the 4-phenyl derivative **6** being the worst of the series (IC_{50}=640 nM). The introduction of the same substituents in the *meta*-position of the benzyl moiety resulted in a general improvement of ADAM-17 inhibitory activity with respect to the unsubstituted analogue **2**, without any significant

improvement in selectivity. Finally, the presence of a 4-quinoline group as in **11** led to a decrease of activity against all tested enzymes, probably due to an excessive bulkiness of the substituent.

At this point the introduction of a second halogen atom on the benzyl moiety was considered and the 3,5-di-halo substituted compounds **12** and **13** were synthesized. Both compounds had the same activity towards ADAM-17 with respect to their 3-mono substituted analogues (**7** and **8**) but presented an increased selectivity over the MMPs tested. In particular, the 3,5-dibromobenzyloxy derivative **13** resulted the most promising of this first series, with a good activity for ADAM-17 (IC_{50}=105 nM) and the best selectivity profile obtained so far over MMPs (a > 500-fold selectivity over MMP-9, -14 and -1), even compared to the reference compound **14**. These data suggested that the 3,5-dibromobenzyloxybenzene group could be a valid alternative to the use of the 4-(but-2-ynyloxy)benzene group as P1' substituent, and therefore **13** became the focus of additional efforts to improve activity towards ADAM-17.

TABLE 1 HERE

Compounds **15-18** (Table 2) were subsequently synthesized as close analogues of **13**, introducing some modifications on this promising scaffold. We first synthesized the *N*-methyl analogue of **13** (**15**), which showed a decrease of ADAM-17 inhibitory activity compared to **13**. Then we tested the carboxylate analogue of **13**, compound **16**, in order to evaluate the effect of a different zinc-binding group (ZBG) on this scaffold but this compound was completely devoid of activity for ADAM-17. This disappointing result discouraged us to change the ZBG at least at this point of the optimization process. Finally, we replaced a bromine atom of **13** with a pyridin-3-yl moiety (**18**) or a 4-methoxyphenyl group (**17**) in order to better explore the S1'-S3' pocket space. Both compounds showed a reduced inhibitory activity against ADAM-17, with **18** being 5-fold less active than **13**.

TABLE 2 HERE

On the basis of these findings, in order to further increase activity on ADAM-17 of this class of inhibitors, we decided to keep the compound **13** scaffold unchanged and to introduce different amidic chains in P1. In fact, as previously shown,[6] an α substituent in this arylsulfonamidic scaffold could highly increase potency against ADAM-17. First, we chose to insert a benzyloxycarbonylamino-ethyl chain in P1 because this was the group originally present in our previous compound **1**. As expected, compound **19** (Table 3) resulted 2.5-fold more active than its unsubstituted analogue **13**, showing an IC_{50}=43 nM on ADAM-17, without any loss in selectivity. Encouraged by this data, we put a longer chain in P1 and synthesized compound **20** (Table 3) with a benzyloxycarbonylamino-propyl chain. We thus obtained a further increase of ADAM-17 inhibitory potency (IC_{50}=11 nM) still maintaining a strong selectivity over MMPs, even compared to **19**. In particular, the selectivity for ADAM-17 over MMP-2 progressively increased from 16-fold for compound **13** to 130-fold for **20**. Finally, as last step of our optimization process we tried to improve the hydrophilicity of our inhibitor by replacing the benzyloxycarbonylamino (Cbz) moiety of **20** with an ureidic group (compound **21**, Table 3).

TABLE 3 HERE

For **21** and its analogue **20**, the physico-chemical properties influencing metabolism, cell permeation, and bioavailability were evaluated with QikProp software (Schrödinger, LLC New York). As shown in Table 4, while **20** possesses an high MW, high number of H-bond acceptors and a logS which is quite far from ideal values, **21** has a lower MW, appropriate number of H-bond donors and acceptors, and a good logS. Thus, the replacement of the Cbz moiety in **20** with an ureidic group (**21**) should lead to an improvement of the physico-chemical properties of the inhibitor.

TABLE 4 HERE

Unpredictably, this ureidic derivative was optimal for this series exhibiting a 6-fold increase in inhibitory potency against ADAM-17 (IC_{50}=1.9 nM) with respect to **20** and a comparable selectivity profile. In fact, **21** displayed a 126-fold selectivity for ADAM-17 over MMP-2, a 860-fold selectivity over MMP-9, a 10000-fold selectivity over MMP-14 and no measurable inhibitory activity toward MMP-1 (IC_{50}>500 μM). Table 5 shows a direct comparison between the selectivity profiles of **21** and of our previous compound **1**. **21** resulted as potent as **1** on ADAM-17 (with an IC_{50}=1.9 nM and 1.6, respectively) but showed a greatly improved selectivity over the tested MMPs.

TABLE 5 HERE

All these P1-substituted compounds (**19-21**) and their unsubstituted analogue **13** were also screened against human recombinant ADAM-10, which has the highest aminoacid sequence homology as compared to ADAM-17 among all known MMPs.[17] ADAM-10 is not implicated in ALCAM ectodomain shedding but is able to cleave another cell-cell adhesion molecule, desmoglein 2 (Dsg-2), together with ADAM-17. [18] Moreover, ADAM-10 is considered a target in anticancer therapy for its ability to shed the human epidermal growth factor receptor-2 (HER-2), a tyrosine kinase receptor that is associated with cancer proliferation.[19] Among the tested compounds, **21** was found to be the most active on this enzyme, with an IC_{50}=150 nM.

Biological Activity on Ovarian Cancer Cell Lines.

The activity of compounds **1**, **13**, **19-21** was then evaluated in living cancer cells. As described before,[6] modulation of soluble sALCAM release can be used as read-out test to score the inhibition of ADAM-17 activity in cells. Ovarian cancer cells (A2774, SKOV3-luc and A2780) express high cell surface levels of ALCAM, which is shed in soluble form constitutively and even more

following tyrosine kinase receptor (e.g. EGFR) activation. sALCAM can be easily assessed by ELISA of cell culture conditioned media.[3] When assayed for inhibition of constitutive or EGF-induced sALCAM release, each compound was tested at 10 μM, 1 μM, 100 nM and 10 nM concentrations with overnight incubation. Multiple tyrosine kinase activation can be mimicked by treatment with pervanadate (PV), which is a general phosphotyrosine phosphatase inhibitor that leads to metalloproteases activation. As controls, cultures treated with the corresponding amounts of solvent (DMSO) were used. Cell morphology was monitored by microscopy and cell viability was assed on parallel cultures by MTT assay (data not shown), which showed no significant toxic effects on cells. As shown in Table 6, among the new derivatives with greatly increased selectivity on isolated enzymes, compound **21** maintained good inhibitory properties on sALCAM shedding in any condition, displaying a similar IC_{50} in the nanomolar range when PV was used as stimulus in the three cell lines (a representative dose/response experiment is shown in Figure 3). Consistent with data obtained on the ADAM-17 isolated enzyme, compounds **1** and **21** showed comparable IC_{50}, while compounds **13**, **19** and **20** showed higher IC_{50}. Similar results were obtained using EGF as stimulus, although the IC_{50} observed were in general higher. The different sensitivity of PV- versus EGF-induced sALCAM release to the compounds studied may reflect the different levels of tyrosine phosphorylation achieved with the two stimuli and the resulting levels of ADAM-17 activation. Indeed, PV behaves as a stronger stimulus, achieved by its broad ability to block phosphotyrosine-phosphatases, resulting in a maximal activation of ADAM-17 and sALCAM shedding in a short time frame (90 min of test). On the other hand, EGF is a more specific stimulus acting on a single TK-receptor, which requires a longer incubation time (18 h), during which redundant and ADAM-17-independent mechanisms of shedding may be operative.

TABLE 6 HERE

FIGURE 3 HERE

4. Molecular Modeling

Docking Studies. To get an insight into the binding mode of **21** into the ADAM-17 active site, ensemble-docking studies were performed by means of Glide 5.5 program[20]. Five X-ray structures representative for the enzyme flexibility (PDB codes: 2DDF, 2I47, 2OI0, 3EDZ, 3EWJ)[6] were used. Docking of **21** in the 2DDF and 2I47 structures resulted in poorly ranked and not fully convincing poses as the 3,5-dibromobenzyloxy P1' group did not occupy the S1' pocket. This is not surprising because in these two structures the S1' loop is in a closed conformation with not enough room to permit the allocation of such a bulky moiety. Otherwise, docking of **21** using 2OI0, 3EDZ and 3EWJ structures converged on similar and more convincing binding poses, where the P1' substituent properly fits in the S1' cavity. Particularly, as shown in Figure 4 (PDB code 2OI0), the hydroxamate group chelates the zinc ion in a bidentate fashion, the phenyl ring stacks with the H405 and interacts with V402 and L348 side chains, while the 3,5-dibromobenzyloxy group is well inserted in the S1' pocket where multiple hydrophobic contacts are detected with V434, L401, A439, V440, L401, N447 side chains and with E398 β- and γ-carbons. Moreover, one sulfonamide oxygen establishes the commonly found bifurcated H-bond with L348 and Gly349 backbone NH. The propyl-urea moiety lies along the S1 pocket establishing hydrophobic contacts with the L350 and V314 side chains, while multiple H-bonds are detected between the two urea NH and T347 hydroxyl group and between the urea carbonyl oxygen and the K315 side chain. Clearly, all these interactions endowed **21** with a low nanomolar inhibitory activity toward ADAM-17.

FIGURE 4 HERE

As regards the activity of **21** towards MMPs, it is well known that differential substitutions on the P1' group can be successfully used to design selective inhibitors for MMPs. Indeed, the analysis of the MMPs X-ray structures reveals that the length of the S1' pocket increases in the following

order: MMP-1 < MMP-14 < MMP-2 ≈ MMP-9. The SAR herein presented (Table 1) corroborates this consideration, in fact the activity on MMP-1 drops abruptly by just introducing a bended P1' group (**2**), while MMP-14 can tolerate *para* and *meta* halogenated phenyl decorations (**2**, **3**, **4**, **8**, **12**). On the contrary, MMP-9 and -2 are able to allocate almost all the inhibitors synthesized except for **13** and its derivatives (**15-21**). Thus, docking studies of **21** into the aforementioned MMPs were performed with the aim to better clarify the structural features responsible for the observed inactivity towards these enzymes. Unsurprisingly, as regards MMP-1 and -14 (1HFC[21] and 3MA2,[22] respectively), the docking program is incapable to find a pose where the P1' group is inserted into the S1' pocket, thus leaving the majority of **21** poses largely solvent exposed. Conversely, in the lowest energy binding poses of **21** into the active sites of MMP-2 and -9 (3AYU,[23] 4H3X,[24] respectively), the 3,5-dibromobenzyloxy group fits in the S1' pocket and the hydroxamate group chelates the zinc ion, although the sulfonamide geometry is gravely distorted. However, the bottom part of the S1' pocket of both enzymes is open and solvent exposed, with a predominance of hydrophilic residues (T143, T145, K146, N147, R149 and S151 in MMP-2; E132, R140, T142, E143 and D150 in MMP-9), consequently, it can be assumed that the large and hydrophobic 3,5-dibromobenzyloxy moiety is not well tolerated in such a region thus accounting for the higher IC_{50} on MMP-2 and -9 found for **21**.

5. Conclusions

Starting with our previously published molecule **1**, we carried out a systematic SAR investigation of the P1' substituent in order to find out a moiety different from the highly exploited 4-(but-2-ynyloxy)benzene group able to improve the selectivity for ADAM-17 over MMPs. Optimization of the benzyloxy-phenyl portion led to the identification of the 3,5-dibromobenzyloxy-phenyl group, which afforded improved ADAM-17 selectivity (compound **13**). Furthermore, introduction of an amidic chain in the α position relative to the hydroxamate (P1 group) greatly increased potency against ADAM-17 (compounds **19-21**). In particular, the ureidic derivative **21** resulted the best of

the series being as potent as **1** on ADAM-17 (IC_{50}=1.9 nM) and showing a greatly improved selectivity over the tested MMPs. In fact, **21** displayed a 126-fold selectivity for ADAM-17 over MMP-2, a 860-fold selectivity over MMP-9, a 10000-fold selectivity over MMP-14 and no measurable inhibitory activity toward MMP-1 (IC_{50}>500 μM). Finally, the compounds, which gave the best results on isolated enzymes (**13**, **19-21**), were tested on human EOC cell lines (A2774, SKOV3-luc and A2780), which express ADAM-17 and its substrate ALCAM. **21** maintained good inhibitory properties on sALCAM shedding in any condition, displaying a similar IC_{50} in the nanomolar range when PV was used as stimulus in the three cell lines. Consistent with data obtained on the ADAM-17 isolated enzyme, compounds **1** and **21** showed comparable IC_{50} also on living cancer cells, suggesting that selective inhibition of ADAM-17 activity might be sufficient to block sALCAM release in cells. Further investigations are necessary to assess the effect of the improved ADAM-17 selectivity in ovarian cancer models in vivo.

6. Experimental Section

Chemistry. Melting points were determined on a Kofler hotstage apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were determined with a Varian Gemini 200 MHz spectrometer. Chemical shifts (δ) are reported in parts per million downfield from tetramethylsilane and referenced from solvent references. Coupling constants *J* are reported in hertz; ^{13}C NMR spectra were fully decoupled. The following abbreviations are used: singlet (s), doublet (d), triplet (t), double-doublet (dd), broad (br) and multiplet (m). Where indicated, the elemental compositions of the compounds agreed to within ±0.4% of the calculated value. Chromatographic separations were performed on silica gel columns by flash column chromatography (Kieselgel 40, 0.040–0.063 mm; Merck) or using ISOLUTE Flash Si II cartridges (Biotage). Reactions were followed by thin-layer chromatography (TLC) on Merck aluminum silica gel (60 F254) sheets that were visualized under a UV lamp and hydroxamic acids were visualized with $FeCl_3$ aqueous solution. Evaporation was performed *in vacuo* (rotating evaporator). Sodium sulfate was always used as the drying agent. D-

Cbz-ornithine (H-D-Orn(Z)-OH) and D-carbamoyl-ornithine (H-D-Cit-OH) were purchased from Bachem (Switzerland). 3,5-Dibromobenzyl bromide and 4-bromomethylbiphenyl were purchased from ABCR (Germany). 1-(bromomethyl)-3-(trifluoromethoxy)benzene, 1-(bromomethyl)-4-fluorobenzene, 1-(bromomethyl)-4-(trifluoromethoxy)benzene, 1-(bromomethyl)-3,5-difluorobenzene, and 8-(bromomethyl)quinoline were purchased from Maybridge (UK). All other commercially available chemicals were purchased from Sigma-Aldrich.

The purity of the final compounds was determined by reverse-phase HPLC on a Merck Hitachi D-7000 liquid chromatograph. HPLC purity was determined to be >95% for all final products using a Discovery C18 column (250 mm × 4.6 mm, 5 μm, Supelco), with a mobile phase of 40% water/60% methanol at a flow rate of 1.4 mL/min, with UV monitoring at the fixed wavelength of 256 nm. See the Supporting Information for compound purity analysis data for final compounds.

General procedure for the synthesis of benzyloxy-benzene sodium salts 22-32. To a suspension of sodium 4-hydroxybenzenesulfonate dihydrate (1.3 g, 5.6 mmol) in isopropanol (25 mL), containing 5.6 mL of NaOH 1N freshly prepared, the appropriate substituted benzylbromide (11.2 mmol) was added. The reaction was refluxed (70 °C) overnight. Isopropanol was evaporated and the precipitate was collected by filtration and washed with isopropanol. The solid was dried *in vacuo*.

Sodium 4-(4-bromobenzyloxy)benzenesulfonate (22). The title compound was prepared from 4-bromobenzyl bromide following the general procedure. White solid (79% yield). ^{1}H-NMR (DMSO-d_6) δ: 5.10 (s, 2H); 7.38-7.42 (m, 4H); 7.49-7.60 (m, 4H).

