Tetrahedron xxx (2014) 1–11

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Combination of click chemistry and sulfonamides to develop three-armed triazole compounds

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ARTICLE INFO

Article history:
Received 7 March 2014
Received in revised form 18 June 2014
Accepted 30 June 2014
Available online xxx

Keywords:
Click chemistry
Azides
Alkynes
Medicinal chemistry
Cycloadditions

ABSTRACT

Fragment-based drug discovery is a valuable tool in hit identification, as well as the combination of different small fragments showing a minimal binding activity against biological receptors or enzymes to give merged hits. A high number of fragments on the same scaffold improve the probability to find a candidate showing single- or multi-target affinities. A rapid and versatile approach for synthesizing libraries of densely fragment-functionalized scaffolds is reported. Many fragments were assembled in few steps around a triazole ring starting from amino alcohols and other readily available building blocks. A binding assay against integrin $\alpha_v\beta_3$ was used as a test-bed in order to demonstrate the potential of such an approach in hit discovery strategies.

1. Introduction

The interactions between biological targets (either receptors or enzymes) and pharmacophoric subunits are the fundamental concept of fragment-based approach to drug discovery issues. Many repeating subunits responsible of such useful interactions can be identified in bioactive molecules.[1–14] Thus, the combination of different 'binder' fragments within a single molecule dramatically increase the probability of finding new promising 'hit' candidates, both for single- and multi-target purposes.[15,16]

Copper (I) catalyzed Azide-Alkyne Cycloaddition (CuAAC), the main tool of the so-called 'click-chemistry', is a versatile reaction allowing for the synthesis of functionalized triazole ring in high yield and mild reaction conditions, and nowadays is one of the most widespread used reaction in chemical synthesis.[17–24] Furthermore, the triazole ring has been proposed as a stable isostere of the peptide bond,[25–27] thus, click chemistry is considered a very promising tool in the synthesis of bioactive ligands.[28,29]

In the present work we propose a versatile 'click-based' approach in developing libraries of densely functionalized scaffolds containing three fragments.[30]

2. Results and discussion

As CuAAC is usually intended to couple an azide and an alkyne, it can be easily exploited in a basic approach in order to achieve a difunctional scaffold. In view of further expanding the combination of multiple fragments in a single molecule, our aim was to develop highly functionalized structures still applying the click chemistry (Fig. 1), as versatility and mild reaction conditions are fundamental requirements in a useful library development technique. Thus, we envisaged the synthesis of building blocks containing three more pharmacophoric moieties in a modular approach, and specifically we focused our attention on single-fragment-bearing alkyne and multi-fragment-bearing azides.

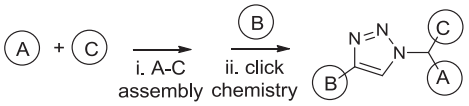

Fig. 1. Schematic of a high-density fragments bearing triazole.

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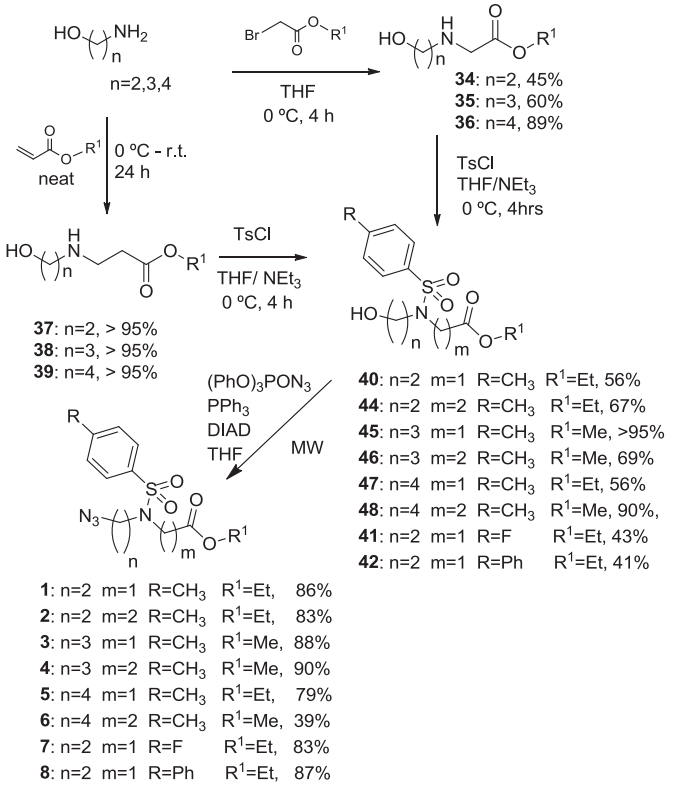
http://dx.doi.org/10.1016/j.tet.2014.06.125
0040-4020/

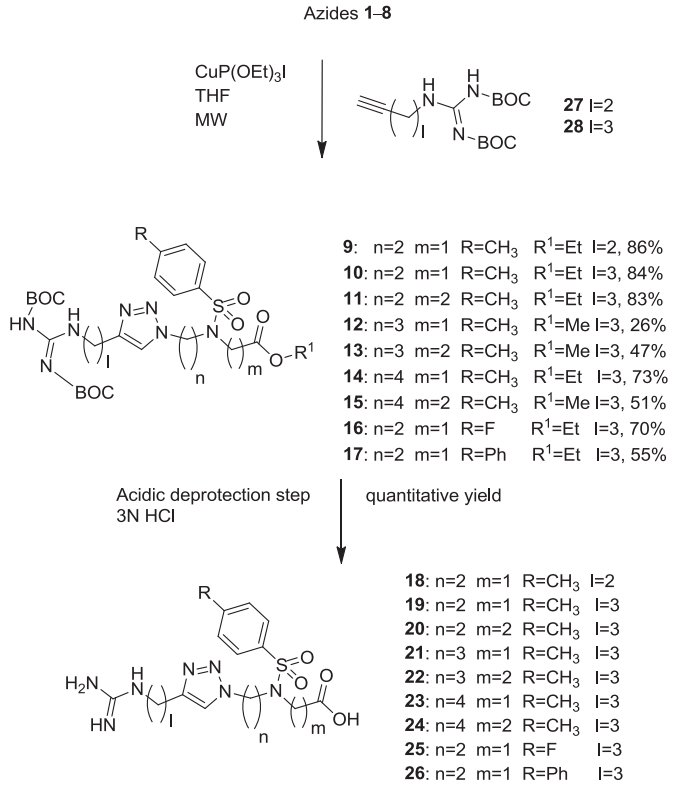
In choosing our model bioactive fragments, we opted for one basic group and one acidic group, which can be found in many peptidic and non-peptidic drug-candidates.[31] Such moieties were used in their protected forms, in order to perform reaction and purification steps with the benefits of organic solvent-soluble substances. Additional common fragments found in enzymes and receptors ligands are π-stacking capable aromatic rings and heteroatom-based bonds, one the most widespread being the sulfonamidic bond. Sulfonamides are widely used in medicinal chemistry for targeting many different receptors, thus, using this versatile scaffold increase the probability to find a multi-target hit, which is a noteworthy additional advantage in designing drug candidates.[32–34]

The first phase of our approach was the synthesis of a sub-library differing only in the size of the backbone-chain (Scheme 1). Intermediates **34**–**39** were synthesized starting from amino alcohols and esters derivatives. Amino alcohols appeared to be the ideal starting materials, taking advantage of the orthogonality of oxygen and nitrogen atoms with respect to the reactivity with α-bromo acetates.[35] Similar profile was exploited for the Michael addition to acrylate ester, which was considered in order to achieve the isolation of the homologous products not achievable from bromo-propionic ester.[36]



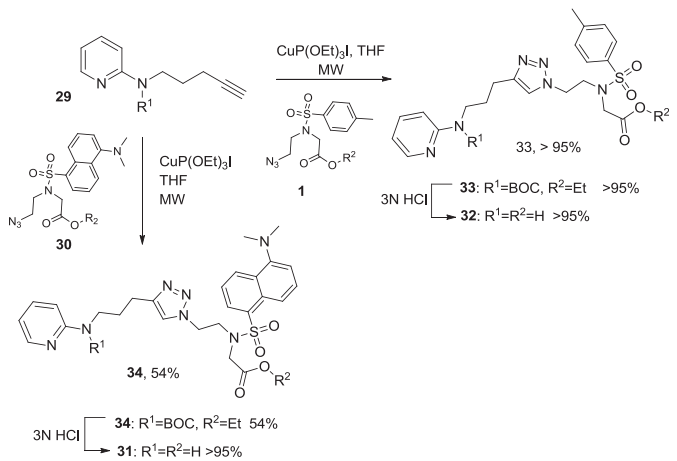
Scheme 1. Modular approach to azides containing two fragments.

The alkylated amino alcohols were subsequently reacted with the chosen aromatic sulfonyl chloride (yields ranging from 40% to quantitative) and converted into azides **1**–**8** via a Mitsunobu reaction. Then, the azides bearing two fragments were reacted via CuAAC with two different Boc-protected alkynes (**27**, **28**) bearing the basic moiety (Scheme 2). Full-organic reaction conditions (THF/CuP(OEt)$_3$I) were preferred for the CuAAC process to minimize the formation of copper-complexes, based upon our previous experience.[37] The cycloaddition step was performed under microwave irradiation in order to shorten the reaction times and improve yields, all being in the medium-to-excellent range after performing the reactions at 120 °C for 60 min. A final acidic deprotection step yielded the water-soluble form of compounds **18**–**26**, without need of further purification, except for solvent evaporation. The overall total yields for the five-step process were about 10–40% starting from the amino alcohols, appearing to be definitely fit for purpose.



Scheme 2. CuAAC and deprotection steps two achieve the three-fragment-bearing scaffolds.

In order to further explore the broad spectrum of functionalities, which can be included in such molecules, another subset of structures was synthesized, varying the basic and the aromatic moieties. Two different groups were chosen, representing many useful characteristics in bioactive candidates, including the fluorogenic dansyl ring and a 2-amino-pyridine as a less basic guanidine isostere (**31** and **32**, see Scheme 3).



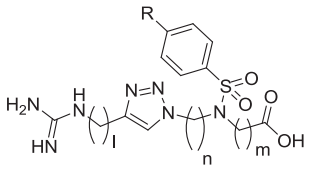
Scheme 3. Scope of the modular approach employing a different alkyne and the dansyl group as additional fragments.

Please cite this article in press as: Fabbrizzi, P.; et al., Tetrahedron (2014), http://dx.doi.org/10.1016/j.tet.2014.06.125

The application of such chemical moieties was accomplished through the same synthetic pathway, with 'click' step yields ranging from 54% to >95%.