Sodium 4-(4-fluorobenzyloxy)benzenesulfonate (23). The title compound was prepared from 1-(bromomethyl)-4-fluorobenzene following the general procedure. White solid (74% yield). ^{1}H-NMR (DMSO-d_6) δ: 5.08 (s, 2H); 6.90-6.96 (m, 2H); 7.18-7.26 (m, 2H); 7.37-7.52 (m, 4H).

Sodium 4-(4-(trifluoromethoxy)benzyloxy)benzenesulfonate (24). The title compound was prepared from 1-(bromomethyl)-4-(trifluoromethoxy)benzene following the general procedure.

White solid (69% yield). ^{1}H-NMR (DMSO-d_6) δ: 5.14 (s, 2H); 7.36-7.42 (m, 4H); 7.50-7.59 (m, 4H).

Sodium 4-(biphenyl-4-ylmethoxy)benzenesulfonate (25). The title compound was prepared from 4-bromomethylbiphenyl following the general procedure. White solid (70% yield). ^{1}H-NMR (DMSO-d_6) δ: 5.17 (s, 2H); 6.94-6.98 (m, 2H); 7.38-7.47 (m, 6H); 7.51-7.55 (m, 5H).

Sodium 4-(3-bromobenzyloxy)benzenesulfonate (26). The title compound was prepared from 1-bromo-3-(bromomethyl)benzene following the general procedure. White solid (76% yield). ^{1}H-NMR (DMSO-d_6) δ: 5.13 (s, 2H); 6.91-6.96 (m, 2H); 7.35-7.39 (m, 1H); 7.42-7.43 (m, 1H); 7.47-7.54 (m, 3H); 7.65-7.66 (m, 1H).

Sodium 4-(3-fluorobenzyloxy)benzenesulfonate (27). The title compound was prepared from 1-(bromomethyl)-3-fluorobenzene following the general procedure. White solid (65% yield). ^{1}H-NMR (DMSO-d_6) δ: 5.14 (s, 2H); 6.92-6.96 (m, 2H); 7.11-7.20 (m, 1H); 7.26-7.30 (m, 2H); 7.38-7.46 (m, 1H); 7.50-7.54 (m, 2H).

Sodium 4-(3-(trifluoromethoxy)benzyloxy)benzenesulfonate (28). The title compound was prepared from 1-(bromomethyl)-3-(trifluoromethoxy)benzene following the general procedure. White solid (61% yield). ^{1}H-NMR (DMSO-d_6) δ: 5.18 (s, 2H); 6.92-6.96 (m, 2H); 7.31-7.34 (m, 1H); 7.44-7.54 (m, 5H).

Sodium 4-(biphenyl-3-ylmethoxy)benzenesulfonate (29). The title compound was prepared from 3-(bromomethyl)biphenyl following the general procedure. White solid (62% yield). ^{1}H-NMR (DMSO-d_6) δ: 5.19 (s, 2H); 6.95-6.99 (m, 2H); 7.34-7.55 (m, 7H); 7.61-7.74 (m, 4H).

Sodium 4-(quinolin-8-ylmethoxy)benzenesulfonate (30). The title compound was prepared from 8-(bromomethyl)quinoline following the general procedure. Yellow solid (80% yield). ^{1}H-NMR (MeOD-d_6) δ: 5.79 (s, 2H); 7.75-8.16 (m, 5H); 8.88-8.96 (m, 1H); 9.01-9.09 (m, 1H).

Sodium 4-(3,5-difluorobenzyloxy)benzenesulfonate (31). The title compound was prepared from 1-(bromomethyl)-3,5-difluorobenzene following the general procedure. White solid (69% yield). ^{1}H-NMR (DMSO-d_6) δ: 5.15 (s, 2H); 6.92-6.96 (m, 2H); 7.15-7.19 (m, 3H); 7.50-7.54 (m, 2H).

Sodium 4-(3,5-dibromobenzyloxy)benzenesulfonate (32). The title compound was prepared from 3,5-dibromobenzyl bromide following the general procedure. White solid (84% yield). ^{1}H-NMR (DMSO-d_6) δ: 5.13 (s, 2H); 6.92-6.96 (m, 2H); 7.50-7.54 (m, 2H); 7.67-7.68 (m, 2H); 7.80-7.81 (m, 2H).

General procedure for the synthesis of benzyloxy-benzene sulfonyl chlorides 33-43. To a 0 °C solution of oxalyl chloride (1.3 mL, 15.3 mmol) in dry CH_2Cl_2 (5.1 mL) under Ar atmosphere, DMF was added dropwise (1.2 mL, 15.3 mmol) followed by the appropriate benzyloxy-benzene sodium salt **22-32** (5.11 mmol). The reaction was stirred at 0 °C for 10 min. and then at rt for 2 days. The crude was poured into ice and the product was extracted with EtOAc. Organic layers were washed with water, dried over Na_2SO_4 and concentrated *in vacuo* to give sulfonyl chlorides **33-43.**

4-(4-Bromobenzyloxy)benzene-1-sulfonyl chloride (33). The title compound was prepared from benzyloxy-benzene sodium salt **22** following the general procedure. The crude product was purified by flash chromatography (*n*-hexane/EtOAc 14:1) using a Isolute Flash Si II cartridge to give **33** as a white solid (32% yield). ^{1}H-NMR ($CDCl_3$) δ: 5.10 (s, 2H); 7.06-7.10 (m, 2H); 7.26-7.30 (m, 2H); 7.51-7.55 (m, 2H); 7.94-7.98 (m, 2H).

4-(4-Fluorobenzyloxy)benzene-1-sulfonyl chloride (34). The title compound was prepared from benzyloxy-benzene sodium salt **23** following the general procedure. The crude product was purified by flash chromatography (*n*-hexane/EtOAc 3:1) using a Isolute Flash Si II cartridge to give **34** as a white solid (68% yield). ^{1}H-NMR ($CDCl_3$) δ: 5.13 (s, 2H); 7.07-7.15 (m, 4H); 7.37-7.44 (m, 2H); 7.95-8.02 (m, 2H).

4-(4-(Trifluoromethoxy)benzyloxy)benzene-1-sulfonyl chloride (35). The title compound was prepared from benzyloxy-benzene sodium salt **24** following the general procedure. White solid (65% yield). ^{1}H-NMR ($CDCl_3$) δ: 5.17 (s, 2H); 7.09-7.14 (m, 2H); 7.26-7.29 (m, 2H); 7.45-7.49 (m, 2H); 7.98-8.02 (m, 2H).

4-(Biphenyl-4-ylmethoxy)benzene-1-sulfonyl chloride (36). The title compound was prepared from benzyloxy-benzene sodium salt **25** following the general procedure. White Solid (70% yield). ^{1}H-NMR ($CDCl_3$) δ: 5.22 (s, 2H); 6.98-7.02 (m, 2H); 7.12-7.16 (m, 2H); 7.37-7.52 (m, 4H); 7.58-7.67 (m, 3H); 7.93-8.02 (m, 2H).

4-(3-Bromobenzyloxy)benzene-1-sulfonyl chloride (37). The title compound was prepared from benzyloxy-benzene sodium salt **26** following the general procedure. Yellow oil (76% yield). ^{1}H-NMR ($CDCl_3$) δ: 5.14 (s, 2H); 7.09-7.14 (m, 2H); 7.28-7.37 (m, 2H); 7.49-7.53 (m, 1H); 7.59-7.60 (m, 1H); 7.97-8.01 (m, 2H).

4-(3-Fluorobenzyloxy)benzene-1-sulfonyl chloride (38). The title compound was prepared from benzyloxy-benzene sodium salt **27** following the general procedure. White solid (92% yield). ^{1}H-NMR ($CDCl_3$) δ: 5.17 (s, 2H); 6.97-7.25 (m, 5H); 7.34-7.44 (m, 1H); 7.91-8.01 (m, 2H).

4-(3-(Trifluoromethoxy)benzyloxy)benzene-1-sulfonyl chloride (39). The title compound was prepared from benzyloxy-benzene sodium salt **28** following the general procedure. White solid (86% yield). ^{1}H-NMR ($CDCl_3$) δ: 5.19 (s, 2H); 7.10-7.14 (m, 2H); 7.22-7.50 (m, 4H); 7.98-8.02 (m, 2H).

4-(Biphenyl-3-ylmethoxy)benzene-1-sulfonyl chloride (40). The title compound was prepared from benzyloxy-benzene sodium salt **29** following the general procedure. Yellow oil (98% yield). ^{1}H-NMR ($CDCl_3$) δ: 5.23 (s, 2H); 6.97-7.02 (m, 2H); 7.11-7.17 (m, 2H); 7.37-7.64 (m, 8H); 7.91-8.01 (m, 2H).

4-(Quinolin-8-ylmethoxy)benzene-1-sulfonyl chloride (41). The title compound was prepared from benzyloxy-benzene sodium salt **30** following the general procedure. The crude product was purified by flash chromatography on silica gel (*n*-hexane/EtOAc 14:1) to give **41** as a white solid (15% yield). ^{1}H-NMR ($CDCl_3$) δ: 5.96 (s, 2H); 7.22-7.26 (m, 2H); 7.46-7.63 (m, 2H); 7.83-7.89 (m, 2H); 7.95-7.99 (m, 2H); 8.20-8.25 (m, 1H); 8.95-8.98 (m, 1H).

4-(3,5-Difluorobenzyloxy)benzene-1-sulfonyl chloride (42). The title compound was prepared from benzyloxy-benzene sodium salt **31** following the general procedure. White solid (68% yield).

^{1}H-NMR ($CDCl_3$) δ: 5.15 (s, 2H); 6.77-6.85 (m, 1H); 6.94-6.97 (m, 2H); 7.08-7.13 (m, 2H); 7.97-8.02 (m, 2H).

4-(3,5-Dibromobenzyloxy)benzene-1-sulfonyl chloride (43). The title compound was prepared from benzyloxy-benzene sodium salt **32** following the general procedure. Yellow oil (82% yield). ^{1}H-NMR ($CDCl_3$) δ: 5.11 (s, 2H); 7.08-7.12 (m, 2H); 7.51-7.52 (m, 2H); 7.67-7.69 (m, 2H); 7.98-8.02 (m, 2H).

General procedure for the synthesis of carboxylic acids 44-53 and 16. To a solution of glycine (68.61 mg, 0.91 mmol), in H_2O (0.9 mL) and dioxane (0.9 mL) containing TEA (0.25 mL, 1.828 mmol), the appropriate sulfonyl chloride (1.83 mmol) was added. The mixture was stirred at RT overnight, dioxane was evaporated and the residue was treated with EtOAc, washed with HCl 1N, brine and with a saturated solution of $NaHCO_3$. Aqueous basic phases were then acidified with HCl 1N until pH=4 and the product was extracted with EtOAc. Organic layers were collected, dried over Na_2SO_4 and concentrated *in vacuo*.

2-(4-(4-Bromobenzyloxy)phenylsulfonamido)acetic acid (44). The title compound was prepared from sulfonyl chloride **33** following the general procedure. White solid (88% yield). ^{1}H-NMR (DMSO-d_6) δ: 3.53 (d, *J*=5.86 Hz, 2H); 5.18 (s, 2H); 7.15-7.19 (m, 2H); 7.41-7.45 (m, 2H); 7.60-7.64 (m, 2H); 7.71-7.75 (m, 2H); 7.89 (t, *J*=5.86 Hz, 1H).

2-(4-(4-Fluorobenzyloxy)phenylsulfonamido)acetic acid (45). The title compound was prepared from sulfonyl chloride **34** following the general procedure. White solid (73% yield). ^{1}H-NMR (DMSO-d_6) δ: 3.53 (d, *J*=6.04 Hz, 2H); 5.16 (s, 2H); 7.14-7.18 (m, 2H); 7.24-7.28 (m, 2H); 7.49-7.56 (m, 2H); 7.70-7.74 (m, 2H); 7.89 (t, *J*=6.04 Hz, 1H).

2-(4-(4-(Trifluoromethoxy)benzyloxy)phenylsulfonamido)acetic acid (46). The title compound was prepared from sulfonyl chloride **35** following the general procedure. White solid (80% yield). ^{1}H-NMR (DMSO-d_6) δ: 3.53 (d, *J*=5.67 Hz, 2H); 5.22 (s, 2H); 7.15-7.19 (m, 2H); 7.39-7.43 (m, 2H); 7.58-7.62 (m, 2H); 7.71-7.75 (m, 2H); 7.89 (t, *J*=5.67 Hz, 1H).

2-(4-(Biphenyl-4-ylmethoxy)phenylsulfonamido)acetic acid (47). The title compound was prepared from sulfonyl chloride **36** following the general procedure. Pinkish solid (45% yield). ^{1}H-NMR (DMSO-d_6) δ: 3.54 (d, *J*=6.02 Hz, 2H); 5.24 (s, 2H); 7.18-7.22 (m, 2H); 7.37-7.57 (m, 5H); 7.66-7.76 (m, 6H); 7.89 (t, *J*=6.02 Hz, 1H).

2-(4-(3-Bromobenzyloxy)phenylsulfonamido)acetic acid (48). The title compound was prepared from sulfonyl chloride **37** following the general procedure. White solid (81% yield). ^{1}H-NMR (DMSO-d_6) δ: 3.54 (d, *J*=6.02 Hz, 2H); 5.19 (s, 2H); 7.16-7.20 (m, 2H); 7.33-7.58 (m, 3H); 7.68-7.72 (m, 2H); 7.75-7.76 (m, 1H); 7.89 (t, *J*=6.02 Hz, 1H).

2-(4-(3-Fluorobenzyloxy)phenylsulfonamido)acetic acid (49). The title compound was prepared from sulfonyl chloride **38** following the general procedure. White solid (76% yield). ^{1}H-NMR (MeOD-d_6) δ: 3.67 (s, 2H); 5.1 (s, 2H); 7.03-7.29 (m, 5H); 7.35-7.46 (m, 1H); 7.78-7.82 (m, 2H).

2-(4-(3-(Trifluoromethoxy)benzyloxy)phenylsulfonamido)acetic acid (50). The title compound was prepared from sulfonyl chloride **39** following the general procedure. White solid (75% yield).^{1}H-NMR (DMSO-d_6) δ: 3.53 (d, *J*=6.04 Hz, 2H); 5.25 (s, 2H); 7.16-7.20 (m, 2H); 7.34-7.37 (m, 1H); 7.49-7.60 (m, 3H); 7.71-7.75 (m, 2H); 7.89 (t, *J*=6.04 Hz, 1H).

2-(4-(Biphenyl-3-ylmethoxy)phenylsulfonamido)acetic acid (51). The title compound was prepared from sulfonyl chloride **40** following the general procedure. White solid (60% yield). ^{1}H-NMR (MeOD-d_6) δ: 3.64 (s, 2H); 5.17 (s, 2H); 7.15-7.19 (m, 2H); 7.29-7.48 (m, 6H); 7.58-7.65 (m, 3H); 7.70 (s, 1H); 7.79-7.83 (m, 2H).

2-(4-(Quinolin-8-ylmethoxy)phenylsulfonamido)acetic acid (52). The title compound was prepared from sulfonyl chloride **41** following the general procedure. White solid (72% yield). ^{1}H-NMR (Acetone-d_6) δ: 3.65 (s, 2H); 5.84 (s, 2H); 7.18-7.22 (m, 2H); 7.57-7.67 (m, 2H); 7.77-7.81 (m, 2H); 7.92-7.97 (m, 2H); 8.40-8.45 (m, 1H); 8.93-8.95 (m, 1H).

2-(4-(3,5-Difluorobenzyloxy)phenylsulfonamido)acetic acid (53). The title compound was prepared from sulfonyl chloride **42** following the general procedure. White solid (81% yield). ^{1}H-

NMR (DMSO-d_6) δ: 3.53 (d, *J*=5.13 Hz, 2H); 5.22 (s, 2H); 7.16-7.22 (m, 5H); 7.72-7.75 (m, 2H); 7.89 (t, *J*=5.13 Hz, 1H).

2-(4-(3,5-Dibromobenzyloxy)phenylsulfonamido)acetic acid (16). The title compound was prepared from sulfonyl chloride **43** following the general procedure. Pale pink solid (79% yield). ^{1}H-NMR (DMSO-d_6) δ: 3.54 (d, *J*=6.02 Hz, 2H); 5.20 (s, 2H); 7.16-7.20 (m, 2H); 7.70-7.71 (m, 2H); 7.72-7.76 (m, 2H); 7.83-7.85 (m, 1H); 7.90 (t, *J*=6.02 Hz, 1H). ^{13}C-NMR ($CDCl_3$) δ: 44.58; 69.079; 115.801; 123.521; 130.021; 130.185; 134.009; 142.275; 162.213; 170.207.

General procedure for the synthesis of Benzyloxy-phenylsulfonamido *N*-(*tert*-butyldimethylsilyloxy)acetamides 54-61. Carboxylates **44-46** and **49-53** (0.32 mmol) were suspended in dry CH_2Cl_2 (8.5 mL) and *O*-(*tert*-butyldimethylsilyl)hydroxylamine (47.9 mg 0.32 mmol) and EDC (93.5 mg, 0.48 mmol) were added. After stirring at RT overnight, the mixture was washed with water and the organic phases were dried and evaporated *in vacuo*.

2-(4-(4-Bromobenzyloxy)phenylsulfonamido)-*N*-(*tert*-butyldimethylsilyloxy)acetamide (54). The title compound was prepared from carboxylic acid **44** following the general procedure. The crude product was purified by flash chromatography (*n*-hexane/EtOAc 2:1) using a Isolute Flash Si II cartridge to give **54** as a white solid (43% yield). ^{1}H-NMR ($CDCl_3$) δ: 0.15 (s, 6H); 0.94 (s, 9H); 3.57 (br s, 1H); 3.77 (br s, 1H); 5.01 (s, 2H); 5.24 (br s, 1H); 7.01-7.05 (m, 2H); 7.27-7.31 (m, 2H); 7.51-7.55 (m, 2H); 7.77-7.81 (m, 2H); 8.43 (br s, 1H).

***N*-(*tert*-Butyldimethylsilyloxy)-2-(4-(4-fluorobenzyloxy)phenylsulfonamido)acetamide (55).** The title compound was prepared from carboxylic acid **45** following the general procedure. The crude product was purified by flash chromatography (*n*-hexane/EtOAc 2:1) using a Isolute Flash Si II cartridge to give **55** as a white solid (26% yield). ^{1}H-NMR ($CDCl_3$) δ: 0.15 (s, 6H); 0.94 (s, 9H); 3.57 (br s, 1H); 3.79 (br s, 1H); 5.08 (s, 2H); 5.28 (br s, 1H); 7.02-7.14 (m, 4H); 7.37-7.43 (m, 2H); 7.77-7.82 (m, 2H); 8.43 (br s, 1H).

***N*-(*tert*-Butyldimethylsilyloxy)-2-(4-(4-(trifluoromethoxy)benzyloxy)phenylsulfonamido)acetamide (56).** The title compound was

prepared from carboxylic acid **46** following the general procedure. The crude product was purified by flash chromatography (*n*-hexane/EtOAc 2:1) using a Isolute Flash Si II cartridge to give **56** as a white solid (52% yield). ^{1}H-NMR ($CDCl_3$) δ:0.15 (s, 6H); 0.94 (s, 9H); 3.58 (br s, 1H); 3.79 (br s, 1H); 5.11 (s, 2H); 7.03-7.07 (m, 2H); 7.24-7.27 (m, 2H); 7.44-7.48 (m, 2H); 7.78-7.82 (m, 2H); 8.41 (br s, 1H).

***N*-(*tert*-Butyldimethylsilyloxy)-2-(4-(3-fluorobenzyloxy)phenylsulfonamido)acetamide (57).** The title compound was prepared from carboxylic acid **49** following the general procedure. The crude product was purified by flash chromatography (*n*-hexane/EtOAc 3:1) using a Isolute Flash Si II cartridge to give **57** as a white solid (57% yield). ^{1}H-NMR ($CDCl_3$) δ:0.14 (s, 6H); 0.94 (s, 9H); 3.57 (br s, 1H); 3.79 (br s, 1H); 5.12 (s, 2H); 5.28 (br s, 1H); 7.02-7.20 (m, 5H); 7.32-7.43 (m, 1H); 7.77-7.82 (m, 2H), 8.53 (br s, 1H).

***N*-(*tert*-Butyldimethylsilyloxy)-2-(4-(3-(trifluoromethoxy)benzyloxy)phenylsulfonamido)acetamide (58).** The title compound was prepared from carboxylic acid **50** following the general procedure. The crude product was purified by flash chromatography (*n*-hexane/EtOAc 2:1) using a Isolute Flash Si II cartridge to give **58** as a colorless oil (41% yield). ^{1}H-NMR ($CDCl_3$) δ: 0.15 (s, 6H); 0.94 (s, 9H); 3.57 (br s, 1H); 3.77 (br s, 1H); 5.13 (s, 2H); 7.03-7.07 (m, 2H); 7.20-7.48 (m, 4H); 7.79-7.83 (m, 2H); 8.44 (brs, 1H).

2-(4-(Biphenyl-3-ylmethoxy)phenylsulfonamido)-*N*-(*tert*-butyldimethylsilyloxy)acetamide (59). The title compound was prepared from carboxylic acid **51** following the general procedure. The crude product was purified by flash chromatography (*n*-hexane/EtOAc 3:1) using a Isolute Flash Si II cartridge to give **59** as a colorless oil (21% yield). ^{1}H-NMR ($CDCl_3$) δ: 0.14 (s, 6H); 0.93 (s, 9H); 3.58 (br s, 1H); 3.78 (br s, 1H); 5.17 (s, 2H); 5.61 (br s, 1H); 7.05-7.09 (m, 2H); 7.36-7.51 (m, 5H); 7.57-7.64 (m, 4H); 7.77-7.81 (m, 2H);8.84 (br s, 1H).

***N*-(*tert*-Butyldimethylsilyloxy)-2-(4-(quinolin-8-ylmethoxy)phenylsulfonamido)acetamide (60).** The title compound was prepared from carboxylic acid **52** following the general procedure. The crude product was purified by flash chromatography (*n*-hexane/EtOAc 1:1) using a Isolute Flash Si

II cartridge to give **60** as a colorless oil (15% yield). ^{1}H-NMR ($CDCl_3$) δ: 0.14 (s, 6H); 0.92 (s, 9H); 3.56 (br s, 1H); 3.80 (br s, 1H); 5.55 (br s, 1H); 5.88 (s, 2H); 7.13-7.17 (m, 2H); 7.43-7.60 (m, 2H); 7.75-7.88 (m, 2H); 8.17-8.22 (m, 1H); 8.85 (br s, 1H); 8.92-8.95 (m, 1H).

***N*-(*tert*-Butyldimethylsilyloxy)-2-(4-(3,5-difluorobenzyloxy)phenylsulfonamido)acetamide (61).** The title compound was prepared from carboxylic acid **53** following the general procedure. The crude product was purified by flash chromatography (*n*-hexane/EtOAc 2:1) using a Isolute Flash Si II cartridge to give **61** as a white solid (53% yield). ^{1}H-NMR ($CDCl_3$) δ: 0.15 (s, 6H); 0.94 (s, 9H); 3.59 (br s, 1H); 3.79 (br s, 1H); 5.10 (s, 2H); 5.23 (br s, 1H); 6.74-6.83 (m, 1H); 6.94-7.06 (m, 4H); 7.79-7.83 (m, 2H); 8.41 (br s, 1H).

General procedure for the synthesis of Benzyloxy-phenylsulfonamido *N*-(tetrahydro-2H-pyran-2-yloxy)acetamides 62-64. Carboxylic acids **47**, **48** and **16** (0.46 mmol) were dissolved in dry DMF (1 mL) and *O*-(tetrahydro-2H-pyran-2-yl)hydroxylamine (166.7 mg, 1.42 mmol), *N*-methylmorpholine redistilled (0.15 mL, 1.38 mmol), HOBT (74.6 mg, 0.55 mmol), and EDC (123.5 mg, 0.64 mmol) were added under N_2 atmosphere. After stirring for 2 h, the reaction mixture was diluted with EtOAc and washed with water, saturated solution of $NaHCO_3$, and brine. The organic phase was dried over Na_2SO_4, filtered and concentrated *in vacuo*.

2-(4-(Biphenyl-4-ylmethoxy)phenylsulfonamido)-*N*-(tetrahydro-2H-pyran-2-yloxy)acetamide (62). The title compound was prepared from carboxylic acid **47** following the general procedure. The crude product was purified by flash chromatography (*n*-hexane/EtOAc 1:1) using a Isolute Flash Si II cartridge to give **62** as a colorless oil (61% yield). ^{1}H-NMR ($CDCl_3$) δ: 1.67-1.81 (m, 6H); 3.62 (s, 2H); 3.81-3.99 (m, 2H); 4.81-4.92 (m, 1H); 5.16 (s, 2H); 7.06-7.10 (m, 2H); 7.36-7.51 (m, 5H); 7.57-7.66 (m, 4H); 7.79-7.83 (m, 2H); 8.91 (br s, 1H).

2-(4-(3-Bromobenzyloxy)phenylsulfonamido)-*N*-(tetrahydro-2H-pyran-2-yloxy)acetamide (63). The title compound was prepared from carboxylic acid **48** following the general procedure. The crude product was purified by flash chromatography (*n*-hexane/EtOAc 1:1) using a Isolute Flash Si II cartridge to give **63** as a colorless oil (86% yield). ^{1}H-NMR ($CDCl_3$) δ: 1.68-1.82 (m,

6H); 3.62 (s, 2H); 3.75-3.93 (m, 2H); 4.81-4.89 (m, 1H); 5.08 (s, 2H); 5.40 (br s, 1H); 7.01-7.05 (m, 2H); 7.29-7.35 (m, 2H); 7.46-7.49 (m, 1H); 7.56-7.57 (m, 1H); 7.78-7.82 (m, 2H); 9.13 (br s, 1H).

2-(4-(3,5-Dibromobenzyloxy)phenylsulfonamido)-*N*-(tetrahydro-2H-pyran-2-yloxy)acetamide (64). The title compound was prepared from carboxylic acid **16** following the general procedure. The crude product was purified by flash chromatography (*n*-hexane/EtOAc 1:1) using a Isolute Flash Si II cartridge to give **64** a colorless oil (82% yield). ^{1}H-NMR ($CDCl_3$) δ: 1.67-1.81 (m, 6H); 3.64 (s, 2H); 3.61-3.99 (m, 2H); 4.79-4.91 (m, 1H); 5.05 (s, 2H); 5.27 (br s, 1H); 7.01-7.05 (m, 2H); 7.51-7.52 (m, 2H); 7.65-7.66 (m, 1H); 7.80-7.84 (m, 2H); 8.98 (br s, 1H).

General procedure for the synthesis of Benzyloxy-phenylesulfonamido *N*-hydroxy acetamides 3-5, 8-12. Silyl precursors (0.05 mmol) were dissolved in dry CH_2Cl_2 (0.4 mL) and TFA (0.22 mL, 2.8 mmol) was added dropwise at 0 °C. After 5h stirring, TFA was evaporated. The crude products were purified by trituration with *n*-hexane/Et_2O to give the desired hydroxamates **3-5** and **8-12**.

2-(4-(4-Bromobenzyloxy)phenylsulfonamido)-*N*-hydroxyacetamide (3). The title compound was prepared from *O*-silylate derivative **54** following the general procedure. White solid (85% yield). Mp: 135-137 °C; ^{1}H-NMR (DMSO-d_6) δ: 3.27 (d, *J*=5.31 Hz, 2H); 5.18 (s, 2H); 7.15-7.19 (m, 2H); 7.41-7.45 (m, 2H); 7.59-7.63 (m, 2H); 7.71-7.75 (m, 2H); 8.86 (br s, 1H); 10.53 (br s, 1H). ^{13}C-NMR (DMSO-d_6) δ: 43.11, 68.78, 114.98, 121.15, 128.70, 129.91, 131.38, 132.18, 135.77, 160.93, 164.28. Elemental Analysis for $C_{15}H_{15}BrN_2O_5S$. Calculated: %C, 43.39; %H, 3.64; %N, 6.75; Found %C, 43.54; %H, 3.81; %N, 6.56.

2-(4-(4-Fluorobenzyloxy)phenylsulfonamido)-*N*-hydroxyacetamide (4). The title compound was prepared from *O*-silylate derivative **55** following the general procedure. The crude product was purified by flash chromatography (*n*-hexane/EtOAc 2:3) using a Isolute Flash Si II cartridge to give **4** as a white solid (36% yield); mp: 128-130°C; ^{1}H-NMR (DMSO-d_6) δ: 3.27 (d, *J*=5.9 Hz, 2H); 5.17 (s, 2H); 7.15-7.19 (m, 2H); 7.24-7.28 (m, 2H); 7.49-7.56(m, 2H); 7.71-7.75 (m, 2H); 7.81 (t, *J*=5.9 Hz, 1H); 8.87 (br s, 1H); 10.53 (br s, 1H). ^{13}C-NMR (DMSO-d_6) δ: 40.71, 70.83, 114.61, 115.73, 128.34, 128.75, 132.09, 136.84, 161.82, 163.74, 166.68. Elemental Analysis for

$C_{15}H_{15}FN_2O_5S$. Calculated: %C, 50.84; %H, 4.27; %N, 7.91; Found %C, 50.97; %H, 4.31; %N, 7.77.

***N*-Hydroxy-2-(4-(4-(trifluoromethoxy)benzyloxy)phenylsulfonamido)acetamide (5).** The title compound was prepared from *O*-silylate derivative **56** following the general procedure. White solid (87% yield); mp: 114-116 °C; ^{1}H-NMR (DMSO-d_6) δ: 3.28 (d, *J*=6.04 Hz, 2H); 5.23 (s, 2H); 7.16-7.20 (m, 2H); 7.39-7.43 (m, 2H); 7.59-7.63 (m, 2H); 7.72-7.76 (m, 2H); 7.82 (t, *J*=6.04 Hz, 1H); 8.88 (br s, 1H); 10.54 (br s, 1H). ^{13}C-NMR (DMSO-d_6) δ: 43.11, 68.65, 114.94, 121.06, 128.72, 129.69, 132.22, 135.82, 147.90, 160.95, 164.28. Elemental Analysis for $C_{16}H_{15}F_3N_2O_6S$. Calculated: %C, 45.72; %H, 3.60; %N, 6.66; Found %C, 45.83; %H, 3.81; %N, 6.30.

2-(4-(3-Fluorobenzyloxy)phenylsulfonamido)-*N*-hydroxyacetamide (8). The title compound was prepared from *O*-silylate derivative **57** following the general procedure. The crude product was purified by flash chromatography (*n*-hexane/EtOAc 2:3) using a Isolute Flash Si II cartridge to give **8** as a yellow solid (45% yield); mp: 98-100 °C; ^{1}H-NMR (DMSO-d_6) δ: 3.27 (s, 2H); 5.21 (s, 2H); 7.16-7.20 (m, 3H); 7.29-7.32 (m, 2H); 7.41-7.51 (m, 1H); 7.72-7.76 (m, 2H); 9.04 (br s, 1H). ^{13}C-NMR (DMSO-d_6) δ: 43.13, 68.73, 114.14, 114.57, 114.97, 123.61, 123.66, 128.70, 130.42, 130.58, 132.22, 139.12, 139.26, 159.64, 160.90, 164.50. Elemental Analysis for $C_{15}H_{15}FN_2O_5S$. Calculated: %C, 50.84; %H, 4.27; %N, 7.91; Found %C, 50.93; %H, 4.34; %N, 7.57.