To demonstrate the value of the reported approach in the field of bioactive molecules, the library was screened using integrin $\alpha_v\beta_3$ as a test-bed in a competition assay versus [^{125}I]-echistatin. The $\alpha_v\beta_3$ is a well-known membrane receptor being subject in many research studies in the fields of cancer research and part of our group expertise.[37–43] Specifically, in vitro solid-phase assays were performed at 10 and 1 μM concentrations of the test compounds for their ability to compete with ^{125}I-echistatin for binding to pure $\alpha_v\beta_3$ integrin isolated from human placenta, and % binding activity is reported in Table 1. Preliminar structure-activity-relationship considerations were formulated starting from the binding assay results. A major influence of the backbone-chain length on the binding activity was easily assessed, with **19** (n=2, m=1, l=3) and **21** (n=3, m=1, l=3) being the most active ligands at one and 10 μM conc. Also, the analysis of all the reported compounds suggested l=3 and m=1 as a must-have requisite for the compounds in order to achieve a good binding affinity towards the integrin. In addition, the comparison between **19** and the inactive pair **26** and **25** readily showed the primary importance of the aromatic moiety in tuning the binding properties. Finally, the amino-pyridine derivatives **32** and **31** showed no activity at 1 μM (37% and 17% at 10 μM, respectively), revealing the significant influence of different basic groups in tuning the binding affinity.

Table 1
Competition studies of triazole ligands versus [^{125}I]-echistatin against $\alpha_v\beta_3$ integrin, reporting % binding activity at 10 and 1 μM conc. of the test compounds



Cmpd	n	m	R	l	% Binding at 1 μM	% Binding at 10 μM
18	2	1	CH_3	2	6.5	15.9
19	2	1	CH_3	3	39	66.5
20	2	2	CH_3	3	11.5	48.8
21	3	1	CH_3	3	31	88
22	3	2	CH_3	3	25	47
23	4	1	CH_3	3	29	47
24	4	2	CH_3	3	18.8	44
25	2	1	F	3	0	0
26	2	1	Ph	3	0	30.3

In order to assess the binding mode of these triazole-containing compounds to $\alpha_v\beta_3$ integrin, a docking simulation was carried out. The crystal structure of the complex formed by *c*[RGDf(Me)V], also known as Cilengitide (Fig. 2, red structure), and the extracellular fragment of $\alpha_v\beta_3$ integrin (PDB code: 1L5G) provides a general mode of interaction between the integrin and its ligands.[44] Asp carboxylate and Arg guanidinium moieties of RGD-based ligands are key structural elements for receptor recognition. Specifically, the carboxylate group interacts with the metal ion-dependent adhesion site (MIDAS) of the Mn^{2+} ion and Ser121/Ser123, whereas the Arg guanidinium group is responsible for salt-bridge interactions with Asp218 and Asp150 side chains. Additional ligand–receptor contacts occur between Tyr122 in hydrophobic π-stacking and Asn215. The docking program Autodock 4.0[45] was used to evaluate the binding energies of selected conformations of ligand **21**. Docked conformations were analyzed by taking into account the binding interactions as observed in the crystal structure of the bound ligand–protein complex. Docking calculations of compound **21** resulted in a cluster of conformations characterized by key interactions with Asp218, Asp150 and the MIDAS site of the integrin (Fig. 2). Specifically, the COOH group was found in the MIDAS site and interacting with Ser121 and Mn^{2+}, and the guanidinium group undergoing a salt-bridge interaction with Asp218 and Asp150. The main conformation of **21** showed two π-stacking interactions experienced by the aromatic ring facing Tyr-122, and the triazole ring interacting with Tyr178. This binding profile was in agreement with the ligand properties of Cilengitide with the exception of the characteristic π-stacking interactions between the triazole ring of **21** and Tyr178. Also, an additional cation–π interaction[46] experienced by Arg214 and the phenyl ring of **21** was found. Such evidences on the binding mode confirmed l=3 and m=1 as a must-have requisite for the compounds in order to achieve a good binding affinity towards the integrin.

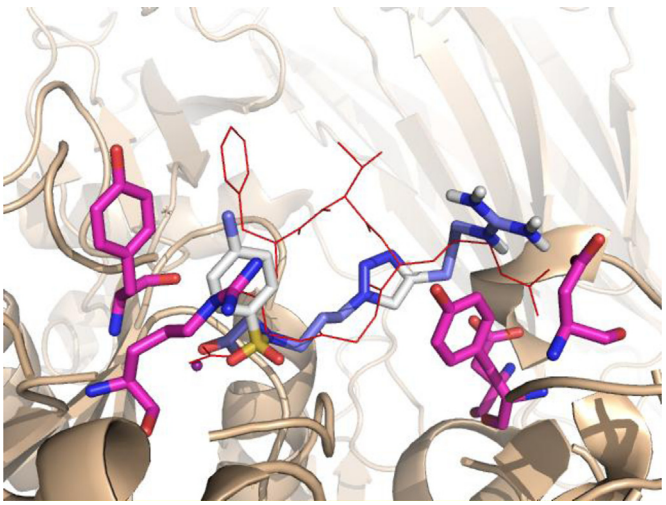


Fig. 2. RGD ligand docked into the binding region of $\alpha_v\beta_3$ integrin, highlighting protein residues (magenta) that form key interactions: Asp218/Asp150 versus guanidine or isostere, Mn^{2+} versus COOH group, Tyr122 and Arg214 versus phenyl moiety, Tyr178 and triazole. Coordinates of reference compound Cilengitide, *c*[RGDf(Me)V] (data from PDB: 1L5G) are shown in red for comparison. Non-polar hydrogen atoms are omitted for clarity.

As regarding to druggability features of the compounds, a preliminar evaluation of the physico-chemical properties of all the compounds was carried out, as shown in Table 2. Specifically, selected physico-chemical parameters (MW, logP, 2D and 3D surface areas, no. of rotatable bonds, and H-bond acceptors/donors) were evaluated with calculator plugins within the framework of MarvinSketch v6.2.2 (Chemaxon Ltd., http://www.chemaxon.com). All compounds but **31** and **32** followed the Lipinski rule of MW <500. Torsional, H-bond capability and 2D surface area values were found to be similar for all library members. These parameters suggested the need of further structural improvement, for example, by inserting structural constraints, so as to reduce the number of rotatable bonds, H-bond acceptors and donors count and polar surface area, according to Veber rules, provided the maintenance of the bioactivity profile.[47]

3. Conclusions

A modular strategy involving only reactions performed in mild conditions starting from readily available building blocks was developed, in order to synthesize highly functionalized molecules

Table 2
Physico-chemical properties prediction of triazole ligands

Cmpd	MW	log *P*	PSA (2D)	VdWSA (3D)	Rot. bonds	H-bond acceptors	H-bond donors
18	409.46	−0.02	167.29	575.43	9	9	4
19	423.49	0.27	167.29	606.56	10	9	4
20	437.52	0.50	167.29	636.39	11	9	4
21	437.52	0.33	167.29	638.04	11	9	4
22	451.54	0.56	167.29	668.22	12	9	4
23	451.54	0.84	167.29	667.93	12	9	4
24	465.57	1.08	167.29	698.54	13	9	4
25	427.45	−0.1	167.29	581.53	10	9	4
26	485.56	1.40	167.29	681.79	11	9	4
31	537.63	2.63	133.55	781.70	12	9	2
32	537.63	2.63	130.31	664.19	11	8	2

suitable as 'hit' candidates for high-throughput screening and multiple receptor targeting in a drug discovery. Those structures were designed around an 1,2,3 triazole ring as a non-classical isostere of the peptide bond. Amino alcohols revealed to be versatile starting materials, due to the wide possibilities of derivatization offered by the concurrent presence of easily functionalizable oxygen and nitrogen atoms. The selected approach did not require any protecting-group strategy, except for the differentiation between organic-soluble intermediates and water-soluble final products. The possibility to readily vary each segment of the model scaffold except for the triazole ring was demonstrated, and the library was screened against integrin $\alpha_v\beta_3$ in order to prove the suitability of the compounds in the field of bioactive ligands. A preliminary SAR analysis showed a major influence of the combination of the type and position of the three selected functional groups on binding properties. Further high-throughput assays against different receptors and enzymes will elucidate the full potential of such a synthetic approach.

4. Experimental section

4.1. General remarks

^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury 400 (^{1}H: 400 MHz, ^{13}C:100 MHz), a Varian Gemini 200 (^{1}H: 200 MHz, ^{13}C: 50 MHz), or a Varian Gemini 300 (^{1}H: 300 MHz, ^{13}C: 75 MHz). The chemical shifts (δ) and coupling constants (J) are expressed in parts per million (ppm) and hertz (Hz), respectively. Flash column chromatography (FCC) purifications were performed manually using glass columns using Merck silica gel (0.040–0.063 mm), or using Biotage Isolera system and SNAP silica cartridges. TLC analyses were performed on Merck silica gel 60 F_{254} plates. Melting points were recorded on a BÜCHI B-540 instrument and were uncorrected. IR spectra were recorded with a FTIR-1600 Perkin–Elmer spectrophotometer. Elemental analyses were recorded on a Perkin Elmer 240 C,H,N analyzer. ESI mass spectra were recorded on a Thermo LCQ-Fleet. Tetrahydrofuran (THF) was distilled over Na/benzophenone, and dichloromethane (DCM) was distilled over CaH_2. Microwave heated reactions were performed in a Biotage Initiator instrument. All commercially available reagents and solvents were used as received, unless otherwise specified. Semipreparative HPLC was performed on a Beckman–Coulter System GOLD (column Grace Alltima C18 10 μm, 250×10 mm), analytical HPLC purity tests were performed on a Dionex UltiMate 3000 (columns: Phenomenex Synergi 4 μm Fusion-RP 80A, 150×4.6 mm and Phenomenex 4 μm Synergi-RP 80A, 150×4.6 mm), using 0.1% TFA-buffered elution gradient CH_3CN–H_2O from 5:95 to 95:5. All tested compounds were >95% pure. Other abbreviations: DPPA: Diphenyl phosphoryl azide, DIAD: Diisopropyl azodicarboxylate.

4.2. General procedure A—alkylation of amino alcohols with bromides

The selected amino alcohol (3 equiv) was dissolved in dry THF (0.25 M), and methyl or ethyl bromoacetate (1 equiv) was slowly added at 0 °C. The reaction was stirred for 4 h at 0 °C, then the solvent was evaporated in vacuo. For further characterization of **35** and **36** see Ref. 35.

*4.2.1. Ethyl N-(2-hydroxyethyl)glycinate (**34**).* Synthesized according to literature procedure.[35,48]

*4.2.2. Methyl N-(3-hydroxypropyl)glycinate (**35**).* Synthesized according to general procedure **A**, starting from propanolamine and methyl bromoacetate. Purified via FCC starting elution with DCM–MeOH 10:1, then increasing polarity to 5:1. 60% yield, colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.80 (t, J=5.7 Hz, 2H), 3.74 (s, 3H), 3.45 (s, 2H), 2.86 (t, J=6 Hz, 2H), 1.78–169 (m, 2H). Anal. Calcd For $C_6H_{13}NO_3$: C, 48.97; H, 8.90; N, 9.52. Found C, 48.86; H, 8.78; N, 9.43.

*4.2.3. Ethyl N-(4-hydroxybutyl)glycinate (**36**).* Synthesized according to general procedure **A**, starting from butanolamine and ethyl bromoacetate. Purified via FCC, eluting with DCM–MeOH 6:1. 89% yield, colorless oil. (TLC DCM–MeOH 5:1, R_f=0.43). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.20 (q, J=7.2 Hz, 2H), 3.63–3.58 (m, 2H), 3.41 (s, 2H), 3,21 (br s, 1H), 2.71–2.66 (m, 2H), 1.68–1.61 (m, 4H), 1.28 (t, J=6.9 Hz, 3H). Anal. Calcd For $C_8H_{17}NO_3$: C, 54.84; H, 9.78; N, 7.99. Found C, 54.69; H, 9.62; N, 7.88.