***N*-Hydroxy-2-(4-(3-(trifluoromethoxy)benzyloxy)phenylsulfonamido)acetamide (9).** The title compound was prepared from *O*-silylate derivative **58** following the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH 36:1) using a Isolute Flash Si II cartridge to give **9** as a white solid (42% yield); mp: 83-85 °C; ^{1}H-NMR (DMSO-d_6) δ: 3.27 (d, *J*=5.31 Hz, 2H); 5.25 (s, 2H); 7.17-7.21 (m, 2H); 7.31-7.38 (m, 1H); 7.48-7.56 (m, 3H); 7.72-7.76 (m, 2H); 9.04 (br s, 2H). ^{13}C-NMR (DMSO-d_6) δ: 3.65, 29.07, 75.44, 80.54, 80.96, 87.20, 89.19, 91.00, 92.77, 99.62, 108.83, 121.34, 124.67. Elemental Analysis for $C_{16}H_{15}F_3N_2O_6S$. Calculated: %C, 45.72; %H, 3.60; %N, 6.66; Found %C, 45.79; %H, 3.76; %N, 6.49.

2-(4-(Biphenyl-3-ylmethoxy)phenylsulfonamido)-*N*-hydroxyacetamide (10). The title compound was prepared from *O*-silylate derivative **59** following the general procedure. The crude product was purified by flash chromatography (*n*-hexane/EtOAc 2:3) using a Isolute Flash Si II cartridge to give **10** as a foaming pink solid (50% yield); mp: 54-55 °C; ^{1}H-NMR (DMSO-d_6) δ: 3.26 (s, 2H); 5.27 (s, 2H); 7.19-7.23 (m, 2H); 7.37-7.51 (m, 5H); 7.62-7.76 (m, 6H). ^{13}C-NMR (DMSO-d_6) δ: 43.13, 69.58, 114.99, 126.19, 126.37, 126.68, 126.86, 127.55, 128.72, 128.92, 129.12, 132.05, 137.03, 139.77, 140.34, 161.15, 164.26. Elemental Analysis for $C_{21}H_{20}N_2O_5S$. Calculated: %C, 61.15; %H, 4.89; %N, 6.79; Found %C, 61.23; %H, 4.96; %N, 6.78.

***N*-Hydroxy-2-(4-(quinolin-8-ylmethoxy)phenylsulfonamido)acetamide (11).** The title compound was prepared from *O*-silylate derivative **60** following the general procedure. The crude product was purified by flash chromatography (CH_2Cl_2 /MeOH 18:1) using a Isolute Flash Si II cartridge to give **11** as a white solid (70% yield); mp: 165-167 °C; ^{1}H-NMR (DMSO-d_6) δ: 3.29 (s, 2H); 5.82 (s, 2H); 7.22-7.26 (m, 2H); 7.59-7.68 (m, 2H); 7.73-7.77 (m, 2H); 7.88-7.92 (m, 1H); 7.99-8.02 (m, 1H); 8.42-8.46 (m, 1H); 8.91 (br s, 1H); 8.96-8.98 (m, 1H); 10.56 (br s, 1H). ^{13}C-NMR (DMSO-d_6) δ:43.14, 66.05, 114.90, 121.73, 126.30, 128.25, 128.43, 128,80, 131.99, 133.78, 136.48, 145.18, 150.15, 161.41, 164.34. Elemental Analysis for $C_{18}H_{17}N_3O_5S$. Calculated: %C, 55.80; %H, 4.42; %N, 10.85; Found %C, 55.63; %H, 4.69; %N, 10.68.

2-(4-(3,5-Difluorobenzyloxy)phenylsulfonamido)-*N*-hydroxyacetamide (12). The title compound was prepared from *O*-silylate derivative **61** following the general procedure. White solid (85% yield); mp: 125-127 °C; ^{1}H-NMR (DMSO-d_6) δ: 3.27 (d, J=6.04 Hz, 2H); 5.23 (s, 2H); 7.16-7.27 (m, 5H); 7.72-7.76 (m, 2H); 7.83 (t, J=6.04 Hz, 2H); 10.54 (br s, 1H). ^{13}C-NMR (DMSO-d_6) δ: 43.11, 68.18, 103.38, 110.33, 110.82, 114.99, 128.74, 132.42, 160.70, 164.26. Elemental Analysis for $C_{15}H_{14}F_2N_2O_5S$. Calculated: %C, 48.39; %H, 3.79; % N, 7.52; Found %C, 48.66; %H, 3.91; %N, 7.46.

General procedure for the synthesis of Benzyloxy-phenylesulfonamido *N*-hydroxy acetamides 6, 7, 13. Protected Hydroxamates **62-64** (0.33 mmol) were dissolved in dioxane (0.6 mL) and a

solution of HCl 4 N (5.94 mL) was added dropwise followed by MeOH (1.32 mL). After stirring for 30 min, the reaction was evaporated *in vacuo*.

2-(4-(Biphenyl-4-ylmethoxy)phenylsulfonamido)-*N*-hydroxyacetamide (6). The title compound was prepared from the THP-protected hydroxamate **62** following the general procedure. The crude product was purified by flash chromatography (*n*-hexane/EtOAc 1:1) using a Isolute Flash Si II cartridge to give **6** as a white solid (39% yield). Mp: 163-165 °C; ^{1}H-NMR (DMSO-d_6) δ: 3.25 (s, 2H); 5.23 (s, 2H); 7.18-7.22 (m, 2H); 7.33-7.57 (m, 5H); 7.66-7.76 (m, 6H); 8.97 (br s, 1H). ^{13}C-NMR (DMSO-d_6) δ: 30.70, 69.29, 114.94, 126.61, 126.76, 127.49, 128.39, 128.70, 128.89, 132.15, 135.46, 139.65, 139.85, 161.10. Elemental Analysis for $C_{21}H_{20}N_2O_5S$. Calculated: %C, 61.15; %H, 4.89; %N, 6.79; Found %C, 61.21; %H, 4.99; %N, 6.66.

2-(4-(3-Bromobenzyloxy)phenylsulfonamido)-*N*-hydroxyacetamide (7). The title compound was prepared from the THP-protected hydroxamate **63** following the general procedure. Hydroxamate **7a** was obtained after trituration with Et_2O as a white crystalline solid (79% yield); mp: 124-125 °C; ^{1}H-NMR (DMSO-d_6) δ: 3.28 (d, *J*=6.02 Hz, 2H); 5.20 (s, 2H); 7.17-7.21 (m, 2H); 7.34-7.58 (m, 3H); 7.68-7.69 (m, 1H); 7.72-7.76 (m, 2H); 7.80-7.86 (t, *J*=6.02 Hz, 1H); 8.88 (br s, 1H); 10.55 (br s, 1H). ^{13}C-NMR (DMSO-d_6) δ: 43.10, 68.60, 114.96, 121.66, 126.68, 128.72, 130.32, 130.67, 130.83, 132.25, 139.10, 160.89, 164.28. Elemental Analysis for $C_{15}H_{15}BrN_2O_5S$. Calculated: %C, 43.39; %H, 3.64; %N, 6.75; Found %C, 43.41; %H, 3.75; %N, 6.49.

2-(4-(3,5-Dibromobenzyloxy)phenylsulfonamido)-*N*-hydroxyacetamide (13). The title compound was prepared from the THP-protected hydroxamate **64** following the general procedure. Hydroxamate **13** was obtained after trituration with Et_2O as a white solid (77% yield). Mp: 94-96 °C; ^{1}H-NMR (DMSO-d_6) δ: 3.28 (d, *J*=6.02 Hz, 2H); 5.20 (s, 2H); 7.17-7.21 (m, 2H); 7.70-7.72 (m, 3H); 7.76-7.77 (m, 1H); 7.80-7.86 (m, 2H); 10.55 (br s, 1H). ^{13}C-NMR (DMSO-d_6) δ: 43.11, 67.80, 114.96, 122.50, 128.72, 129.47, 132.42, 132.87, 141.10, 160.68, 164.25. Elemental Analysis for $C_{15}H_{14}Br_2N_2O_5S$. Calculated: %C, 36.46; %H, 2.86; %N, 5.67; Found %C, 36.51; %H, 2.93; %N, 5.43.

***tert*-Butyl 2-(4-(3,5-dibromobenzyloxy)phenylsulfonamido)acetate (65).** To a solution of glycine *tert*-butyl ester hydrochloride (176 mg, 1.05 mmol), in H_2O (1.0 mL) and dioxane (5.0 mL) containing TEA (0.44 mL, 3.15 mmol), sulfonyl chloride **43** (500 mg, 1.05 mmol) was added. The mixture was stirred at RT overnight, dioxane was evaporated and the residue was diluited with EtOAc and washed with H_2O and brine. The organic phase was dried over Na_2SO_4, filtered and concentrated *in vacuo* to give a white solid (512 mg, 90% yield). ^{1}H-NMR ($CDCl_3$) δ: 1.34 (s, 9H); 3.65 (d, *J*=5.3 Hz, 2H); 4.99 (t, *J*=5.4 Hz, 1H); 5.04 (s, 2H); 6.99-7.04 (m, 2H); 7.50 (m, 2H); 7.65 (s, 1H); 7.79-7.83 (m, 2H).

General procedure for the synthesis of *tert*-butyl esters 66 and 67. A solution of aryl bromide **65** (250 mg, 0.48 mmol) in dioxane (4.8 mL)/H_2O (1.0 mL) was sequentially treated under nitrogen with K_3PO_4 (306 mg, 1.4 mmol), the appropriate arylboronic acid (0.52 mmol), and $Pd(PPh_3)_4$ (22 mg, 0.02 mmol). The resulting mixture was stirred for 1 h at 85 °C. After being cooled to RT, the mixture was treated with $NaHCO_3$ and extracted with $CHCl_3$. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. The crude was purified by flash chromatography on silica gel column.

***tert*-Butyl 2-(4-((5-bromo-4'-methoxybiphenyl-3-yl)methoxy)phenylsulfonamido)acetate (66).** The title compound was prepared from 4-methoxyphenylboronic acid following the general procedure. Purified with *n*-hexane/EtOAc 7:2 (40% yield) as colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.33 (s, 9H); 3.65 (d, *J*=5.5 Hz, 2H); 3.85 (s, 3H); 4.96 (t, *J*=5.5 Hz, 1H); 5.12 (s, 2H); 6.94-7.00 (m, 2H); 7.02-7.07 (m, 2H); 7.47-7.51 (m, 4H); 7.66 (s, 1H); 7.77-7.83 (m, 2H).

***tert*-Butyl 2-(4-(3-bromo-5-(pyridin-3-yl)benzyloxy)phenylsulfonamido)acetate (67).** The title compound was prepared from 3-pyridinylboronic acid following the general procedure. Purified with *n*-hexane/EtOAc 2:1 (36% yield) as white solid. ^{1}H-NMR ($CDCl_3$) δ: 1.32 (s, 9H); 3.63 (d, *J*= 5.6 Hz, 2H); 5.14 (s, 2H); 5.29 (t, *J*=5.4 Hz, 1H); 7.02-7.06 (m, 2H); 7.39-7.71 (m, 4H); 7.78-7.83 (m, 2H); 7.91 (dt, J_1= 8 Hz, J_2= 2 Hz, 1H); 8.64 (d, *J*=3.6 Hz, 1H); 8.84 (s, 1H).

General Procedure for the Preparation of Carboxylic Acids 68 and 69. TFA (10.44 mmol, 0.8 mL) was added dropwise to a stirred, ice-chilled solution of *tert*-butyl esters **66** and **67** (0.18 mmol) in freshly distilled dichloromethane (2.0 mL). The mixture was stirred under these reaction conditions for 5 h, and the solvent was removed *in vacuo* to give the corresponding carboxylic acids **68** and **69**. The crude products were purified by trituration with Et_2O.

2-(4-((5-Bromo-4'-methoxybiphenyl-3-yl)methoxy)phenylsulfonamido)acetic acid (68). The title compound was prepared from *tert*-butyl ester **66** following the general procedure. White solid (87% yield). ^{1}H-NMR (CD_3OD) δ: 3.65 (s, 2H); 3.82 (s, 3H); 5.12 (s, 2H); 6.93-6.97 (m, 2H); 7.03-7.08 (m, 2H); 7.45-7.50 (m, 4H); 7.63 (s, 1H); 7.76-7.80 (m, 2H).

3-(3-Bromo-5-((4-(*N*-(carboxymethyl)sulfamoyl)phenoxy)methyl)phenyl)pyridinium 2,2,2-trifluoroacetate (69). The title compound was prepared from *tert*-butyl ester **67** following the general procedure. White solid (58% yield). ^{1}H-NMR (DMSO-d_6) δ: 3.53 (d, *J*=6.0 Hz, 2H); 5.26 (s, 2H); 7.19-7.23 (m, 2H); 7.57-7.63 (m, 2H); 7.72-7.76 (m, 3H); 7.88-7.97 (m, 3H); 8.24 (d, *J*=7.8 Hz, 1H); 8.67 (s, 1H); 8.98 (s, 1H).

General Procedure for the Synthesis of Hydroxamates 17 and 18. EDC was added portionwise (92 mg, 0.48 mmol) to a stirred and cooled solution (0 °C) of the appropriate carboxylic acid **68, 69** (0.19 mmol) and *O*-(*tert*-butyldimethylsilyl)hydroxylamine (113 mg, 0.77 mmol) in dry CH_2Cl_2 (10 mL). After stirring at RT overnight, the mixture was washed with water and the organic phase was dried and evaporated *in vacuo*. Silyl precursors (0.12 mmol) were then dissolved in dry CH_2Cl_2 (2 mL) and TFA (0.5 mL, 6.56 mmol) was added dropwise at 0 °C. After 5 h of stirring, TFA was evaporated. The crude products were purified by trituration with *n*-hexane/Et_2O to give the desired hydroxamates **17** and **18**.

2-(4-((5-Bromo-4'-methoxybiphenyl-3-yl)methoxy)phenylsulfonamido)-*N*-hydroxyacetamide (17). The title compound was prepared from carboxylic acid **68** following the general procedure. *O*-Silylate derivative was purified by flash chromatography on silica gel (*n*-hexane/EtOAc 1:1) to give a white solid (60% yield). ^{1}H-NMR ($CDCl_3$) δ: 0.02 (s, 6H); 0.85 (s, 9H); 3.65 (s, 2H); 3.82 (s, 3H);

5.16 (s, 2H); 6.93-6.97 (m, 2H); 7.03-7.08 (m, 2H); 7.45-7.50 (m, 4H); 7.63 (s, 1H); 7.76-7.80 (m, 2H); 10.39 (s, 1H).

Hydroxamic acid **17** was obtained as white solid (87% yield) after trituration with Et_2O. Mp: 175-177 °C; ^{1}H-NMR ($CDCl_3$) δ: 3.52 (s, 2H); 3.85 (s, 3H); 5.31 (s, 2H); 7.02-7.08 (m, 2H); 7.20-7.25 (m, 2H); 7.62-7.68 (m, 4H); 7.75 (s, 1H); 7.81-7.86 (m, 2H); 10.20 (br s, 1H). ^{13}C-NMR (DMSO-d_6) δ: 42.10; 57.90; 69.30; 114.95; 122.49; 127.90; 128.72; 129.47; 130.10; 132.42; 132.87; 138.70; 142.20; 159.15; 161.67; 165.21. Elemental Analysis for $C_{22}H_{21}BrN_2O_6S$. Calculated: %C, 50.68; %H, 4.06; %N, 5.37; Found %C, 50.75; %H, 4.19; %N, 5.29.

3-(3-Bromo-5-((4-(*N*-(2-(hydroxyamino)-2-oxoethyl)sulfamoyl)phenoxy)methyl)phenyl)pyridinium 2,2,2-trifluoroacetate (18). The title compound was prepared from carboxylic acid **69** following the general procedure. *O*-Silylate derivative was purified by flash chromatography on silica gel (*n*-hexane/EtOAc 1:1) to give a white solid (67% yield). ^{1}H-NMR (DMSO-d_6) δ: 0,03 (s, 6H); 0,86 (s, 9H); 5,27 (s, 2H); 7.20-7.24 (m, 2H); 7.48-7.63 (m, 3H); 7.72-7.76 (m, 3H); 7.86-7.94 (m, 2H); 8.13 (dt, J_1=8 Hz, J_2=1.6 Hz, 1H); 8.61 (dd, J_1=4.7 Hz, J_2=1.4 Hz, 1H); 8.92 (d, J=1.6 Hz, 1H); 10.75 (s, 1H).

Hydroxamic acid **18** was obtained as pale yellow solid (37% yield) after a flash chromatography ($CHCl_3$/MeOH 25:1) using a Isolute Flash Si II cartridge. Mp: 135 °C (dec.); ^{1}H-NMR (DMSO-d_6) δ: 3.28 (d, 2H); 5.27 (s, 2H); 7.20-7.24 (m, 2H); 7.47-7.54 (m, 1H); 7.72-7.94 (m, 6H); 8.13 (dt, J_1=8 Hz, J_2=1.6 Hz, 1H); 8.61 (dd, J_1=4.8 Hz, J_2=1.4 Hz, 1H); 8.88 (s, 1H); 8.92 (d, J=1.6 Hz, 1H). ^{13}C-NMR (CD_3OD) δ: 44.57; 70.30; 116.37; 124.27; 125.42; 125.98; 130.33; 130.64; 131.13; 136.54; 136.83; 141.33; 148.39; 149.49; 163.23; 167.64. Elemental Analysis for $C_{22}H_{19}BrF_3N_3O_7S$. Calculated: %C, 43.58; %H, 3.16; %N, 6.93; Found %C, 43.75; %H, 3.29; %N, 6.69.

General procedure for the synthesis of sulfonamides 70, 72 and 73. To a solution of sarcosine *tert*-butyl ester hydrochloride or H-D-Orn(Z)-OH or H-D-Orn(carbamoyl)-OH (0.59 mmol) in H_2O (1 mL) and dioxane (1 mL) containing TEA (1.76 mmol, 0.25 mL) 4-(3,5-dibromobenzyloxy)benzene-1-sulfonyl chloride **43** (0.59 mmol, 280mg) was added, and the

reaction was stirred at RT overnight. Dioxane was then evaporated and the residue was taken up with EtOAc, washed with HCl 1N and brine. Organic layers were then dried over Na_2SO_4, and evaporated *in vacuo* to furnish derivatives **70, 72** and **73**.

***tert*-Butyl 2-(4-(3,5-dibromobenzyloxy)-*N*-methylphenylsulfonamido)acetate (70).** The title compound was prepared from sarcosine *tert*-butyl ester hydrochloride following the general procedure. White solid (93% yield). ^{1}H-NMR ($CDCl_3$) δ: 1.40 (s, 9H); 2.89 (s, 3H); 3.88 (s, 2H); 5.05 (s, 2H): 7.00-7.04 (m, 2H); 7.51-7.52 (m, 2H); 7.64-7.65 (m, 1H); 7.75-7.80 (m, 2H).

(*R*)-5-(Benzyloxycarbonylamino)-2-(4-(3,5-dibromobenzyloxy)phenylsulfonamido)pentanoic acid (72). The title compound was prepared from H-D-Orn(Z)-OH following the general procedure. Carboxylic acid **72** was triturated with Et_2O and *n*-hexane to yield a pink solid (62% yield). ^{1}H-NMR (DMSO-d_6) δ: 1.21-1.58 (m, 4H); 2.81-2.96 (m, 2H); 3.57-3.67 (m, 1H); 4.99 (s, 2H); 5.21 (s, 2H); 6.91-6.93 (m, 1H); 7.15-7.19 (m, 2H); 7.33 (s, 5H); 7.70-7.74 (m, 4H); 7.82-7.84 (m, 1H); 7.89-8.15 (m, 1H).

(*R*)-2-(4-(3,5-Dibromobenzyloxy)phenylsulfonamido)-5-ureidopentanoic acid (73). The title compound was prepared from H-D-Orn(carbamoyl)-OH (H-D-Cit-OH) following the general procedure. White solid (47% yield). ^{1}H-NMR (DMSO-d_6) δ: 1.22-1.60 (m, 4H); 2.84 (dd, J_1= 5.8 Hz, J_2=6.6 Hz, 2H); 3.56-3.67 (m, 1H); 5.18 (s, 2H); 5.36 (s, 2H); 5.88 (t, *J*= 5.8 Hz, 1 H); 7.13-7.17 (m, 2H); 7.69-7.73 (m, 4H); 7.83 (t, *J*=1.4 Hz, 1H); 8.00 (d, *J*=8.6 Hz,1H).

2-(4-(3,5-Dibromobenzyloxy)-*N*-methylphenylsulfonamido)acetic acid (71). TFA (2.25 mL, 29.24 mmol) was added dropwise to a stirred, ice-chilled solution of *tert*-butyl ester **70** (300 mg, 0.51 mmol) in dry dichloromethane (4.0 mL). The mixture was stirred under these reaction conditions for 5 h and the solvent was removed *in vacuo* to give the carboxylic acid **71**. White solid (253 mg, 0.51 mmol, quantitative yield). ^{1}H-NMR (Acetone-d_6) δ: 2.85 (s, 3H); 3.97 (s, 2H); 5.29 (s, 2H); 7.21-7.25 (m, 2H); 7.73-7.74 (m, 2H); 7.75-7.76 (m, 1H); 7.80-7.84 (m, 2H).

General procedure for the synthesis of 3,5-dibromobenzyloxy-phenylsulfonamido-*N*-(tetrahydro-2H-pyran-2-yloxy)acetamides 74 and 75. Protected hydroxamates **74** and **75** were obtained from carboxylates **71** and **72** following the procedure previously described for **62-64.**

2-(4-(3,5-Dibromobenzyloxy)-*N*-methylphenylsulfonamido)-*N*-(tetrahydro-2H-pyran-2-yloxy)acetamide (74). The crude product was purified by flash chromatography (*n*-hexane/EtOAc 1:1) using a Isolute Flash Si II cartridge to give **74** as a white solid (39% yield). ^{1}H-NMR ($CDCl_3$) δ: 1.52-1.68 (m, 4H); 1.76-1.88 (m, 2H); 2.82 (s, 3H); 3.67 (s, 2H); 3.90-4.08 (m, 2H); 4.96-5.02 (m, 1H); 5.07 (s, 2H); 7.05-7.09 (m, 2H); 7.52 (s, 2H); 7.66 (s, 1H); 7.74-7.79 (m, 2H); 9.13 (br s, 1H).

Benzyl (*R*)-4-(4-(3,5-dibromobenzyloxy)phenylsulfonamido)-5-oxo-5-(tetrahydro-2H-pyran-2-yloxyamino)pentylcarbamate (75). The crude product was purified by flash chromatography (*n*-hexane/EtOAc 3:2) using a Isolute Flash Si II cartridge to give **75** as a white solid (36% yield). ^{1}H-NMR ($CDCl_3$) δ: 1.76-1.88 (m, 2H); 1.52-1.68 (m, 2H); 3.06-3.14 (m, 2H); 3.54-3.60 (m, 2H); 3.74-3.82 (m, 2H); 3.98-4.02 (m, 2H); 4.35 (s, 1H); 4.95-5.12 (m, 1H); 5.01 (s, 2H); 5.09 (s, 2H); 5.51 (d, *J*= 9 Hz, 1H); 6.98-7.03 (m, 2H); 7.33-7.34 (m, 5H); 7.51 (s, 2H); 7.66 (s, 1H); 7.78-7.82 (m, 2H); 9.47 (br s, 1H).

General procedure for the synthesis of 3,5-dibromobenzyloxy-phenylsulfonamido-*N*-hydroxyacetamides 15 and 20. Hydroxamates **15** and **20** were obtained from THP-protected hydroxamates **74** and **75** following the procedure previously described for **6, 7** and **13.**

2-(4-(3,5-Dibromobenzyloxy)-*N*-methylphenylsulfonamido)-*N*-hydroxyacetamide (15). The crude product was purified by trituration with Et_2O, *n*-hexane and acetone to give **15** as a white solid (55% yield); mp: 163-165 °C; ^{1}H-NMR (DMSO-d_6) δ: 2.67 (s, 3H); 3.52 (s, 2H); 5.21 (s, 2H); 7.20-7.24 (m, 2H); 7.72 (s, 2H); 7.72-7.76 (m, 2H); 7.84 (s, 1H); 8.95 (br s, 1H); 10.65 (br s, 1H). ^{13}C-NMR (DMSO-d_6) δ: 35.81, 50.10, 67.89, 115.18, 122.50, 129.05, 129.43, 129.52, 132.91, 141.01, 161.15, 163.74. Elemental Analysis for $C_{16}H_{16}Br_2N_2O_5S$. Calculated: %C, 37.82; %H, 3.17; %N, 5.51; Found %C, 37.93; %H, 3.19; %N, 5.47.

(*R*)-Benzyl 4-(4-(3,5-dibromobenzyloxy)phenylsulfonamido)-5-(hydroxyamino)-5-oxopentylcarbamate (20). The crude product was purified by trituration with Et_2O and *n*-hexane to give **20** as a white solid (95% yield); mp: 88-90 °C; ^{1}H-NMR (DMSO-d_6) δ: 1.26-1.48 (m, 4H); 2.84 (dd, J_1= 8Hz, J_2= 14Hz, 2H); 3.43-3.55 (m, 1H); 4.98 (s, 2H); 5.18 (s, 2H); 7.12-7.16 (m, 2H); 7.22 (t, *J*= 8Hz, 1H); 7.33 (s, 5H); 7.68-7.72 (m, 2H); 7.70 (s, 2H); 7.88 (s, 2H); 7.90 (d, *J*= 8Hz, 1H); 8.83 (br s, 1H); 10.54 (br s, 1H). ^{13}C-NMR (DMSO-d_6) δ: 25.22; 29.70; 53.08; 64.57; 67.21; 114.24; 121.94; 127.10; 127.73; 127.90; 128.92; 132.30; 133.07; 136.56; 140.53; 155.37; 159.94; 166.52. Elemental Analysis for $C_{26}H_{27}Br_2N_3O_7S$. Calculated: %C, 45.56; %H, 3.97; %N, 6.13; Found %C, 45.71; %H, 3.99; %N, 6.26.

***tert*-Butyl 4-(3,5-dibromobenzyloxy)phenylsulfonylcarbamate (76).** Aqueous ammonia (30% solution) (0.67 mL) was added dropwise to a stirred, ice-chilled solution of sulfonyl chloride **43** (1 g, 2.1 mmol) in acetonitrile (1.05 mL). The resulting mixture was stirred for 10 min at RT, and then it was diluted with water and the precipitate was filtered under vacuum. 4-(3,5-dibromobenzyloxy)benzenesulfonamide was obtained as a white solid (900 mg, 2.11 mmol, quantitative yield). ^{1}H-NMR ($CDCl_3$) δ: 4.70 (br s, 2H); 5.10 (s, 2H); 7.01-7.05 (m, 2H); 7.51 (s, 2H); 7.65 (s, 1H); 7.86-7.90 (m, 2H).

4-(3,5-Dibromobenzyloxy)benzenesulfonamide (892 mg, 2.11 mmol) was then suspended in dry CH_2Cl_2 (2.65 mL) containing DMAP (25.9 mg, 0.21 mmol) and TEA (0.33 mL, 2.33 mmol). A solution of di-(*tert*-butyl)dicarbonate (531.6 mg, 2.44 mmol) in dry CH_2Cl_2 (3.69 mL) was added dropwise, and the reaction was stirred at RT overnight. The solution was concentrated *in vacuo*, and the residue was diluited with CH_2Cl_2 and treated with HCl 1N. The organic phase was then washed with water and brine, dried over Na_2SO_4, and evaporated. The crude product was purified by flash chromatography (*n*-hexane/EtOAc 7:1) using a Isolute Flash Si II cartridge to give **76** as a colorless oil (933 mg, 1.80 mmol, 84% yield). ^{1}H-NMR ($CDCl_3$) δ: 1.39 (s, 9H); 5.08 (s, 2H); 7.03-7.08 (m, 2H); 7.27 (s, 1H); 7.51 (s, 2H); 7.65 (s, 1H); 7.95-7.99 (m, 2H).

(*R*)-*tert*-Butyl 8-(4-(3,5-dibromobenzyloxy)phenylsulfonyl)-11,11-dimethyl-3,9-dioxo-1-phenyl-2,10-dioxa-4,8-diazadodecane-7-carboxylate (78). To a solution of Boc-sulfonamide **76** (500mg, 0.96 mmol), in dry THF (9.6 mL), alcohol **77** (197.84 mg, 0.64 mmol), triphenylphosphine (503.6 mg, 1.92 mmol) and DIAD (0.32 mL, 1.6 mmol) were added. The mixture was stirred under Argon atmosphere for 4h at RT, and then it was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (*n*-hexane/EtOAc 4:1) to give **78** as a colorless oil (431 mg, 0.53 mmol, 83% yield). ^{1}H-NMR ($CDCl_3$) δ: 1.32 (s, 9H); 1.39 (s, 9H); 2.34-2.48 (m, 2H); 3.18-3.27 (m, 1H); 3.51-3.65 (m, 1H); 4.95 (dd, J_1=4.76 Hz, J_2=4.76 Hz, 1H); 5.08 (s, 2H); 5.11 (s, 2H); 5.45 (br s, 1H); 6.99-7.03 (m, 2H); 7.31-7.37 (m, 5H); 7.50 (d, *J*=1.6 Hz, 2H); 7.65 (t, *J*=1.6 Hz, 1H); 7.95-8.00 (m, 2H).

(*R*)-8-(4-(3,5-Dibromobenzyloxy)phenylsulfonyl)-11,11-dimethyl-3,9-dioxo-1-phenyl-2,10-dioxa-4,8-diazadodecane-7-carboxylic acid (79). TFA (2.29 mL, 29.67 mmol) was added dropwise to a stirred, ice-chilled solution of *tert*-butyl ester **78** (423 mg, 0.52 mmol) in dry CH_2Cl_2 (4.0 mL). The mixture was stirred under these reaction conditions for 5 h and the solvent was removed *in vacuo* to give the carboxylic acid **79** as a white solid (291.4 mg, 0.44 mmol, 85% yield) after trituration with Et_2O and *n*-hexane. ^{1}H-NMR (DMSO-d_6) δ: 1.56-1.67 (m, 1H); 1.68-1.80 (m, 1H); 2.92-3.02 (m, 2H); 3.68-3.82 (m, 1H); 4.99 (s, 2H); 5.17 (s, 2H); 7.13-7.17 (m, 2H); 7.22 (t, *J*=5 Hz, 1H); 7.35 (s. 5H); 7.69 (s, 2H); 7.70-7.74 (m, 2H); 8.07 (d, *J*=8.9 Hz, 1H).