4.3. General procedure B—Michael addition to amino alcohols

The amino alcohol (1 equiv) was cooled to 0 °C and methyl acrylate (1 equiv) was slowly added. The reaction was stirred 1 h at 0 °C, then the mixture was allowed to warm up to room temperature and stirred for additional 24 h, to afford the corresponding pure product.

*4.3.1. Ethyl N-(2-hydroxyethyl)-β-alaninate (**37**).* Synthesized according to general procedure **B**, starting from ethanolamine and ethyl acrylate. Quantitative yield, white oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.44 (q, J=7.2 Hz, 2H), 3.62 (t, J=5.4 Hz, 2H), 2.90 (t, J=6.3 Hz, 2H), 2.78 (t, J=5.4 Hz, 2H), 2.50 (t, J=6.3 Hz, 2H), 1.26 (t, J=7.2 Hz, 3H). Anal. Calcd For $C_7H_{15}NO_3$: C, 52.16; H, 9.38; N, 8.69. Found C, 51.95; H, 9.32; N, 8.63.

*4.3.2. Methyl N-(3-hydroxypropyl)-β-alaninate (**38**).* Synthesized according to general procedure **B**, starting from propanolamine and methyl acrylate. Quantitative yield, white oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.79 (t, J=5.4 Hz, 2H), 3.68 (s, 3H), 2.95–2.82 (m, 4H), 2.51 (t, J=6.2 Hz, 2H), 1.77–1.61 (m, 2H). Anal. Calcd For $C_7H_{15}NO_3$: C, 52.16; H, 9.38; N, 8.69. Found C, 51.92; H, 9.34; N, 8.66.

*4.3.3. Methyl N-(4-hydroxybutyl)-β-alaninate (**39**).* Synthesized general procedure **B**, starting from buthanolamine and methyl acrylate. Quantitative yield, white oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.56 (s, 3H), 3.48–3.40 (m, 2H), 2.76 (t, J=6.3 Hz, 2H), 2.67 (t, J=7.2 Hz, 2H), 2.44–2.40 (m, 2H), 1.51–1.49 (m, 2H), 1.48–1.44 (m, 2H). Anal. Calcd for $C_8H_{17}NO_3$: C, 54.84; H, 9.78; N, 7.99. Found C, 54.65; H, 9.69; N, 7.87.

4.4. General procedure C—synthesis of sulfonamides

The alkylated amino alcohol (1 equiv) was dissolved in THF (0.75 M), and Et_3N was added (1 equiv). Then, the solution was

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cooled to 0 °C and the corresponding sulfonyl chloride (1 equiv) was added portionwise. The mixture was stirred for 4 h at 0 °C, then water was added (0.4 mL for each mmol of sulfonyl chloride) and 2 N HCl was added until pH≈2 was reached. The solution was extracted twice with ethyl acetate (5 mL for each mmol of sulfonyl chloride), the organic phase was dried over Na_2SO_4 and the solvent evaporated under vacuum. Flash column chromatography afforded the desired products.

*4.4.1. Ethyl N-(2-hydroxyethyl)-N-[(4-methylphenyl)sulfonyl]glycinate (**40**).* Obtained from general procedure C, starting from **34** and tosyl chloride. Purified via FCC using Biotage Isolera system (P. E.–EtOAc gradient). 56% yield, colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 (d, J=8.4 Hz, 2H), 7.32 (d, J=8.4 Hz, 2H), 4.18 (q, J=7.2 Hz, 2H), 4.04 (s, 2H), 3.71 (t, J=4.8 Hz, 2H), 3.34 (t, J=4.8 Hz), 2.43 (s, 3H), 1.26 (t, J=7.2 Hz, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.3, 143.8, 136, 130.2, 127, 61.7, 60.5, 51.9, 49.9, 21.4, 13.9. MS (ESI): m/z (%) 324 [M+Na^+] (100), 318.8 (85), 302 (50) [M+H^+]. IR ($CHCl_3$) 3622 (s), 2943 (m), 2835 (m), 2253 (m), 1750 (s), 1604 (m), 1341 (s), 1217 (m), 1158 (s), 1088 (m), 1072 (m), 1024 (s), 814 (m) cm^{-1}. Anal. Calcd For $C_{13}H_{19}NO_5S$: C, 51.81; H, 6.35; N, 4.65. Found C, 51.65; H, 6.29; N, 4.62.

*4.4.2. Ethyl N-[(4-fluorophenyl)sulfonyl]-N-(2-hydroxyethyl)glycinate (**41**).* Obtained from general procedure C, starting from **34** and 4-fluorobenzenesulfonyl chloride. Purified via FCC using Biotage Isolera system (TLC to gradient, TLC eluant P. E.–EtOAc 1:1, R_f=0.36). 43% Yield, white solid. Mp 83.5–84.5 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90–7.84 (m, 2H), 7.22–7.15 (m, 2H), 4.17 (q, J=7.2 Hz, 2H), 4.08 (s, 2H), 3.72–3.68 (m, 2H), 3.36 (t, J=4.8 Hz, 2H), 2.99–2.97 (m, 1H), 1.25 (t, J=7.2 Hz, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.3, 167.8, 135.4, 130.3, 116.5, 116, 61.9, 60.6, 52.2, 49.8, 14. MS (ESI): m/z (%) 328 (100) [M+Na^+], 322.9 (48), 305.9 (18) [M+H^+]. IR ($CHCl_3$) 3622 (s), 2954 (m), 2911 (m), 2835 (w), 2253 (s), 1744 (m), 1604 (m), 1497 (w), 1373 (w), 1346 (w), 1160 (w), 1154 (w), 1094 (m), 1018 (s), 809 (m) cm^{-1}. Anal. Calcd For $C_{12}H_{16}FNO_5S$: C, 47.21; H, 5.28; N, 4.59. Found C, 47.09; H, 5.22; N, 4.63.

*4.4.3. Ethyl N-(biphenyl-4-ylsulfonyl)-N-(2-hydroxyethyl)glycinate (**42**).* Obtained from general procedure C, starting from **34** and [1,1′-biphenyl]-4-sulfonyl chloride. Purified via FCC using Biotage Isolera system (TLC to gradient, TLC eluant P. E.–EtOAc 1:1, R_f=0.55). 41% Yield, white solid. Mp 92.6–94.5 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93–7.89 (m, 2H), 7.74–7.70 (m, 2H), 7.63–7.58 (m, 2H), 7.52–7.45 (m, 3H), 4.23–4.11 (m, 2H), 4.09 (s 2H), 3.76–3.71 (m, 2H), 3.41 (t, J=4.8 Hz, 2H), 3.11 (t, J=6.3 Hz, 2H), 1.26 (t, J=7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4, 145.7, 137.6, 132.3,129, 128.5, 127.9, 127.7, 127.3, 61.9, 60.7, 52.4, 50.1, 14. MS (ESI): m/z (%)386.1 (100) (M+Na^+), 363.1 (14) [M^+]. IR ($CHCl_3$) 3622 (s), 2943 (m), 2921 (m), 2835, 2253, 1740 (w), 1599 (w), 1500 (w), 1330 (m), 1158 (w), 1072 (m), 1018 (s), 809 (m) cm^{-1}. Anal. Calcd For $C_{18}H_{21}NO_5S$: C, 59.49; H, 5.82; N, 3.85. Found C, 59.25; H, 5.73; N, 3.79.

*4.4.4. Ethyl N-{[5-(dimethylamino)-1-naphthyl]sulfonyl}-N-(2-hydroxyethyl)glycinate (**43**).* Obtained from general procedure C, starting from **34** and dansyl chloride. Purified via FCC using Biotage Isolera system (TLC to gradient, TLC eluant P. E.–EtOAc 1:1, R_f=0.43). 49% Yield, yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.55 (d, J=8.4 Hz, 1H), 8.32 (d, J=8.4 Hz, 1H), 8.23 (dd, J=7.5, 1.2 Hz, 1H), 7.59–7.48 (m, 2H), 7.18 (d, J=7.8 Hz, 1H), 4.16 (s, 2H), 4.11 (q, J=7.2 Hz, 2H), 3.70–3.65 (m, 2H), 3.46 (t, J=5.1 Hz, 2H), 2.89 (s, 6H), 1.19 (t, J=7.2 Hz, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.1, 151.7, 134.7, 130.5, 130, 129.3, 128.2, 123.1, 119.2, 115.2, 61.5, 60.5, 51.6, 49.5, 45.3, 13.8. MS (ESI): m/z (%) 381.1 (100) [M+H^+], 335.1 (34). IR ($CHCl_3$) 3638 (s), 3477 (bm), 2948 (m), 2851 (m), 2247 (m), 1602 (m), 1467 (m), 1333 (m), 1075 (w), 1021 (s) cm^{-1}. Anal. Calcd For $C_{18}H_{24}N_2O_5S$: C, 56.82; H, 6.36; N, 7.36. Found C, 56.67; H, 6.30; N, 7.32.

*4.4.5. Ethyl N-(2-hydroxyethyl)-N-[(4-methylphenyl)sulfonyl]-β-alaninate (**44**).* Obtained from general procedure C, starting from **37** and tosyl chloride. Purified via FCC using Biotage Isolera system (TLC to gradient, TLC eluant P. E.–EtOAc 1:1, R_f=0.39). 67% Yield, colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.70 (d, J=8.4 Hz, 2H), 7.32 (d, J=8.4 Hz, 2H), 4.14 (q, J=7.2 Hz, 2H), 3.81–3.74 (m, 2H), 3.43 (t, J=7.2 Hz, 2H), 3.23 (t, J=5.1 Hz, 2H), 2.71 (t, J=7.2 Hz, 2H), 2.43 (s, 3H), 1.26 (t, J=7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.8, 143.7, 135.5, 129.8, 127.2, 61.4, 60.9, 52.1, 45.9, 34.5, 21.4, 14.1. MS (ESI): m/z (%) 338 (100) [M+Na^+], 316 (12) [M+H^+]. IR ($CHCl_3$) 3626 (s), 2943 (m), 2835 (m), 2253 (m), 1750 (s), 1604 (m), 1341 (s), 1217 (m), 1159 (s), 1088 (m), 1072 (m), 1022 (s), 814 (m) cm^{-1}. Anal. Calcd For $C_{14}H_{21}NO_5S$: C, 53.32; H, 6.71; N, 4.44. Found C, 53.26; H, 6.67; N, 4.41.

*4.4.6. Methyl N-(3-hydroxypropyl)-N-[(4-methylphenyl)sulfonyl]glycinate (**45**).* Obtained from general procedure C with addition of 20% methanol to solubilize the product, starting from **35** and tosyl chloride. Purified via FCC using Biotage Isolera system (TLC to gradient, TLC eluant P. E.–EtOAc 1:1, R_f=0.32). >95% Yield, colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.69 (d, J=8.2 Hz, 2H), 7.28 (d, J=8.2 Hz), 4.00 (s, 2H), 3.75–3.70 (m, 2H), 3.60 (s, 3H), 3.33 (t, J=6.6 Hz, 2H), 2.0 (s, 3H), 1.75–1.68 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 169.5, 143.7, 136.1, 129.6, 127.3, 58.8, 52.1, 48.4, 45.4, 30.4, 21.5. MS (ESI): m/z (%) 302.1 (100) [M+H^+]. IR ($CHCl_3$) 3623 (s), 2940 (m), 2835 (m), 2253 (m), 1751 (s), 1604 (m), 1341 (s), 1217 (m), 1158 (s), 1090 (m), 1072 (m), 1024 (s), 814 (m) cm^{-1}. Anal. Calcd For $C_{13}H_{19}NO_5S$: C, 51.81; H, 6.35; N, 4.65. Found C, 51.64; H, 6.28; N, 4.62.