(*R*)-Benzyl 3-(4-(3,5-dibromobenzyloxy)phenylsulfonamido)-4-(hydroxyamino)-4-oxobutylcarbamate (19). The THP-protected hydroxamate was obtained from carboxylic acid **79** following the procedure previously described for compounds **62-64**. The crude product was purified by flash chromatography (*n*-hexane/EtOAc 1:1) using a Isolute Flash Si II cartridge to give a white solid (130 mg, 0.17 mmol, 44% yield). ^{1}H-NMR ($CDCl_3$) δ: 1.67-1.81 (m, 6H); 3.07-3.18 (m, 2H); 3.32-3.61 (m, 2H); 3.70-3.86 (m, 2H); 4.42-4.51 (m, 1H); 4.98 (s, 2H); 5.12 (s, 2H); 5.33 (br s, 1H); 5.75 (m, 1H); 6.91-6.95 (m, 2H); 7.36 (s, 5H); 7.49 (s, 2H); 7.65-7.69 (m, 2H); 7.73 (s, 1H); 10.10 (br s, 1H).

Hydroxamate **19** was obtained from its THP-protected precursor following the procedure previously described for compounds **6**, **7** and **13**. The crude product was purified by trituration with Et_2O and *n*-hexane to give **19** as a white solid (97.3 mg, 0.14 mmol, 91% yield). Mp: 98-100 °C; ^{1}H-NMR (DMSO-d_6) δ: 1.40-1.76 (m, 2H); 2.84 (dd, J_1=6.7 Hz, J_2=12.8 Hz, 2H); 3.59-3.61 (m, 1H); 4.98 (s, 2H); 5.18 (s, 2H); 7.12-7.16 (m, 2H); 7.33 (s, 5H); 7.68-7.72 (m, 2H); 7.70 (s, 2H); 7.83 (s, 1H); 8.00 (d, *J*=8.6 Hz, 2H); 10.53 (br s, 1H). ^{13}C-NMR (DMSO-d_6) δ: 33.00, 37.15, 51.88, 65.23, 67.82, 114.85, 122.50, 127.68, 128.29, 128.45, 132.87, 133.53, 137.03, 141.09, 155.78, 160.57, 166.70. Elemental Analysis for $C_{25}H_{25}Br_2N_3O_7S$. Calculated: %C, 44.73; %H, 3.75; %N, 6.26. Found %C, 44.81; %H, 3.99; %N, 6.19.

(*R*)-*N*-(*tert*-Butyldimethylsilyloxy)-2-(4-(3,5-dibromobenzyloxy)phenylsulfonamido)-5-ureidopentanamide (80). EDC was added portionwise (250 mg, 1.30 mmol) to a stirred and cooled solution (0 °C) of the carboxylic acid **73** (300 mg, 0.52 mmol), and *O*-(*tert*-butyldimethylsilyl)hydroxylamine (305 mg, 2.07 mmol) in freshly distilled CH_2Cl_2 (13 mL). After stirring at RT overnight, the mixture was washed with water and the organic phase was dried and evaporated *in vacuo*. The crude was purified by flash chromatography ($CHCl_3$/MeOH 50:1) using a ISOLUTE Flash Si II cartridge to afford the *O*-silylate derivative **80** as a white solid (296 mg, 80% yield). ^{1}H-NMR (DMSO-d_6) δ: 0.03 (s, 6H); 0.85 (s, 9H); 1.25-1.39 (m, 4H); 2.81 (dd, *J*= 6.0 Hz, 2H); 3.53 (m, 1H); 5.18 (s, 2H); 5.37 (s, 2H); 5.87 (t, *J*=6.0 Hz, 1H); 7.12-7.16 (m, 2H); 7.69-7.72 (m, 4H); 7.82 (s, 1H); 7.98 (brs, 1H); 10,72 (brs, 1H).

(*R*)-2-(4-(3,5-Dibromobenzyloxy)phenylsulfonamido)-*N*-hydroxy-5-ureidopentanamide (21). TFA (1.8 mL, 23.3 mmol) was added dropwise to a stirred and ice-chilled solution of *O*-silylate derivative **80** (290 mg, 0.41 mmol) in CH_2Cl_2 (10 mL). The solution was stirred under these reaction conditions for 5 h and the solvent was removed *in vacuo*. The crude product was purified by trituration with Et_2O and *n*-hexane to give the hydroxamate **21** as a white solid (230 mg, 94.6% yield). Mp: 80-82 °C; ^{1}H-NMR (DMSO-d_6) δ: 1.16-1.39 (m, 4H); 2.80 (m, 2H); 3.50 (dd, J_1= 7.6 Hz, J_2= 7.6 Hz, 1H); 5.20 (s, 2H); 5.89 (brs, 1H); 7.13-7.18 (m, 2H); 7.69-7.74 (m, 4H); 7.84 (t, *J*=

1.8 Hz, 1 H); 7.90 (d, *J*= 8.8 Hz, 1H); 10.54 (s, 1H). ^{13}C-NMR (DMSO-d_6) δ: 26.15; 30.25; 53.55; 67.72; 114.70; 122.27; 128.30; 129.23; 132.65; 133.69; 141.01; 158.40; 160.38; 167.03. Elemental Analysis for $C_{19}H_{22}Br_2N_4O_6S$. Calculated: %C, 38.40; %H, 3.73; %N, 9.43. Found: %C, 38.55; %H, 3.96; %N, 9.20.

MMPs and ADAMs inhibition assays. Recombinant human MMP-14 catalytic domain was a kind gift of Prof. Gillian Murphy (Department of Oncology, University of Cambridge, UK). Pro-MMP-1, pro-MMP-2, pro-MMP-9, and recombinant human ADAM-17 (PF133) were purchased from Calbiochem. Recombinant human ADAM-10 was purchased from R&D Systems. Pro-enzymes were activated immediately prior to use with *p*-aminophenylmercuric acetate (APMA 2 mM for 1 h at 37 °C for MMP-2, APMA 2 mM for 2 h at 37 °C for MMP-1 and 1 mM for 1 h at 37 °C for MMP-9). For assay measurements, the inhibitor stock solutions (DMSO, 10 mM) were further diluted in the fluorometric assay buffer (FAB: Tris 50 mM, pH = 7.5, NaCl 150 mM, $CaCl_2$ 10 mM, Brij-35 0.05% and DMSO 1%). Activated enzyme (final concentration 0.56 nM for MMP-2, 1.3 nM for MMP-9, 1.0 nM for MMP-14cd, 2.0 nM for MMP-1, 5 nM for ADAM-17 and 20 nM for ADAM-10) and inhibitor solutions were incubated in the assay buffer for 4 h at 25 °C. ADAM-17 was incubated for 30 min at 37 °C and ADAM-10 for 1 h at 37 °C in a different buffer at pH = 9 (Tris 25 mM, $ZnCl_2$ 25 μM, Brij-35 0.005%). After the addition of 200 μM solution of the fluorogenic substrate Mca-Lys-Pro-Leu-Gly-Leu-Dap(Dnp)-Ala-Arg-NH_2[15] (Bachem) for all the enzymes in DMSO (final concentration 2 μM for all enzymes, 10 μM for ADAM-10), the hydrolysis was monitored every 15 sec for 20 min recording the increase in fluorescence (λ_{ex} = 325 nm, λ_{em} = 400 nm) with a Molecular Devices SpectraMax Gemini XS plate reader. The assays were performed in duplicate in a total volume of 200 μL per well in 96-well microtitre plates (Corning black, NBS). Control wells lack inhibitor. The MMP inhibition activity was expressed in relative fluorescent units (RFU). Percent of inhibition was calculated from control reactions without the inhibitor. IC_{50} was determined using the formula: $v_i/v_o = 1/(1 + [I]/ IC_{50})$, where v_i is the initial velocity of substrate cleavage in the presence of the inhibitor at concentration [I] and v_o is the initial

velocity in the absence of the inhibitor. Results were analyzed using SoftMax Pro software[25] and GraFit software.[26]

Cells and reagents. The human EOC cell lines SKOV3-luc (INT, Milan, Italy), A2774 (IST Genoa) and A2780 (ICLC Cell Bank, Genoa, Italy) were grown in RPMI 1640, with 2 mM L-glutamine, 10% heat-inactivated fetal calf serum and 100 μg/mL penicillin-streptomycin (Lonza, Milan, Italy) at 37 °C in a 5% CO_2 incubator. Cell lines identity was confirmed by genotyping through the Cell IDTM System (Promega) and the GeneMapper® software, version 4.0. Recombinant human EGF was from PeproTech EC (London, UK). PV was prepared by mixing sodium orthovanadate (0.1 M) and H_2O_2 (0.1 M) and was used within 20 min of preparation. Metalloproteinase inhibitors were dissolved in DMSO as 10 mM stock solution and stored in aliquots at -20 °C.

Cell treatments and ELISA. Cells were seeded in 24-well plates and assays performed in duplicates. Subconfluent cultures were challenged with the indicated amounts of metalloproteinase inhibitors or the equivalent amount of their solvent as control. After 30 minutes PV (200 μM) or EGF (100 ng/mL) were added and incubation prolonged for 90 min or 18 h, respectively. Conditioned media were collected, centrifuged at 1000*g* and used undiluted for sALCAM detection by ELISA (DuoSet ELISA Development kit, R&D Systems). After background subtraction, data were expressed as the mean ± SD and analyzed by two-tailed Student's *t* test.

MTT assay. To test cell viability during treatment with the metalloproteinase inhibitors, parallel cultures were set up for evaluation with a microculture tetrazolium reduction assay using MTT [3-(4,5-dimethyltiazol-2-yl)2,5-diphenyl-tetrazolium bromide; Sigma], as previously described.[6]

X-ray Structures Selection for Docking Studies. The superposition of the fourteen ADAM-17 X-ray structures available in the PDB, on the alpha-carbon atoms, reveals that the overall fold of the catalytic domain is well conserved, but a noteworthy flexibility of the S1' and S2 (Tyr352-Gly363) loops exists. Thus, as already described in an our previous work,[6] a deeper analysis based on the RMSD values calculated for every possible combination of alignment and on the S1' and S2 loops

shapes, identified five different clusters. From each identified cluster, the X-ray structure with the highest resolution (2I47, 3EDZ, 3EWJ, 2DDF, 2OI0) was chosen for subsequent molecular docking calculations.

As regards MMP-1, -2, -9 and -14, the analysis of the available X-ray structures reveals that the overall fold of the catalytic domains is strictly conserved, thus for each MMP, the structure having the highest resolution, preferring those co-crystallized with organic or peptidic inhibitors, was chosen (PDB codes: 1HFC, 3AYU, 4H3X and 3MA2, respectively).

Docking Simulations. Molecular docking of **21** into the X-ray structures of ADAM-17 (PDB codes: 2I47, 2FV5, 2OI0, 3EWJ, 2DDF) was carried out using the Glide 5.5 program.[20] Maestro 9.1.[27] was employed as the graphical user interface, and Figure 4 representing the most probable binding mode of **21** was rendered by the Chimera software package.[28]

Ligand and Protein Setup. The 3D structures of the ligands were generated with the Maestro fragment Build tool,[27] then, a geometry-optimized ligand was prepared using Lig-Prep 2.3 as implemented in Maestro. The target proteins were prepared using the protein preparation wizard in Maestro 9.1. Water molecules were removed, and the resulting proteins were aligned based on the R-carbon trace. Hydrogen atoms were added, a +2 charge was assigned to the zinc ion in the active site, and the binding pocket was identified by placing a 20Å cube around the catalytic ion.

Docking Setting. Molecular docking calculations were performed with the aid of Glide 5.5 in extra-precision (XP)[29,30] mode, using Glidescore for ligand ranking. A constraint that forced the interaction with the metal ion was included. The selected docking pose was further minimized using OPLSA2005 as force field, the PRCG methods until a gradient of 0.001 kcal/mol*Å^2 and the implicit water model implemented in Macromodel.

Supporting Information available: A table reporting the HPLC purity analysis data of the final products. This material is available free of charge via the Internet at http://pubs.acs.org.

Corresponding Author

*Phone: +390502219562. E-mail: aros@farm.unipi.it

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

Acknowledgment. This work was supported by grants awarded by AIRC (Italian Association for Cancer Research to SF and MF), by Fondazione Compagnia di San Paolo, Torino and by MIUR (Prin 2007 to AR). We thank Miss Simona Calcagnini for technical help in cell assays.

Abbreviations used

ADAM, a disintegrin and metalloproteinase; MMP, matrix metalloproteinase; TNF, tumor necrosis factor; TACE, tumor necrosis factor-α converting enzyme; EGFR, epidermal growth factor receptor; CAM, cell adhesion molecule; ALCAM, activated leukocyte cell adhesion molecule; EOC, epithelial ovarian cancer; MMPI, MMP inhibitor; MSS, musculoskeletal syndrome; sALCAM, soluble ALCAM; ZBG, zinc-binding group; NMM, *N*-methylmorpholine; DIAD, diisopropyl azodicarboxylate; DMAP, 4-(dimethylamino)pyridine; EDC, *N*-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride; SAR, structure-activity relationship; PV, pervanadate; EGF, epidermal growth factor; TK, tyrosine kinase; MTT, 3-(4,5-dimethyltiazol-2-yl) 2,5-diphenyltetrazolium bromide; APMA, *p*-aminophenylmercuric acetate; THP, tetrahydro-2H-pyran-2-yl; TEA, triethylamine; SD, standard deviation.

References

(1) Ge, L.; Baskic, D.; Basse, P.; Vujanovic, L.; Unlu, S.; Yoneyama, T.; Vujanovic, A.; Han, J.; Bankovic, D.; Szczepanski, M. J.; Hunt, J. L.; Herberman, R. B.; Gollin, S. M.; Ferris, R. L.; Whiteside, T. L.; Myers, E. N.; Vujanovic, N. L. Sheddase activity of tumor necrosis factor-alpha

converting enzyme is increased and prognostically valuable in head and neck cancer. *Cancer Epidemiol. Biomarkers Prev.* **2009**, *18*, 2913-2922.

(2) Piazza, T.; Cha, E.; Bongarzone, I.; Canevari, S.; Bolognesi, A.; Polito, L.; Bargellesi, A.; Sassi, F.; Ferrini, S.; Fabbi, M. Internalization and recycling of ALCAM/CD166 detected by a fully human single chain recombinant antibody. *J. Cell Sci.* **2005**, *118*, 1515-1525.

(3) Rosso, O.; Piazza, T.; Bongarzone, I.; Rossello, A.; Mezzanzanica, D.; Canevari, S.; Orengo, A. M.; Puppo, A.; Ferrini, S.; Fabbi, M. The ALCAM shedding by the metalloprotease ADAM17/TACE is a tyrosine kinase-inducible process involved in motility of ovarian carcinoma cells. *Mol Cancer Res.* **2007**, *5*, 1246-1253.

(4) Santiago-Josefat, B.; Esselens, C.; Bech-Serra, J. J.; Arribas, J. Post-transcriptional up-regulation of ADAM17 upon epidermal growth factor receptor activation and in breast tumors. *J. Biol. Chem.* **2007**, *282*, 8325–8331.

(5) Kenny, P. A. TACE: a new target in epidermal growth factor receptor dependent tumors. *Differentiation* **2007***, 75,* 800–808.

(6) Nuti, E.; Casalini, F.; Avramova, S. I.; Santamaria, S.; Fabbi, M.; Ferrini, S.; Marinelli, L.; La Pietra, V.; Limongelli, V.; Novellino, E.; Cercignani, G.; Orlandini, E.; Nencetti, S.; Rossello, A. Potent arylsulfonamide inhibitors of tumor necrosis factor-alpha converting enzyme able to reduce activated leukocyte cell adhesion molecule shedding in cancer cell models. *J. Med. Chem.* **2010**, *53*, 2622-2635.