*4.4.7. Methyl N-(3-hydroxypropyl)-N-[(4-methylphenyl)sulfonyl]-β-alaninate (**46**).* Obtained from general procedure C starting from **38** and tosyl chloride. Purified via FCC using Biotage Isolera system (TLC to gradient, TLC eluant P. E.–EtOAc 1:1, R_f=0.66). 69% Yield, colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.69 (d, J=8.4 Hz, 2H), 7.31 (d, J=8.4 Hz, 2H), 3.78–3.69 (m, 2H), 3.66 (s, 3H), 3.41 (t, J=7.2 Hz, 2H), 3.24 (t, J=6.6 Hz, 2H), 2.63 (t, J=7.2 Hz, 2H), 2.42 (s, 3H), 1.78–1.72 (m, 2H). MS (ESI): m/z (%) 301.6 (96) [M+H^+]. IR ($CHCl_3$) 3623 (s), 2943 (m), 2835 (m), 2253 (m), 1755 (s), 1601 (m), 1344 (s), 1217 (m), 1158 (s), 1088 (m), 1072 (m), 1024 (s), 814 (m) cm^{-1}.

Anal. Calcd for $C_{14}H_{21}NO_5S$: C, 53.32; H, 6.71; N, 4.44. Found C, 53.27; H, 6.68; N, 4.40.

*4.4.8. Methyl N-(4-hydroxybutyl)-N-[(4-methylphenyl)sulfonyl]-β-alaninate (**48**).* Obtained from general procedure C starting from **39** and tosyl chloride. Purified via FCC using Biotage Isolera system (TLC to gradient, TLC eluant P. E.–EtOAc 1:1, R_f=0.60). 56% Yield, colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69 (d, J=8.4 Hz, 2H), 7.30 (d, J=8.4 Hz, 2H), 3.67–3.62 (m, 4H), 3.40 (t, J=7.2 Hz, 2H), 3.15 (t, J=7.2 Hz, 2H), 2.65 (t, J=7.2 Hz, 2H), 2.42 (s, 3H), 1.65–1.56 (m, 4H). MS (ESI): m/z (%) 330.3 (100) [M+H^+]. IR ($CHCl_3$) 3621 (s), 2943 (m), 2838 (m), 2253 (m), 1751 (s), 1605 (m), 1341 (s), 1218 (m), 1158 (s), 1089 (m), 1072 (m), 1024 (s), 814 (m) cm^{-1}. Anal. Calcd for $C_{15}H_{23}NO_5S$: C, 54.69; H, 7.04; N, 4.25. Found C, 54.45; H, 6.98; N, 4.23.

*4.4.9. Ethyl N-(4-hydroxybutyl)-N-[(4-methylphenyl)sulfonyl]glycinate (**47**).* Obtained from general procedure C starting from **36** and tosyl chloride. Purified via FCC using Biotage Isolera system (TLC to gradient, TLC eluant P. E.–EtOAc 1:1, R_f=0.34). 90% Yield, colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.13–4.01 (m, 4H), 3.68–3.62 (m, 2H), 3.27 (d, J=6.9 Hz, 2H), 2.42 (s, 3H), 1.63–1.59 (m, 4H). MS (ESI): m/z (%) 352.3 (47) [M+Na^+], 329.3 (100) [M+H^+]. IR ($CHCl_3$) 3627

Please cite this article in press as: Fabbrizzi, P.; et al., Tetrahedron (2014), http://dx.doi.org/10.1016/j.tet.2014.06.125

(s), 2943 (m), 2835 (m), 2255 (m), 1751 (s), 1607 (m), 1341 (s), 1218 (m), 1158 (s), 1088 (m), 1072 (m), 1026 (s), 814 (m) cm^{-1}. Anal. Calcd for $C_{15}H_{23}NO_5S$: C, 54.69; H, 7.04; N, 4.25. Found C, 54.45; H, 6.99; N, 4.25.

4.5. General procedure D—conversion to azides (Mitsunobu reaction)

Sulfonamidic adduct (1 equiv) was dissolved in anhydrous THF (0.1 M) in a microwave vial, and the solution cooled to −10 °C, then PPh_3 (1.2 equiv), DIAD (1.2 equiv), and DPPA (1.2 equiv) were successively added. The mixture was stirred for 10 min at −10 °C, then heated under microwave irradiation at 60 °C for 30 min. Solvent was evaporated under vacuum and the product purified via FCC.

*4.5.1. Ethyl N-(2-azidoethyl)-N-[(4-methylphenyl)sulfonyl]glycinate (**1**).* Obtained from general procedure D, starting from **40**. Purified via FCC using Biotage Isolera system (TLC to gradient, TLC eluant P. E.−EtOAc 3:1, R_f=0.35). 86% Yield, colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72 (d, *J*=8.4 Hz, 2H), 7.31 (d, *J*=8.4 Hz, 2H), 4.15 (s, 2H), 4.09 (q, *J*=7.2 Hz, 2H), 3.56 (t, *J*=6 Hz, 2H), 3.39 (t, *J*=6 Hz, 2H), 2.43 (s, 3H), 1.21 (t, *J*=7.2 Hz, 3H). MS (ESI): *m*/*z* (%)365 [M+K^+] (25), 349 [M+Na^+] (100), 343.9 (25), 326.8 (5) [M^+]. IR ($CHCl_3$) 3628 (s), 2959 (m), 2916 (m), 2830 (m), 2247 (m), 1741 (w), 1603 (w), 1338 (m), 1161 (w), 1096 (m), 1016 (s), 811 (m) cm^{-1}. Anal. Calcd for $C_{13}H_{18}N_4O_4S$: C, 47.84; H, 5.56; N, 17.17. Found C, 47.72; H, 5.49; N, 17.08.

*4.5.2. Ethyl N-(2-azidoethyl)-N-(biphenyl-4-ylsulfonyl)glycinate (**8**).* Obtained from general procedure D, starting from **42**. Purified via FCC using Biotage Isolera system (TLC to gradient, TLC eluant P. E.−EtOAc 3:1, R_f=0.56). 87% Yield, waxy solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92−7.88 (m, 2H), 7.75−7.69 (m, 2H), 7.63−7.58 (m, 2H), 7.51−7.42 (m, 3H), 4.19 (s, 2H), 4.10 (q, *J*=7.2 Hz, 2H), 3.59 (t, *J*=6 Hz, 2H), 3.43 (t, *J*=6 Hz, 2H), 1.20 (t, *J*=7.2 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.7, 145.9, 139.2, 137.8, 132.3, 129, 128.9, 128.5, 127.9, 127.6, 127.3, 61.5, 50.8, 49.7, 47.8, 14. MS (ESI): *m*/*z* (%) 388.7 [M+H^+] (95). IR ($CHCl_3$) 3681 (m), 3628 (m), 2948 (m), 2840 (m), 2247 (s), 2097 (s), 1747 (s), 1596 (m), 1478 (w), 1376 (w), 1349 (m), 1215 (w), 1155 (s), 1096 (m), 1016 (s). Anal. Calcd For $C_{18}H_{20}N_4O_4S$: C, 55.66; H, 5.19; N, 14.42. Found C, 55.51; H, 5.13; N, 14.37.

*4.5.3. Ethyl N-(2-azidoethyl)-N-[(4-fluorophenyl)sulfonyl]glycinate (**7**).* Obtained from general procedure D, starting from **41**. Purified via FCC using Biotage Isolera system (TLC to gradient, TLC eluant P. E.−EtOAc 3:1, R_f=0.51). 83% Yield, colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90−7.84 (m, 2H), 7.23−7.15 (m, 2H), 4.18 (s, 2H), 4.10 (q, *J*=7.2 Hz, 2H), 3.56 (t, *J*=5.7 Hz, 2H), 3.40 (t, *J*=5.7 Hz, 2H), 1.22 (t, *J*=7.2 Hz, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 168.6, 162.7, 135.5, 130.2, 116.4, 116, 61.5, 50.7, 49.5, 47.6, 14. MS (ESI): *m*/*z* (%) 353.1 (100) [M+Na^+]. IR ($CHCl_3$) 3611 (s), 2943 (m), 2835 (m), 2253 (m), 2102 (m), 1744 (m), 1644 (m), 1336 (m), 1163 (w), 1153 (w), 1088 (m), 811 (m). Anal. Calcd for $C_{12}H_{15}FN_4O_4S$: C, 43.63; H, 4.58; N, 16.96. Found C, 43.75; H, 4.62; N, 16.83.

*4.5.4. Ethyl N-(2-azidoethyl)-N-{[5-(dimethylamino)-1-naphthyl] sulfonyl}glycinate (**30**).* Obtained from general procedure D, starting from **43**. Purified via FCC using Biotage Isolera system (TLC to gradient, TLC eluant P. E.−EtOAc 4:1, R_f=0.29). 67% Yield, yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.55 (d, *J*=8.7 Hz, 1), 8.30−8.24 (m, 2H), 7.59−7.48 (m, 2H), 7.18 (d, *J*=7.8 Hz, 1H), 4.25 (s, 2H), 3.99 (q, *J*=7.2 Hz, 2H), 3.53−3.47 (m, 2H), 2.87 (s, 6H), 1.10 (t, *J*=7.2 Hz, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 168.7, 151.9, 134.9, 130.7, 130.1, 129.5, 128.3, 123.2, 119.2, 115.3, 61.2, 50.4, 49.3, 47.5, 45.3, 14.2, 13.8. MS (ESI): *m*/*z* (%) 428 (96) [M+Na^+], 406.2 (100) [M+H^+]. IR ($CHCl_3$) 3622 (s), 2943 (m), 2835 (m), 2253 (m), 2102 (m), 1750 (m), 1604 (m), 1459 (m), 1330, 1206 (w), 1158 (m), 1142 (m), 1013 (s). Anal. Calcd For $C_{18}H_{23}N_5O_4S$: C, 53.32; H, 5.72; N, 17.27. Found C, 53.44; H, 5.78; N, 17.14.

*4.5.5. Ethyl N-(2-azidoethyl)-N-[(4-methylphenyl)sulfonyl]-β-alaninate (**2**).* Obtained from general procedure D, starting from **44**. Purified via FCC using Biotage Isolera system (TLC to gradient, TLC eluant P. E.−EtOAc 2:1, R_f=0.51). 83% Yield, pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71 (d, *J*=8.4 Hz, 2H), 7.32 (d, *J*=8.4 Hz, 2H), 4.13 (q, *J*=7.2 Hz, 2H), 3.54−3.41 (m, 4H), 2.43 (s, 3H), 1.27 (t, *J*=7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171, 143.6, 137.7, 129.7, 129.6, 127, 60.4, 50.2, 48.3, 45.2, 34.2, 21.1, 13.9. MS (ESI): *m*/*z* (%) 363.1 (100) [M+Na^+], 357.8 (92), 340.7 (42) [M+H^+], 290.8 (62), 287.1 (40). IR ($CHCl_3$) 3628 (s), 2958 (m), 2916 (m), 2830 (m), 2247 (m), 1742 (w), 1601 (w), 1338 (m), 1161 (w), 1097 (m), 1015 (s), 811 (m) cm^{-1}. Anal. Calcd For $C_{14}H_{20}N_4O_4S$: C, 49.40; H, 5.92; N, 16.46. Found C, 49.51; H, 5.99; N, 16.37.

*4.5.6. Methyl N-(3-azidopropyl)-N-[(4-methylphenyl)sulfonyl]glycinate (**3**).* Obtained from general procedure D, starting from **45**. Purified via FCC using Biotage Isolera system (TLC to gradient, TLC eluant P. E.−EtOAc 2:1, R_f=0.47). 88% Yield, pale yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.65 (d, *J*=8 Hz, 2H), 7.24 (d, *J*=8 Hz, 2H), 3.96 (s, 2H), 3.57 (s, 3H), 3.32 (t, *J*=6.4 Hz, 2H), 3.23 (t, *J*=6.4 Hz, 2H), 2.35 (s, 3H), 1.82−1.65 (m, 2H). MS (ESI): *m*/*z* (%) 327.4 (100) [M+H^+]. IR ($CHCl_3$) 3627 (s), 2960 (m), 2917 (m), 2831 (m), 2247 (m), 1736 (w), 1602 (w), 1338 (m), 1161 (w), 1094 (m), 1016 (s), 811 (m) cm^{-1}. Anal. Calcd For $C_{13}H_{18}N_4O_4S$: C, 47.84; H, 5.56; N, 17.17. Found C, 47.99; H, 5.68; N, 17.04.

*4.5.7. Methyl N-(3-azidopropyl)-N-[(4-methylphenyl)sulfonyl]-β-alaninate (**4**).* Obtained from general procedure D, starting from **46**. Purified via FCC using Biotage Isolera system (TLC to gradient, TLC eluant P. E.−EtOAc 3:1, R_f=0.47). 90% Yield, pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69 (d, *J*=8.4 Hz, 2H), 7.32 (d, *J*=8.4 Hz, 2H), 3.68 (s, 3H), 3.43−3.35 (m, 4H), 3.18 (t, *J*=7.2 Hz, 2H), 2.64 (t, *J*=7.2 Hz, 2H), 2.43 (s, 3H), 1.85−1.79 (m, 2H). MS (ESI): *m*/*z* (%) 341.3 (100) [M+H^+]. IR ($CHCl_3$) 3626 (s), 2959 (m), 2916 (m), 2828 (m), 2245 (m), 1743 (w), 1601 (w), 1336 (m), 1161 (w), 1094 (m), 1016 (s), 811 (m) cm^{-1}. Anal. Calcd For $C_{14}H_{20}N_4O_4S$: C, 49.40; H, 5.92; N, 16.46. Found C, 49.32; H, 5.89; N, 16.43.

*4.5.8. Methyl N-(4-azidobutyl)-N-[(4-methylphenyl)sulfonyl]-β-alaninate (**6**).* Obtained from general procedure D, starting from **47**. Purified via FCC using Biotage Isolera system (TLC to gradient, TLC eluant P. E.−EtOAc 3:1, R_f=0.42). 39% Yield, colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.68 (d, *J*=8.4 Hz, 2H), 7.32 (d, *J*=8.4 Hz, 2H), 3.63 (s, 3H), 3.40−3.25 (m, 4H), 3.20−3.12 (m, 2H), 2.64 (t, *J*=6.6 Hz, 2H), 2.39 (s, 3H), 1.71−1.55 (m, 4H). MS (ESI): *m*/*z* (%) 355.2 (100) [M+H^+]. IR ($CHCl_3$) 3628 (s), 2958 (m), 2917 (m), 2831 (m), 2247 (m), 1741 (w), 1601 (w), 1335 (m), 1161 (w), 1098 (m), 1016 (s) cm^{-1}. Anal. Calcd For $C_{15}H_{22}N_4O_4S$: C, 50.83; H, 6.26; N, 15.81. Found C, 51.08.72; H, 6.32; N, 15.72.

*4.5.9. Ethyl N-(4-azidobutyl)-N-[(4-methylphenyl)sulfonyl]glycinate (**5**).* Obtained from general procedure D, starting from **47**. Purified via FCC using Biotage Isolera system (TLC to gradient, TLC eluant P. E.−EtOAc 3:1, R_f=0.61). 79% Yield, colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.70 (d, *J*=8.4 Hz, 2H), 7.28 (d, *J*=8.4 Hz, 2H), 4.09−3.95 (m, 4H), 3.33−3.17 (m, 4H), 2.40 (s, 3H), 1.67−1.51 (m, 4H), 1.18 (t, *J*=7.2 Hz, 3H). MS (ESI): *m*/*z* (%) 354.8 (100) [M+H^+]. IR ($CHCl_3$) 3628 (s), 2957 (m), 2914 (m), 2830 (m), 2247 (m), 1739 (w), 1601 (w), 1336 (m), 1159 (w), 1096 (m), 1013 (s), 809 (m) cm^{-1}. Anal. Calcd For $C_{15}H_{22}N_4O_4S$: C, 50.83; H, 6.26; N, 15.81. Found C, 50.96; H, 6.31; N, 15.64.

4.6. General procedure E—CuAAC

Azide (1.1 equiv) was dissolved in THF (0.15 M solution). Alkyne (1 equiv) and $CuP(OEt)_3I$ (0.11 equiv) were added. The reaction was

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performed under microwave irradiation (temperature 120 °C, time 1 h). THF was evaporated, the crude dissolved in AcOEt or DCM, washed twice with concentrated ammonia solution (40 mL for each mmol of $CuP(OEt)_3I$) and brine. The organic layer was dried over Na_2SO_4, evaporated under vacuum and purified via flash column chromatography.

*4.6.1. Ethyl N-(2-{4-[3-({[(tert-butoxycarbonyl)amino][(tert-butoxycarbonyl)imino]methyl}amino)propyl]-1H-1,2,3-triazol-1-yl}ethyl)-N-[(4-methylphenyl)sulfonyl]glycinate (**10**).* Obtained from general procedure E starting from **1** and **28** via FCC using Biotage Isolera system (TLC to gradient, TLC eluant P. E.–EtOAc 1:2, R_f=0.38). 84% Yield, pale yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.62 (d, *J*=8.4 Hz, 2H), 7.57 (s, 1H), 7.29 (d, *J*=8.4 Hz, 2H), 4.61 (t, *J*=6.2 Hz, 2H), 4.03–3.92 (m, 4H), 2.05–1.82 (m, 2H), 1.50 (s, 18H), 1.15 (t, *J*=7.2 Hz, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 168.4, 163.7, 160.6, 154.9, 143.9, 135.6, 129.7, 127.3, 83.8, 78.7, 61.4, 49.8, 49.6, 48.9, 44.1, 28.3, 28.1, 28, 23, 21.5, 21, 14. MS (ESI): *m*/*z* (%)673.9 [M+Na$^+$] (15), 651.9 (100) [M$^+$]. IR ($CHCl_3$) 3686 (m), 3619 (m), 3046 (s), 2979 (m), 2939 (m), 2826 (w), 2257 (m), 1724 (m), 1601 (s), 1424 (m), 1367 (m), 1329 (m), 1152 (s), 1020 (s) cm^{-1}. Anal. Calcd For $C_{29}H_{45}N_7O_8S$: C, 53.44; H, 6.96; N, 15.04. Found C, 53.32; H, 6.89; N, 14.98.

*4.6.2. Ethyl N-(2-{4-[2-({[(tert-butoxycarbonyl)amino][(tert-butoxycarbonyl)imino]methyl}amino)ethyl]-1H-1,2,3-triazol-1-yl}ethyl)-N-[(4-methylphenyl)sulfonyl]glycinate (**9**).* Obtained from general procedure E starting from **1** and **27**. Purified via FCC using Biotage Isolera system (TLC to gradient, TLC eluant P. E.–EtOAc 1:1, R_f=0.25). 86% Yield, pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.68 (d, *J*=2.1 Hz, 2H), 7.54 (s, 1H), 7.30 (d, *J*=2.1 Hz, 2H), 4.53 (t, *J*=6.4 Hz, 2H), 4.25–4.08 (m, 4H), 3.93 (s, 3H), 3.70 (d, *J*=6.4 Hz, 2H), 3.09–2.95 (m, 2H), 2.43 (s, 3H), 1.60–1.43 (m, 18H), 1.28 (t, *J*=8.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.5, 160.3, 154.8, 144.1, 135.6, 129.7, 127.4, 127.4, 84, 78.8, 77.3, 77, 76.7, 61.5, 50, 49.7, 49, 44.3, 28.3, 28.3, 27.9, 25.3, 21.5, 13.9. MS (ESI): *m*/*z* (%) 660 (46) [M+Na$^+$], 638.1 (100) [M+H$^+$]. IR ($CHCl_3$) 3684 (m), 3619 (m), 3045 (s), 2980 (m), 2942 (m), 2838 (w), 2255 (m), 1724 (m), 1599 (s), 1424 (m), 1367 (m), 1329 (m), 1152 (s), 1020 (s) cm^{-1}. Anal. Calcd For $C_{28}H_{43}N_7O_8S$: C, 52.73; H, 6.80; N, 15.37. Found C, 52.84; H, 6.85; N, 15.35.

*4.6.3. Ethyl N-[2-(4-{3-[(tert-butoxycarbonyl)(pyridin-2-yl)amino]propyl}-1H-1,2,3-triazol-1-yl)ethyl]-N-[(4-methylphenyl)sulfonyl]glycinate (**33**).* Obtained from general procedure E starting from **1** and **29**. Pale yellow oil >95% Yield. FCC using Biotage Isolera system (TLC to gradient, TLC eluant P. E.–EtOAc 1:2, R_f=0.26). ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.36 (d, *J*=5.6 Hz, 1H), 7.71–7.58 (m, 4H), 7.41 (s, 1H), 7.27 (d, *J*=8.2 Hz, 2H), 7.05–6.84 (m, 1H), 4.58 (t, *J*=6.2 Hz, 2H), 4.11–3.90 (m, 4H), 3.78 (s, 2H), 3.67 (t, *J*=6.2 Hz, 2H), 2.73 (t, *J*=7.8 Hz, 2H), 2.39 (s, 3H), 2.10–1.92 (m, 2H), 1.50 (s, 9H), 1.12 (t, *J*=7.1 Hz, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 168.5, 154.6, 154.1, 147.6, 144, 136.9, 135.7, 129.7, 127.4, 120.1, 119.6, 81, 67.1, 61.4, 50, 49.7, 49, 46.3, 28.3, 23.1, 21.5, 13.9. MS (ESI): *m*/*z* (%) 609 (100) [M+Na$^+$], 587 (28) [M+H$^+$]. IR ($CHCl_3$) 3685 (m), 3619 (m), 3044 (s), 2980 (m), 2942 (m), 2838 (w), 2257 (m), 1724 (m), 1600 (s), 1422 (m), 1367 (m), 1329 (m), 1149 (s), 1020 (s) cm^{-1}. Anal. Calcd for $C_{28}H_{38}N_6O_6S$: C, 57.32; H, 6.53; N, 14.32. Found C, 57.38; H, 6.56; N, 14.30.