(7) Carbotti, G.; Orengo, A. M.; Mezzanzanica, D.; Bagnoli, M.; Brizzolara, A.; Emionite, L.; Puppo, A.; Centurioni, M.G.; Bruzzone, M.; Marroni, P.; Rossello, A.; Canevari, S.; Ferrini, S.; Fabbi, M. Activated leukocyte cell adhesion molecule soluble form: a potential biomarker of epithelial ovarian cancer is increased in type II tumors. *Int. J. Cancer*. **2013**, *132*, 2597-2605.

(8) Becker, D. P.; Barta, T. E.; Bedell, L. J.; Boehm, T. L.; Bond, B. R.; Carroll, J.; Carron, C. P.; DeCrescenzo, G. A.; Easton, A. M.; Freskos, J. N.; Funckes-Shippy, C. L.; Heron, M.; Hockerman,

S.; Howard, C. P.; Kiefer, J. R.; Li, M. H.; Mathis, K. J.; McDonald, J. J.; Mehta, P. P.; Munie, G. E.; Sunyer, T.; Swearingen, C. A.; Villamil, C. I.; Welsch, D.; Williams, J. M.; Yu, Y.; Yao, J. Orally active MMP-1 sparing α-tetrahydropyranyl and α-piperidinyl sulfone matrix metalloproteinase (MMP) inhibitors with efficacy in cancer, arthritis, and cardiovascular disease. *J. Med. Chem.* **2010**, *53*, 6653–6680.

(9) (a) Rossello, A.; Nuti, E.; Maresca, A. ADAMs and ADAMTs Selective Synthetic Inhibitors. In *Drug Design of Zinc-Enzyme Inhibitors: Functional, Structural, and Disease Applications*; Wiley Series in Drug Discovery and Development, Binghe Wang, Series Ed., John Wiley & Sons, Inc. 2009; pp 591-645; (b) Fisher, J. F.; Mobashery, S. Recent advances in MMP inhibitor design. *Cancer Metastasis Rev.* **2006**, *25*, 115–136. (c) Georgiadis, D.; Yiotakis, A. Specific targeting of metzincin family members with small-molecule inhibitors: Progress toward a multifarious challenge. *Bioorg. Med. Chem.* **2008**, *16*, 8781–8794.

(10) Rossello, A.; Nuti, E.; Casalini, F.; Orlandini, E. Inibitori altamente selettivi dell'attivita' di ADAMs. Italian Patent Application, PI2011A000007, **2011**.

(11) Maskos K., Fernandez-Catalan C., Huber R., Bourenkov G.P., Bartunik H., Ellestad G.A., Reddy P., Wolfson M.F., Rauch C. T., Castner B. J., Davis R., Clarke H. R. G., Petersen M., Fitzner J. N., Cerretti D. P., March C.J., Paxton R. J., Black R. A., Bode W. Crystal structure of the catalytic domain of human tumor necrosis factor-α-converting enzyme. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 3408-3412.

(12) (a) Grootveld, M.; McDermott, M. F. BMS-561392 (Bristol-Myers Squibb). *Curr. Opin. In*V*est. Drugs* **2003**, *4*, 598–602. (b) Liu, R.; Magolda, R.; Newton, R.; Duan, J.; Vaddi, K.; Madskuie, T.; Qian, M.; Collins, R.; Taylor, T.; Giannaris, J. Pharmacological profile of DPC 333, a selective TACE inhibitor. *Inflammation Res.* **2002**, *51*, A27. (c) Zhang, Y.; Xu, J.; Udata, C.; McDevitt, J.; Cummons, T.; Sun, L.; Zhu, Y.; Li, G.; Rao, V.; Wang, Q; Gibbons, J. Indentification

and characterization of Aprarastat (TMI-005), a potent, dual TACE/MMP inhibitor for the treatment of RA. *Ann. Rheum. Dis.* **2005**, *64*, 462–463.

(13) (a) Levin, J.; Li, Z.; Diamantidis, G.; Lovering, F. E.; Wang, W.; Condon, J. S.; Lin, Y.; Skotnicki, J. S.; Park, K. β-Sulfonamide Hydroxamic Acid Inhibitors of TACE/Matrix Metalloproteinase. U.S. Patent Appl. Pub. 0211730, **2006**. (b) Wang, Y.; Papamichelakis, M.; Chew, W.; Sellstedt, J.; Noureldin, R.; Tadayon, S.; Daigneault, S. Development of a suitable process for the preparation of a TNF-α Converting Enzyme inhibitor, WAY-281418. *Org. Process Res. Dev.* **2008**, *12,* 1253–1260.

(14) Nuti, E.; Santamaria, S.; Casalini, F.; Yamamoto, K.; Marinelli, L.; La Pietra, V.; Novellino, E.; Orlandini, E.; Nencetti, S.; Marini, A. M.; Salerno, S.; Taliani, S.; Da Settimo, F.; Nagase, H.; Rossello, A. Arylsulfonamide inhibitors of aggrecanases as potential therapeutic agents for osteoarthritis: Synthesis and biological evaluation. *Eur. J. Med. Chem.* **2013**, *62*, 379-394.

(15) Neumann, U.; Kubota, H.; Frei, K.; Ganu, V.; Leppert, D. Characterization of Mca-Lys-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH2, a fluorogenic substrate with increased specificity constants for collagenases and tumor necrosis factor converting enzyme. *Anal. Biochem.* **2004**, *328*, 166–173.

(16) Levin, J. I.; Chen, J. M.; Cheung, K.; Cole, D.; Crago, C.; Santos, E. D.; Du, X.; Khafizova, G.; MacEwan, G.; Niu, C.; Salaski, E. J.; Zask, A.; Cummons, T.; Sung, A.; Xu, J.; Zhang, Y.; Xu, W.; Ayral-Kaloustian, S.; Jin, G.; Cowling, R.; Barone, D.; Mohler, K. M.; Black, R. A.; Skotnicki, J. S. Acetylenic TACE inhibitors. Part 1. SAR of the acyclic sulfonamide hydroxamates. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2799–2803.

(17) Brocker, C.N.; Vasiliou, V.; Nebert, D.W. Evolutionary divergence and functions of the ADAM and ADAMTS gene families. *Hum. Genomics.* **2009**, *4*, 43-55.

(18) Bech-Serra, J. J.; Santiago-Josefat, B.; Esselens, C.; Saftig, P.; Baselga, J.; Arribas, J.; Canals, F. Proteomic Identification of Desmoglein 2 and Activated Leukocyte Cell Adhesion Molecule as

Substrates of ADAM17 and ADAM10 by Difference Gel Electrophoresis. *Mol. Cell Biol.* **2006**, *26*, 5086–5095.

(19) (a) Crawford, H. C.; Dempsey, P. J.; Brown, G.; Adam, L.; Moss, M. L. ADAM10 as a therapeutic target for cancer and inflammation. *Curr. Pharm. Des.* **2009**, *15*, 2288–2299. (b) Moss, M. L.; Stoeck, A.; Yan, W.; Dempsey, P. J. ADAM10 as a target for anticancer therapy. *Curr. Pharm. Biotechnol.* **2008**, *9*, 2–8.

(20) Glide, version 5.5; Schrödinger, LLC: New York, 2009.

(21) Spurlino J.C.; Smallwood, A. M.; Carlton, D. D.; Banks, T. M.; Vavra, K. J.; Johnson, J. S.; Cook, E. R.; Falvo, J.; Wahl, R. C.; Pulvino, T. A.; Wendoloski, J. J.; Smith, D. L. 1.56 A structure of mature truncated human fibroblast collagenase. *Proteins*. **1994**, *19*, 98-109.

(22) Grossman, M.; Tworowski, D.; Dym, O.; Lee, M. H.; Levy, Y.; Murphy, G.; Sagi, I. The intrinsic protein flexibility of endogenous protease inhibitor TIMP-1 controls its binding interface and affects its function. *Biochemistry* **2010**, *49*, 6184-6192.

(23) Hashimoto, H.; Takeuchi, T.; Komatsu, K.; Miyazaki, K.; Sato, M.; Higashi, S. Structural basis for matrix metalloproteinase-2 (MMP-2)-selective inhibitory action of β-amyloid precursor protein-derived inhibitor. *J Biol Chem.* **2011**, *286*, 33236-33243.

(24) Nuti, E.; Antoni, C.; Vera, L.; Devel, L.; Beau, F.; Da Pozzo, E.; Costa, B.; Giacomelli, C.; Orlandini, E.; Nencetti, S.; Rosalia, L.; Dive, V.; Stura, E. A.; Martini, C.; Rossello, A. Powerful twin carboxylic inhibitors for MMP homodimerisation. To be Published.

(25) SoftMax Pro 4.7.1 by Molecular Devices.

(26) GraFit version 4 by Erithecus Software.

(27) Schrödinger, Maestro version 9.1 Schrödinger, LLC, New York 2009.

(28) Pettersen, E. F.; Goddard, T. D.; Huang, C. C.; Couch, G. S.; Greenblatt, D. M.; Meng, E. C.; Ferrin, T. E. UCSF Chimera; A Visualization System for Exploratory Research and Analysis. *J. Comput. Chem.* **2004**, *25*, 1605–1612.

(29) Friesner, R. A.; Banks, J. L.; Murphy, R. B.; Halgren, T. A.; Klicic, J. J.; Mainz, D. T.; Repasky,M.P.; Knoll, E. H.; Shelley, M.; Perry, J. K.; Shaw, D. E.; Francis, P.; Shenkin, P. S. Glide: A new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. *J. Med. Chem.* **2004**, *47*, 1739–1749.

(30) Friesner, R. A.; Murphy, R. B.; Repasky, M. P.; Frye, L. L.; Greenwood, J. R.; Halgren, T. A.; Sanschagrin, P. C.; Mainz, D. T. Extra Precision Glide: Docking and Scoring Incorporating a Model of Hydrophobic Enclosure for Protein-Ligand Complexes. *J. Med. Chem.* **2006**, *49*, 6177–6196.

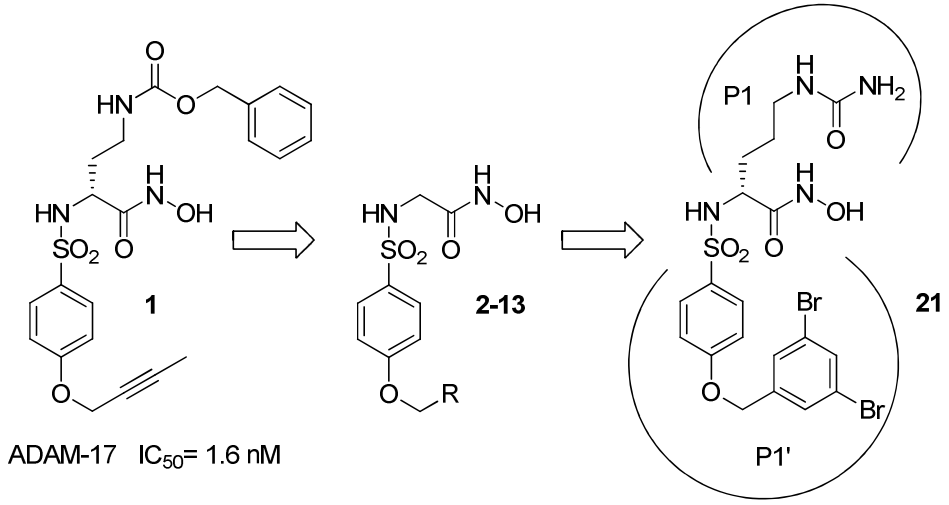


Figure 1.

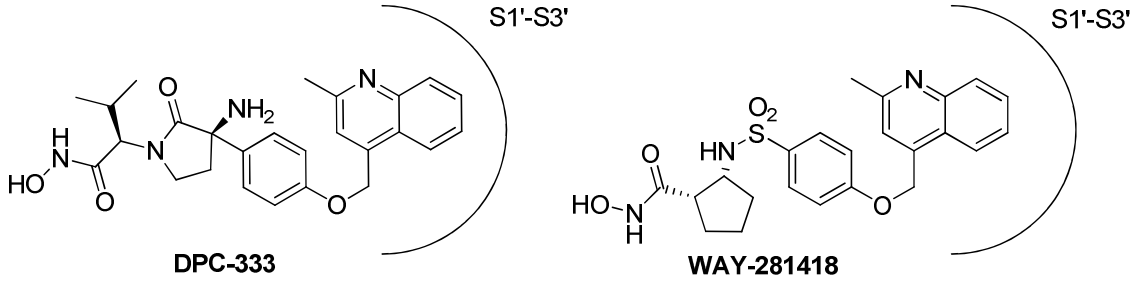


Figure 2.

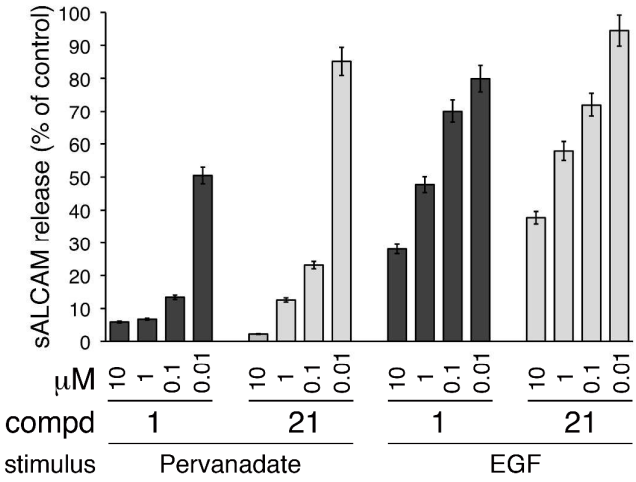


Figure 3.

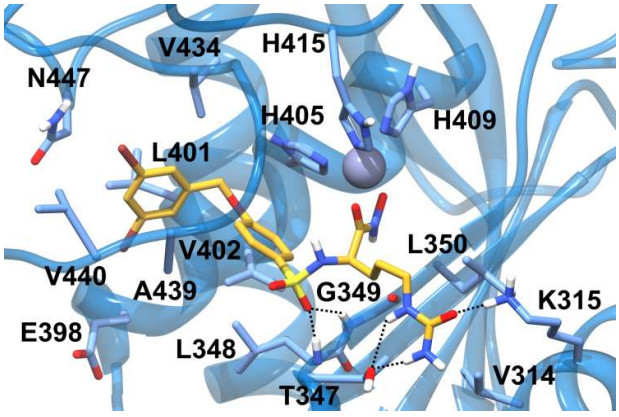


Figure 4.

Figure Captions.

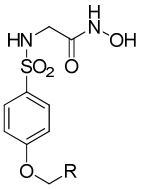
Figure 1. Progression of the SAR: from the hit compound **1** to the ADAM-17 selective inhibitor **21**.

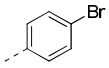
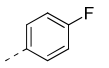
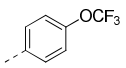
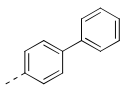
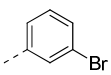
Figure 2. Examples of ADAM-17 selective inhibitors.

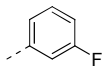
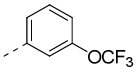
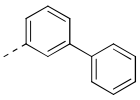
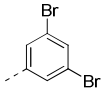
Figure 3. Inhibition of soluble ALCAM release. SKOV3-luc EOC cells were treated with the indicated amounts of compound **1** or **21** and then stimulated with pervanadate or EGF for 90 min or 18 h, respectively. Conditioned media were then harvested and tested by ELISA for sALCAM levels. Data are expressed as % of control cultures treated with solvent (DMSO). Whole cell assays were performed and tested in duplicates. A representative experiment is shown.

Figure 4. Binding mode of **21** (gold sticks) within the active site of ADAM-17 as resulted from docking calculations (PDB code 2OI0). H-bonds are depicted as dashed black lines.

Table 1. In Vitro Enzymatic[a] Activity (IC_{50} nM values) of glycine-like compounds **2-13** and the reference compound **14**[16].