*4.6.4. Ethyl N-(2-{4-[3-({[(tert-butoxycarbonyl)amino][(tert-butoxycarbonyl)imino]methyl}amino)propyl]-1H-1,2,3-triazol-1-yl}ethyl)-N-[(4-fluorophenyl)sulfonyl]glycinate (**16**).* Obtained from general procedure E starting from **7** and **28**. Purified via FCC using Biotage Isolera system (TLC to gradient, TLC eluant P. E.–EtOAc 1:1, R_f=0.22). 70% Yield, colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85–7.78 (m, 2H), 7.60 (s, 1H), 7.22–7.13 (m, 2H), 4.60 (t, *J*=6.3 Hz, 2H), 4.06–3.93 (m, 4H), 3.86 (s, 2H), 3.75 (t, *J*=6.3 Hz, 2H), 2.76 (t, *J*=7.5 Hz, 2H), 2.01–1.96 (m, 2H), 1.51 (s, 9H), 1.17 (t, *J*=7.2 Hz, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 168.3, 167.8, 163.8, 162.7, 160.7, 154.9, 147.6, 134.9, 130.2, 130, 122.3, 116.5, 116, 83.8, 78.7, 61.5, 49.6, 49.4, 48.7, 44.1, 28.3, 28.1, 27.9, 22.9, 13.9. MS (ESI): *m*/*z* (%) 677.9 (22) [M+Na$^+$], 655.9 (100) [M+H$^+$]. IR ($CHCl_3$) 3682 (m), 3617 (m), 3044 (s), 2980 (m), 2942 (m), 2836 (w), 2257 (m), 1722 (m), 1603 (s), 1421 (m), 1367 (m), 1329 (m), 1153 (s), 1021 (s) cm^{-1}. Anal. Calcd for $C_{28}H_{42}FN_7O_8S$: C, 51.29; H, 6.46; N, 14.95. Found C, 51.36; H, 6.48; N, 14.90.

*4.6.5. Ethyl N-(biphenyl-4-ylsulfonyl)-N-(2-{4-[3-({(Z)-[(tert-butoxycarbonyl)amino][(tert-butoxycarbonyl)imino]methyl}amino)propyl]-1H-1,2,3-triazol-1-yl}ethyl)glycinate (**17**).* Obtained from general procedure E starting from **8** and **28**. Purified via FCC using Biotage Isolera system (TLC to gradient, TLC eluant P. E.–EtOAc 1:1, R_f=0.29). 55% Yield, colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88–7.83 (m, 2H), 7.73–7.68 (m, 2H), 7.61–7.56 (m, 3H), 7.50–7.40 (m, 3H), 4.63 (t, *J*=6.3 Hz, 2H), 4.11 (q, *J*=7.2 Hz, 2H), 3.88 (s, 2H), 3.79 (t, *J*=6.3 Hz, 2H), 2.75 (t, *J*=7.5 Hz, 2H), 1.99–1.94 (m, 2H), 1.51 (s, 18H), 1.14 (t, *J*=7.2 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.4, 163.8, 160.7, 155, 147.6, 146, 139, 137.2, 129, 128.6, 127.9, 127.7, 127.3, 122.4, 83.8, 78.8, 61.5, 44.1, 30.9, 28.4, 28, 23, 14. MS (ESI): *m*/*z* (%) 736.1 (100) [M+Na$^+$], 714.2 (94) [M+H$^+$]. IR ($CHCl_3$) 3940 (m), 3757 (w), 3692 (m), 3056 (s), 2981 (s), 2679 (m), 2528 (w), 2398 (w), 2301 (m), 2247 (m), 2150 (w), 2053 (w), 1790 (w), 1602 (w), 1548 (w), 1446 (m), 1424 (s), 1370 (m), 1263 (vs), 1155 (m), 1096 (w), 1010 (w) cm^{-1}. Anal. Calcd For $C_{34}H_{47}N_7O_8S$: C, 57.21; H, 6.64; N, 13.74. Found C, 57.43; H, 6.71; N, 13.64.

*4.6.6. Ethyl N-[2-(4-{3-[(tert-butoxycarbonyl)(pyridin-2-yl)amino]propyl}-1H-1,2,3-triazol-1-yl)ethyl]-N-{[5-(dimethylamino)-1-naphthyl]sulfonyl}glycinate (**34**).* Obtained from general procedure E starting from **30** and **29**. Purified via FCC using Biotage Isolera system (TLC to gradient, TLC eluant P. E.–EtOAc 1:2, R_f=0.30). 54% Yield, yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.54 (d, *J*=8.4 Hz, 1H), 8.39–8.35 (m, 1H), 8.26–8.19 (m, 2H), 7.66–7.46 (m, 4H), 7.21 (s, 1H), 7.16 (d, *J*=7.5 Hz, 1H), 7.02–6.97 (m, 1H), 4.50 (t, *J*=6.3 Hz, 2H), 3.99 (t, *J*=7.2 Hz, 2H), 3.95–3.80 (m, 6H), 2.85 (s, 6H), 2.68 (t, *J*=7.8 Hz, 2H), 2.01–1.94 (m, 2H), 1.49 (s, 18H), 1.03 (t, *J*=7.2 Hz, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 168.4, 154.5, 154.1, 151.9, 147.5, 136.8, 134.4, 130.8, 130, 129.6, 128.3, 123.1, 121.9, 119.9, 119.5, 119, 115.3, 81, 61.3, 49.6, 49.1, 48.7, 46.2, 45.3, 28.4, 28.2, 23, 13.8. MS (ESI): *m*/*z* (%) 688.1 (100) [M+Na$^+$], 666 (56) [M+H$^+$]. IR ($CHCl_3$) 3687 (m), 3617 (m), 3045 (s), 2982 (s), 2942 (m), 2838 (w), 2254 (m), 1724 (m), 1605 (s), 1424 (m), 1368 (m), 1329 (m), 1153 (s), 1021 (s) cm^{-1}. Anal. Calcd For $C_{33}H_{43}N_7O_6S$: C, 59.53; H, 6.51; N, 14.73. Found C, 59.46; H, 6.48; N, 14.68.

*4.6.7. Ethyl N-(2-{4-[3-({[(tert-butoxycarbonyl)amino][(tert-butoxycarbonyl)imino]methyl}amino)propyl]-1H-1,2,3-triazol-1-yl}ethyl)-N-[(4-methylphenyl)sulfonyl]-β-alaninate (**11**).* Obtained from general procedure E starting from **2** and **28**. Purified via FCC using Biotage Isolera system (TLC to gradient, TLC eluant P. E.–EtOAc 1:1, R_f=0.26). 83% Yield, colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.66 (d, *J*=8.4 Hz, 2H), 7.31 (d, *J*=8.4 Hz, 2H), 4.57 (t, *J*=6.6 Hz, 2H), 4.13–3.92 (m, 4H), 3.56 (t, *J*=6.6 Hz, 2H), 3.29 (t, *J*=7.2 Hz, 2H), 2.74 (t, *J*=7.8 Hz, 2H), 2.42–2.32 (m, 5H), 2.01–1.92 (m, 2H), 1.49 (s, 18H), 1.27–1.17 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171, 163.8, 160.7, 155, 144, 135.2, 129.9, 127.3, 83.8, 78.7, 77.4, 77.1, 76.7, 60.8, 49.6, 49.5, 45.9, 44.1, 33.9, 28.3, 28.2, 28, 23, 21.5, 14.1. MS (ESI): *m*/*z* (%) 688 (24) [M+Na$^+$], 665.9 (100) [M+H$^+$]. IR ($CHCl_3$) 3684 (m), 3618 (m), 3047 (s), 2980 (m), 2935 (m), 2838 (w), 2257 (m), 1723 (m), 1598 (s), 1424 (m), 1367 (m), 1329 (m), 1153 (s), 1021 (s) cm^{-1}.

Please cite this article in press as: Fabbrizzi, P.; et al., Tetrahedron (2014), http://dx.doi.org/10.1016/j.tet.2014.06.125

Anal. Calcd For $C_{30}H_{47}N_7O_8S$: C, 54.12; H, 7.12; N, 14.73. Found C, 54.21; H, 7.20; N, 14.52.

*4.6.8. Methyl N-(3-{4-[3-({[(tert-butoxycarbonyl)amino][(tert-butoxycarbonyl)imino]methyl}amino)propyl]-1H-1,2,3-triazol-1-yl}propyl)-N-[(4-methylphenyl)sulfonyl]glycinate (**12**).* Obtained from general procedure E starting from **3** and **28**. Purified via FCC using Biotage Isolera system (TLC to gradient, TLC eluant P. E.–EtOAc 1:2, R_f=0.38). 26% Yield, colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.70–7.62 (m, 3H), 7.58 (d, J=8.4 Hz, 2H), 4.46 (t, J=6.9 Hz, 2H), 4.00–3.64 (m, 4H), 3.63 (s, 2H), 3.20 (t, J=6.6 Hz, 2H), 2.77 (t, J=7.2 Hz, 2H), 2.41 (s, 3H), 2.20 (t, J=6.6 Hz, 2H), 2.00–1.55 (m, 2H), 1.49 (s, 18H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 169.3, 154.9, 143.8, 135.8, 129.7, 127.3, 127, 83.8, 52.2, 49.1, 47.1, 46.4, 44.1, 29, 28.3, 27.9, 27.8, 23, 21.5. MS (ESI): m/z (%) 651.9 (100) [M^+]. IR ($CHCl_3$) 3688 (m), 3615 (m), 3044 (s), 2979 (m), 2939 (m), 2831 (w), 2254 (m), 1721 (m), 1598 (s), 1422 (m), 1361 (m), 1322 (m), 1156 (s), 1022 (s) cm^{-1}. Anal. Calcd For $C_{29}H_{45}N_7O_8S$: C, 53.44; H, 6.96; N, 15.04. Found C, 53.48; H, 6.97; N, 15.02.

*4.6.9. Methyl N-(3-{4-[3-({[(tert-butoxycarbonyl)amino][(tert-butoxycarbonyl)imino]methyl}amino)propyl]-1H-1,2,3-triazol-1-yl}propyl)-N-[(4-methylphenyl)sulfonyl]-β-alaninate (**13**).* Obtained from general procedure E starting from **4** and **28**. Purified via FCC using Biotage Isolera system (TLC to gradient, TLC eluant P. E.–EtOAc 1:2, R_f=0.58). 47% Yield, colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.68–7.55 (m, 2H), 7.28 (d, J=8.2 Hz, 2H), 4.37 (t, J=6.6 Hz, 2H), 3.94 (t, J=6.6 Hz, 2H), 3.64 (s, 3H), 3.35 (d, J=7.2 Hz, 2H), 3.10 (t, J=6.6 Hz, 2H), 2.75 (t, J=7.2 Hz, 2H), 2.59 (t, J=7.8 Hz, 2H), 2.40 (s, 3H), 2.27–2.12 (m, 2H), 2.03–1.88 (m, 2H), 1.48 (s, 18H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 171.6, 163.8, 160.7, 155, 147.5, 144, 135.4, 129.8, 127.1, 126.9, 83.8, 78.7, 51.8, 47.2, 46.8, 44.9, 44.1, 34.2, 29.5, 28.3, 28.0, 27.9, 22.9, 21.4. MS (ESI): m/z (%) 665.4 (100) [M^+]. IR ($CHCl_3$) 3686 (m), 3619 (m), 3044 (s), 2982 (m), 2941 (m), 2837 (w), 2255 (m), 1721 (m), 1601 (s), 1424 (m), 1366 (m), 1329 (m), 1151 (s), 1019 (s) cm^{-1}. Anal. Calcd For $C_{30}H_{47}N_7O_8S$: C, 54.12; H, 7.12; N, 14.73. Found C, 54.09; H, 7.11; N, 14.74.