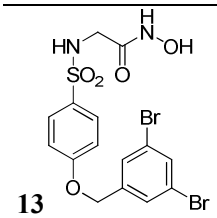
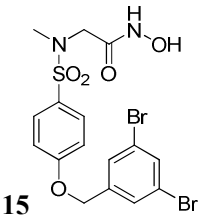


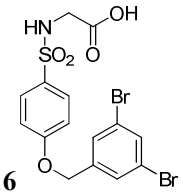
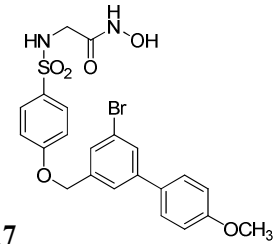
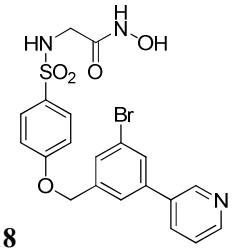
Compd	R	ADAM-17	MMP-1	MMP-2	MMP-9	MMP-14
2		590	27000	3.8	96	310
3		16	120000	1.2	1.4	630
4		145	32000	3.0	42	590
5		200	74000	7.5	37	7200
6		640	>500000	48	690	72000
7		104	>500000	15	60	1600

8		71	61000	7.8	110	940
9		280	>500000	20	76	2900
10		76	100000	41	920	1700
11		650	620000	670	29000	120000
12		82	181000	18	330	890
13		105	>500000	1750	51000	113000
14[b]		17	28000	110	890	2900

[a] The IC_{50} values are the average of three determinations with SD <10%. [b] Data from our lab.

Table 2. In Vitro Enzymatic activity[a] (IC_{50} nM values) of close analogues of **13**.

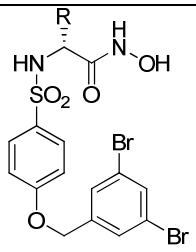
Compd	ADAM-17	MMP-1	MMP-2	MMP-14
 **13**	105	>500000	1750	113000
 **15**	160	>200000	1870	123000

 **16**	>50000	nd	125000	nd
 **17**	320	>200000	3800	170000
 **18**	500	>500000	1045	21400

[a] The IC_{50} values are the average of three determinations with SD <10%.

Nd: not determined.

Table 3. In Vitro Enzymatic[a] Activity (IC_{50} nM values) of P1-substituted analogues of **13**.



Compd	R	ADAM-17	ADAM-10	MMP-1	MMP-2	MMP-9	MMP-14
13	H	105	39000	>500000	1750	51000	113000
19	$(CH_2)_2NHCbz$	43	8200	400000	4100	11000	104000
20	$(CH_2)_3NHCbz$	11	300	370000	1440	4400	68000
21	$(CH_2)_3NHCONH_2$	1.9	150	>500000	240	1630	19500

[a] The IC_{50} values are the average of three determinations with SD <10%.

Table 4. Properties of Tested Compounds Calculated by QikProp

Compd	MW[a]	QPlogPo/w[b]	QPlogS[c]	# of primary metabolites[d]	# H-bond donor[e]	# H-bond acceptor[f]
20	685.4	4.03	-7.5	4	3	11
21	594.3	0.68	-3.7	4	5	10

[a] Lipinski's rule of 5 (<500). [b] Log of the octanol/water partition coefficient, range 95% of drugs (-2/6.5). [c] Log of aqueous solubility S (mol/L), range 95% of drugs (-6.5/0.5). [d] Range 95% of drugs (1/8). [e] Lipinski's rule of 5 (<5). [f] Lipinski's rule of 5 (<10).

Table 5. Selectivity profile of compound **21** in comparison with **1**[6].

Compd	IC_{50}[a] (nM)					
	MMP-1	MMP-2	MMP-9	MMP-14	ADAM-17	ADAM-10
1	1100	17	46	210	1.6	240
21	>500000	240	1630	19500	1.9	150

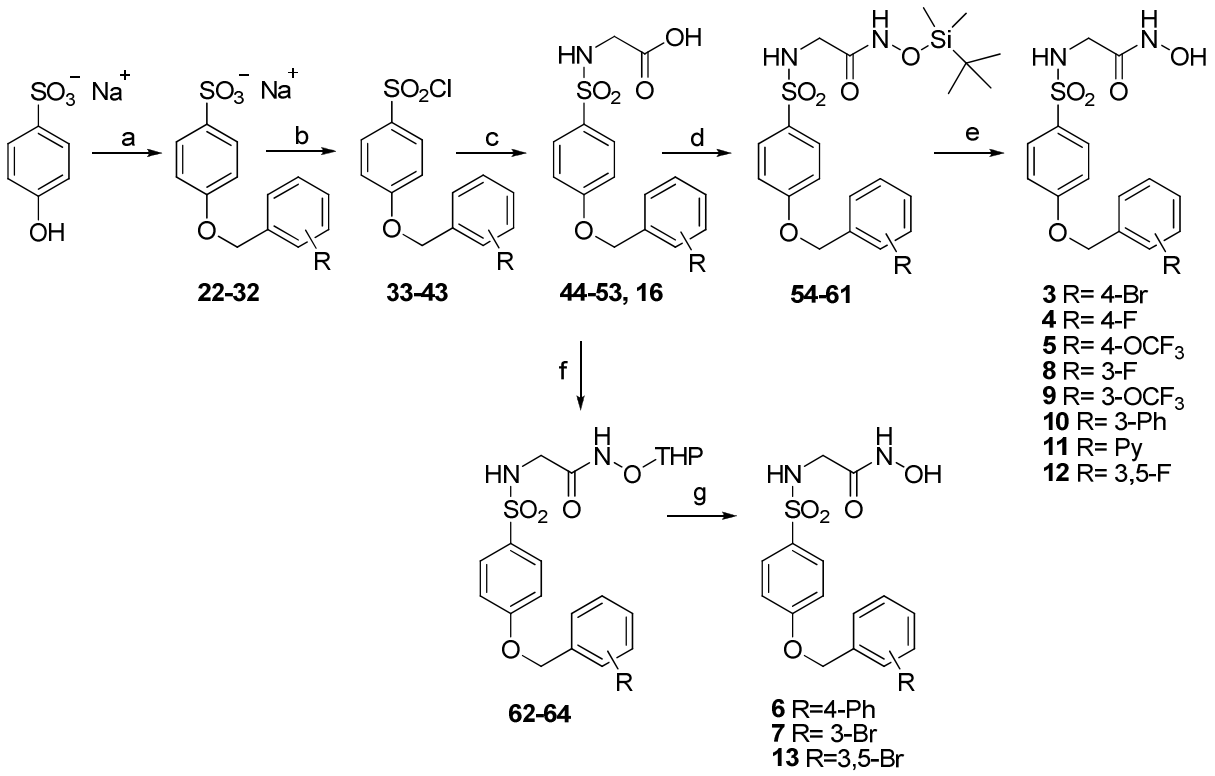
[a] The IC_{50} values are the average of three determinations with SD <10%.

Table 6. In vitro cellular activity[a] of synthesized compounds **13**, **19-21** and the reference compound **1**.

Inhibition of sALCAM release, IC_{50} (nM)						
	A2774		SKOV3-luc		A2780	
compd	PV[b]	EGF[c]	PV[b]	EGF[c]	PV[b]	EGF[c]
13	>10000	>10000	5301	>10000	840	>10000
19	1391	>10000	2096	9519	825	>10000
20	274	7626	350	5641	349	10000
21	45	651	58	4272	<10	845
1	26	419	14	966	<10	1436

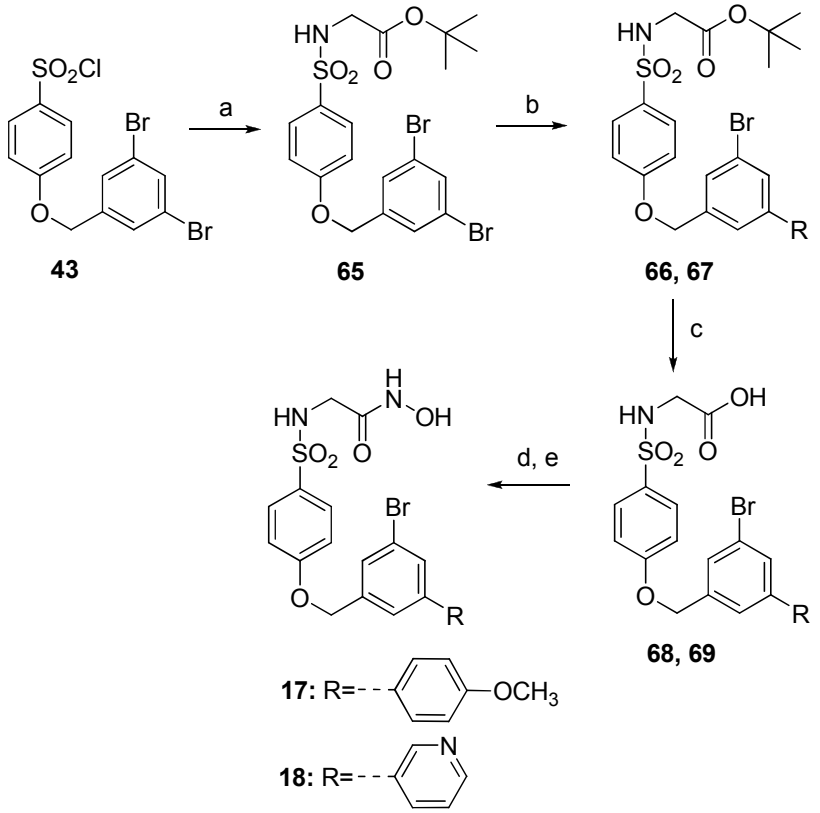
[a] Whole cells assays were performed in duplicates. SD was generally within ± 10%. One experiment, representative of three, is shown. [b] Inhibition of pervanadate-induced sALCAM release. [c] Inhibition of EGF-induced sALCAM release.

Scheme 1. Synthesis of compounds **3-13** and **16**[a]



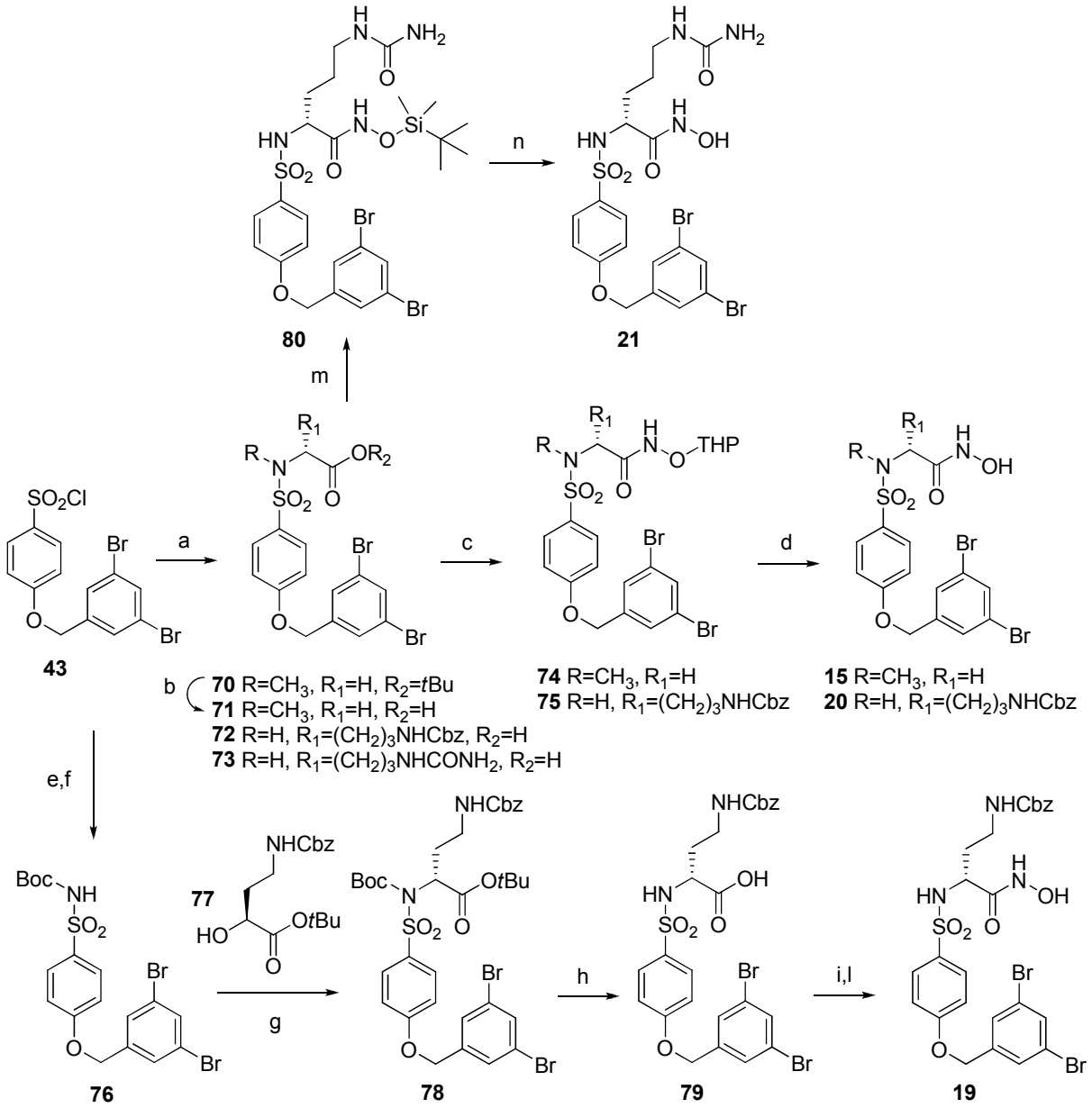
[a]Reagents and conditions: (a) the appropriate substituted benzylbromide, NaOH, *i*-PrOH, 70 °C; (b) $(COCl)_2$, DMF, CH_2Cl_2; (c) glycine, TEA, H_2O, dioxane; (d) $TBDMSiONH_2$, EDC, CH_2Cl_2; (e) TFA, CH_2Cl_2, 0 °C; (f) $THPONH_2$, HOBT, NMM, EDC, DMF; (g) HCl 4N, dioxane, MeOH.

Scheme 2. Synthesis of compounds **17** and **18**[a]



[a]Reagents and conditions: (a) glycine *tert*-butyl ester hydrochloride, TEA, H_2O, dioxane; (b) 3-pyridinylboronic acid or 4-methoxyphenylboronic acid, $Pd(PPh_3)_4$, K_3PO_4, dioxane/H_2O, 85 °C; (c) TFA, CH_2Cl_2, 0 °C; (d) $TBDMSiONH_2$, EDC, CH_2Cl_2; (e) TFA, CH_2Cl_2, 0 °C.

Scheme 3. Synthesis of compounds **15** and **19-21**[a]



[a]Reagents and conditions: (a) sarcosine *tert*-butyl ester hydrochloride or H-D-Orn(Z)-OH or H-D-Orn(carbamoyl)-OH, TEA, H_2O, dioxane; (b) TFA, CH_2Cl_2, 0 °C; (c) $THPONH_2$, HOBT, NMM, EDC, DMF; (d) HCl 4N, dioxane, MeOH; (e) NH_3 aq, CH_3CN, 0°C to rt; (f) $(Boc)_2O$, TEA, DMAP, CH_2Cl_2; (g) **77**, PPh_3, DIAD, THF; (h) TFA, CH_2Cl_2, 0 °C; (i) $THPONH_2$, HOBT, NMM, EDC, DMF; (l) HCl 4N, dioxane, MeOH; (m) $TBDMSiONH_2$, EDC, CH_2Cl_2; (n) TFA, CH_2Cl_2, 0 °C.

Table of Contents Graphic.