*4.6.10. Methyl N-(4-{4-[3-({[(tert-butoxycarbonyl)amino][(tert-butoxycarbonyl)imino]methyl}amino)propyl]-1H-1,2,3-triazol-1-yl}butyl)-N-[(4-methylphenyl)sulfonyl]-β-alaninate (**15**).* Obtained from general procedure E starting from **6** and **28**. Purified via FCC using Biotage Isolera system (TLC to gradient, TLC eluant P. E.–EtOAc 1:2, R_f=0.29). 51% Yield, colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.66 (d, J=8 Hz, 2H), 7.56 (s, 1H), 7.30 (d, J=8 Hz, 2H), 4.34 (t, J=6.6 Hz, 2H), 3.95 (t, J=7.2 Hz, 2H), 3.64 (s, 2H), 3.34 (t, J=7.2 Hz, 2H), 3.12 (t, J=7.2 Hz, 2H), 2.75 (t, J=7.2 Hz, 2H), 2.41 (s, 3H), 2.06–1.89 (m, 4H), 1.55–1.40 (m, 20H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 171.6, 160.7, 155, 147.6, 143.5, 136.1, 129.8, 127.1, 121.1, 83.8, 78.7, 51.8, 49.2, 48.4, 44.3, 44.1, 34.3, 28.4, 28, 27.2, 25.4, 23, 21.5. MS (ESI): m/z (%) 702.4 (100) [M+Na$^+$], 680.2 (100) [M+H$^+$]. IR ($CHCl_3$) 3687 (m), 3621 (m), 3047 (s), 2980 (m), 2943 (m), 2839 (w), 2254 (m), 1724 (m), 1605 (s), 1424 (m), 1365 (m), 1323 (m), 1153 (s), 1021 (s) cm^{-1}. Anal. Calcd For $C_{31}H_{49}N_7O_8S$: C, 54.77; H, 7.26; N, 14.42. Found C, 54.82; H, 7.23; N, 14.38.

*4.6.11. Ethyl N-(4-{4-[3-({[(tert-butoxycarbonyl)amino][(tert-butoxycarbonyl)imino]methyl}amino)propyl]-1H-1,2,3-triazol-1-yl}butyl)-N-[(4-methylphenyl)sulfonyl]glycinate (**14**).* Obtained from general procedure E starting from **6** and **28**. Purified via FCC using Biotage Isolera system (TLC to gradient, TLC eluant P. E.–EtOAc 1:2, R_f=0.47). 73% Yield, colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.64 (d, J=8.1 Hz, 2H), 7.52 (s, 1H), 7.24 (d, J=8.1 Hz, 2H), 4.29 (t, J=6.9 Hz, 2H), 4.30–3.88 (m, 6H), 3.20 (t, J=6.9 Hz, 2H), 2.71 (t, J=7.2 Hz, 2H), 2.36 (s, 3H), 1.96–1.88 (m, 4H), 1.53–1.44 (m, 20H), 1.13 (t, J=7.2 Hz, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 168.7, 163.8, 160.7, 155, 144.3, 143.5, 136.4, 129.5, 127.3, 83.8, 78.6, 61.3, 49.3, 48.2, 47.6, 44.1, 28.3, 27.9, 27.1, 24.9, 23, 21.4, 13.9. MS (ESI): m/z (%) 680.2 (100) [M+H$^+$]. IR ($CHCl_3$) 3689 (m), 3621 (m), 3046 (s), 2980 (m), 2942 (m), 2838 (w), 2256 (m), 1726 (m), 1600 (s), 1424 (m), 1366 (m), 1325 (m), 1153 (s), 1025 (s) cm^{-1}. Anal. Calcd for $C_{31}H_{49}N_7O_8S$: C, 54.77; H, 7.26; N, 14.42. Found C, 54.72; H, 7.24; N, 14.39.

4.7. General procedure F—deprotection

The protected adduct was suspended in 3 N HCl (10 mL/mmol) and stirred at 30 °C overnight. The solution was evaporated to dryness in vacuo to afford the pure product in quantitative yield.

*4.7.1. N-{2-[4-(3-{[Amino(imino)methyl]amino}propyl)-1H-1,2,3-triazol-1-yl]ethyl}-N-[(4-methylphenyl)sulfonyl]glycine monohydrochloride (**19**).* Obtained from general procedure F starting from **10**. Pale yellow solid. ^{1}H NMR (300 MHz, D_2O) δ 7.67 (s, 1H), 7.23 (d, J=8.4 Hz, 2H), 7.21 (d, J=8.4 Hz, 2H), 4.43 (t, J=3.4 Hz, 2H), 4.00 (s, 2H), 3.67 (d, J=3.4 Hz, 2H), 3.04 (d, J=7.2 Hz, 2H), 2.57 (d, J=7.2 Hz, 2H), 2.27 (s, 3H), 1.82–1.71 (m, 2H). ^{13}C NMR (100 MHz, D_2O) δ 172.4, 156.7, 145.3, 134.2, 129.9, 126.8, 48.7, 40, 30.2, 27.1, 21.1, 20.6. MS (ESI): m/z (%) 424.1 (100) [M^+]; 410 (28). IR (KBr) 3396 (br s), 3191 (s), 2964 (m), 2921 (m), 2846 (w), 1744 (m), 1733 (m), 1663 (s), 1647 (s), 1626 (m), 1465 (m), 1448 (m), 1379 (m), 1330 (s), 1293 (m), 1260 (m), 1158 (s), 1088 (m), 1045 (m), 932 (w), 814 (m), 803 (m), 663 (m), 572 (m), 551 (m) cm^{-1}. Anal. Calcd For $C_{17}H_{26}ClN_7O_4S$: C, 44.39; H, 5.70; N, 21.32. Found C, 44.30; H, 5.74; N, 21.29.

*4.7.2. N-{2-[4-(2-{[Amino(imino)methyl]amino}ethyl)-1H-1,2,3-triazol-1-yl]ethyl}-N-[(4-methylphenyl)sulfonyl]glycine monohydrochloride (**18**).* Obtained from general procedure F starting from **9**. Pale yellow solid. ^{1}H NMR (300 MHz, D_2O) δ 7.70 (s, 1H), 7.44 (d, J=6.9 Hz, 2H), 7.24 (d, J=6.9 Hz, 2H), 4.44 (t, J=5.1 Hz, 2H), 3.98 (s, 3H), 3.75–3.62 (m, 2H), 3.33 (d, J=6.3 Hz, 2H), 2.79 (d, J=6.3 Hz, 2H), 2.28 (s, 3H). ^{13}C NMR (100 MHz, D_2O) δ 172.4, 156.7, 145.3, 134.2, 129.9, 129.9, 126.8, 125.0, 48.7, 48.5, 48.3, 40.4, 24.1, 20.6. MS (ESI): m/z (%) 410.2 (100) [M^+], 303.7 (26). IR (KBr) 3396 (br s), 3191 (s), 2964 (m), 2922 (m), 2846 (w), 1744 (m), 1733 (m), 1663 (s), 1647 (s), 1626 (m), 1465 (m), 1448 (m), 1379 (m), 1330 (s), 1293 (m), 1260 (m), 1158 (s), 1081 (m), 1039 (m), 932 (w), 814 (m), 803 (m), 663 (m), 572 (m), 551 (m) cm^{-1}. Anal. Calcd for $C_{16}H_{24}ClN_7O_4S$: C, 43.10; H, 5.42; N, 21.99. Found C, 43.05; H, 5.48; N, 21.95.

*4.7.3. N-[(4-Methylphenyl)sulfonyl]-N-(2-{4-[3-(pyridinium-2-ylamino)propyl]-1H-1,2,3-triazol-1-yl}ethyl)glycine chloride (**32**).* Obtained from general procedure F starting from **33**. Pale yellow solid. ^{1}H NMR (200 MHz, D_2O) δ 7.81 (s, 1H), 7.72–7.61 (m, 1H), 7.55 (d, J=7 Hz, 1H), 7.39 (d, J=7.8 Hz, 2H), 7.21 (d, J=7.8 Hz, 2H), 6.81 (d, J=9.2 Hz, 1H), 6.66 (t, J=7 Hz, 1H), 4.52–4.43 (m, 2H), 4.00 (s, 2H), 3.72–3.65 (m, 2H), 3.22 (t, J=7 Hz, 2H), 2.70 (t, J=7 Hz, 2H), 2.26 (s, 3H), 2.05–1.96 (m, 2H). ^{13}C NMR (50 MHz, D_2O) δ 152.4, 145.3, 143.5, 134.5, 134.1, 129.9, 126.8, 113, 112.4, 49.9, 48.9, 40.5, 26.4, 20.8, 20.7. MS (ESI): m/z (%) 459.2 (100) [M^+]. IR (KBr) 3415 (br s), 3191 (s), 2964 (m), 2921 (m), 2846 (w), 1744 (m), 1733 (m), 1662 (s), 1647 (s), 1625 (m), 1465 (m), 1448 (m), 1379 (m), 1330 (s), 1293 (m), 1260 (m), 1157 (s), 1088 (m), 1045 (m), 932 (w), 814 (m), 803 (m), 793 (m), 771 (m), 668 (w), 647 (w), 665 (m), 588 (m) 572 (m), 551 (m) cm^{-1}. Anal. Calcd For $C_{21}H_{27}ClN_6O_4S$: C, 50.96; H, 5.50; N, 16.98. Found C, 50.89; H, 5.55; N, 16.94.

*4.7.4. N-{2-[4-(3-{[Amino(imino)methyl]amino}propyl)-1H-1,2,3-triazol-1-yl]ethyl}-N-[(4-fluorophenyl)sulfonyl]glycine monohydrochloride (**25**).* Obtained from general procedure F starting from **16**. Pale yellow solid. ^{1}H NMR (300 MHz, D_2O) δ 7.85 (s, 1H), 7.66–7.60 (m, 2H), 7.19–7.11 (m, 2H), 4.51 (t, J=5.4 Hz, 2H), 4.05 (s, 2H), 3.73–3.68 (m, 2H), 3.08 (t, J=6.9 Hz, 2H), 2.66 (t, J=7.5 Hz, 2H),

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1.86–1.75 (m, 2H). ^{13}C NMR (50 MHz, D_2O) δ 172.2, 167.9, 162.8, 156.7, 144.5, 133.5, 130, 129.8, 126.3, 116.9, 116.4, 49.8, 48.7, 40, 26.9, 20.6. MS (ESI): *m*/*z* (%) 428.1 (100) [M^+]. IR (KBr) 3396 (br s), 3192 (s), 2964 (m), 2921 (m), 2846 (w), 1743 (m), 1733 (m), 1663 (s), 1647 (s), 1616 (m), 1465 (m), 1448 (m), 1379 (m), 1330 (s), 1293 (m), 1260 (m), 1158 (s), 1088 (m), 1045 (m), 932 (w), 814 (m), 803 (m), 663 (m), 572 (m), 551 (m) cm^{-1}. Anal. Calcd for $C_{16}H_{23}ClN_7O_4S$: C, 41.42; H, 5.00; N, 21.13. Found C, 41.32; H, 5.11; N, 21.05.

*4.7.5. N-{2-[4-(3-{[Amino(imino)methyl]amino}propyl)-1H-1,2,3-triazol-1-yl]ethyl}-N-(biphenyl-4-ylsulfonyl)glycine monohydrochloride (**26**).* Obtained from general procedure F starting from **17**. White solid. ^{1}H NMR (200 MHz, D_2O) δ 7.50–7.29 (m, 8H), 7.26–7.21 (m, 3H), 4.40–4.30 (m, 2H), 4.01 (s, 2H), 3.70–3.55 (m, 2H), 2.77 (t, *J*=6 Hz, 2H), 2.29 (t, *J*=6 Hz, 2H), 1.62–1.49 (m, 2H). ^{13}C NMR (50 MHz, D_2O) δ 171.1, 155.7, 144.6, 143.8, 136.8, 135.5, 128, 127.7, 126.5, 126.2, 125.8, 124, 48.4, 39.4, 26.3, 20.4. MS (ESI): *m*/*z* (%) 509.2 (44) [M+Na^+], 486.2 (100) [M^+]. IR (KBr) 3396 (br s), 3191 (s), 2966 (m), 2923 (m), 2846 (w), 1741 (m), 1733 (m), 1661 (s), 1646 (s), 1623 (m), 1465 (m), 1448 (m), 1379 (m), 1330 (s), 1291 (m), 1260 (m), 1158 (s), 1083 (m), 1041 (m), 932 (w), 814 (m), 803 (m), 663 (m), 571 (m), 551 (m) cm^{-1}. Anal. Calcd For $C_{22}H_{28}ClN_7O_4S$: C, 50.62; H, 5.41; N, 18.78. Found C, 50.55; H, 5.52; N, 18.69.

*4.7.6. N-{[5-(Dimethylamino)-1-naphthyl]sulfonyl}-N-(2-{4-[3-(pyridin-2-ylamino)propyl]-1H-1,2,3-triazol-1-yl}ethyl)glycine monohydrochloride (**31**).* Obtained from general procedure F starting from **34**. Yellow solid. ^{1}H NMR (300 MHz, D_2O) δ 8.44–8.34 (m, 2H), 8.18 (d, *J*=7.2 Hz, 1H), 8.06 (d, *J*=7.2 Hz, 1H), 8.00 (s, 1H), 7.86–7.67 (m, 3H), 7.59 (d, *J*=6.6 Hz, 1H), 6.88 (d, *J*=9 Hz, 1H), 6.68 (t, *J*=6.6 Hz, 1H), 4.65–4.60 (m, 2H), 4.31 (s, 2H), 3.97–3.92 (m, 2H), 3.50 (s, 6H), 3.29 (t, *J*=6.9 Hz, 2H), 2.71 (t, *J*=6.9 Hz, 2H), 2.01–1.94 (m, 2H). ^{13}C NMR (50 MHz, D_2O) δ 171.1, 152.1, 143.3, 138, 134.5, 134, 130.4, 128.5, 128, 126.9, 126.3, 126, 125.3, 119.7, 112.9, 112.2, 50.1, 48.5, 48.2, 46.9, 40.4, 26.2, 20.3. MS (ESI): *m*/*z* (%) 538.2 (100) [M^+]. IR (KBr) 3428 (br s), 3137 (m), 2954 (m), 2921 (m), 2857 (w), 1733 (m), 1653 (s), 1626 (m), 1454 (m), 1379 (m), 1336 (s), 1206 (w), 1169 (w), 1142 (s), 1099 (m), 1045 (m), 1024 (w), 991 (w), 879 (m), 793 (m), 771 (m), 669 (w), 647 (w), 588 (m) cm^{-1}. Anal. Calcd For $C_{26}H_{32}ClN_7O_4S$: C, 54.39; H, 5.62; N, 17.08. Found C, 54.32; H, 5.70; N, 17.01.

*4.7.7. Ethyl N-{2-[4-(3-{[amino(imino)methyl]amino}propyl)-1H-1,2,3-triazol-1-yl]ethyl}-N-[(4-methylphenyl)sulfonyl]-β-alaninate monohydrochloride (**20**).* Obtained from general procedure F starting from **11**. Pale yellow solid. ^{1}H NMR (300 MHz, D_2O) δ 7.71 (s, 1H), 7.48 (d, *J*=7.8 Hz, 2H), 7.28 (d, *J*=7.8 Hz, 2H), 4.53–4.49 (m, 2H), 3.60–3.58 (m, 2H), 3.33 (t, *J*=6.9 Hz, 2H), 3.06 (t, *J*=6.9 Hz, 2H), 2.65 (t, *J*=7.5 Hz, 2H), 2.40–2.34 (m, 2H), 2.29 (s, 3H), 1.84–1.67 (m, 2H). ^{13}C NMR (100 MHz, D_2O) δ 174.9, 156.6, 145.2, 133.8, 130.1, 126.8, 50.3, 48.3, 44.4, 40, 33, 30.2, 26.9, 20.7. MS (ESI): *m*/*z* (%) 438.2 (100) [M^+]. IR (KBr) 3391 (br s), 3191 (s), 2964 (m), 2921 (m), 2846 (w), 1744 (m), 1733 (m), 1663 (s), 1647 (s), 1628 (m), 1465 (m), 1448 (m), 1379 (m), 1333 (s), 1293 (m), 1260 (m), 1158 (s), 1088 (m), 1045 (m), 933 (w), 814 (m), 803 (m), 668 (m), 572 (m), 551 (m) cm^{-1}. Anal. Calcd For $C_{18}H_{28}ClN_7O_4S$: C, 45.61; H, 5.95; N, 20.69. Found C, 45.51; H, 6.01; N, 20.49.

*4.7.8. N-{3-[4-(3-{[Amino(imino)methyl]amino}propyl)-1H-1,2,3-triazol-1-yl]propyl}-N-[(4-methylphenyl)sulfonyl]glycine monohydrochloride (**21**).* Obtained from general procedure F starting from **12**. Partially unstable compound, a small aliquot was isolated via semipreparative RP-HPLC and used in screening assay and ^{1}H spectroscopy. ^{1}H NMR (300 MHz, D_2O) δ 7.61 (s, 1H), 7.48 (d, *J*=8.4 Hz, 2H), 7.26 (d, *J*=8.4 Hz, 2H), 4.29–2.5 (m, 2H), 3.89 (s, 2H), 3.07–3.01 (m, 4H), 2.67–2.61 (m, 2H), 2.27 (s, 3H), 1.96–1.85 (m, 2H), 1.84–1.77 (m, 2H). MS (ESI): *m*/*z* (%) 438.2 (100) [M^+], 380.2 (38). IR (KBr) 3394 (br s), 3192 (s), 2961 (m), 2921 (m), 2847 (w), 1744 (m), 1733 (m), 1663 (s), 1649 (s), 1626 (m), 1464 (m), 1448 (m), 1379 (m), 1330 (s), 1293 (m), 1260 (m), 1158 (s), 1088 (m), 1045 (m), 932 (w), 814 (m), 803 (m), 663 (m), 572 (m), 551 (m) cm^{-1}. Anal. Calcd For $C_{18}H_{28}ClN_7O_4S$: C, 45.61; H, 5.95; N, 20.69. Found C, 45.30; H, 5.98; N, 20.65.

*4.7.9. N-{3-[4-(3-{[Amino(imino)methyl]amino}propyl)-1H-1,2,3-triazol-1-yl]propyl}-N-[(4-methylphenyl)sulfonyl]-β-alanine monohydrochloride (**22**).* Obtained from general procedure F starting from **13**. Pale yellow solid. ^{1}H NMR (200 MHz, D_2O) δ 7.87 (s, 1H), 7.46 (d, *J*=8.4 Hz, 2H), 7.25 (d, *J*=8.4 Hz, 2H), 4.34 (t, *J*=6.6 Hz, 2H), 3.28 (d, *J*=6.6 Hz, 2H), 3.14–2.94 (m, 2H), 2.70 (d, *J*=7.4 Hz, 2H), 2.42 (d, *J*=6.9 Hz, 2H), 2.25 (s, 3H), 2.06–2.00 (m, 2H), 1.86–1.79. ^{13}C NMR (50 MHz, D_2O) δ 174.9, 156.2, 144.8, 133.2, 129.6, 126.4, 126.1, 48.3, 45.1, 43.7, 39.7, 39, 33.1, 27.2, 26.7, 25.7, 21.8, 20.6, 20.3. MS (ESI): *m*/*z* (%) 452.2 (100) (M^+). IR (KBr) 3396 (br s), 3191 (s), 2964 (m), 2928 (m), 2841 (w), 1744 (m), 1733 (m), 1664 (s), 1647 (s), 1626 (m), 1468 (m), 1448 (m), 1379 (m), 1330 (s), 1293 (m), 1259 (m), 1147 (s), 1088 (m), 1041 (m), 932 (w), 814 (m), 88 (m), 664 (m), 575 (m), 542 (m) cm^{-1}. Anal. Calcd For $C_{19}H_{30}ClN_7O_4S$: C, 46.76; H, 6.20; N, 20.09. Found C, 46.66; H, 6.32; N, 20.01.

*4.7.10. N-{4-[4-(3-{[Amino(imino)methyl]amino}propyl)-1H-1,2,3-triazol-1-yl]butyl}-N-[(4-methylphenyl)sulfonyl]-β-alanine monohydrochloride (**24**).* Obtained from general procedure F starting from **15**. Pale yellow solid. ^{1}H NMR (300 MHz, D_2O) δ 7.83 (s, 1H), 7.44 (d, *J*=8.2 Hz, 2H), 7.18 (d, *J*=8.2 Hz, 2H), 4.24 (t, *J*=6.6 Hz, 2H), 3.19 (t, *J*=6.9 Hz, 2H), 3.02 (t, *J*=6.9 Hz, 2H), 2.94 (t, *J*=7.2 Hz, 2H), 2.18 (s, 3H), 1.79–1.74 (m, 2H), 1.65–1.60 (m, 2H), 1.31–1.22 (m, 2H). ^{13}C NMR (50 MHz, D_2O) δ 175.4, 145.1, 134.4, 130, 126.8, 124.7, 51, 47.6, 43.8, 40.2, 33.7, 27, 26.1, 24.4, 20.9, 20.6. MS (ESI): *m*/*z* (%) 466.2 (100) (M^+). IR (KBr) 3398 (br s), 3191 (s), 2965 (m), 2919 (m), 2846 (w), 1738 (m), 1736 (m), 1662 (s), 1642 (s), 1621 (m), 14,657 (m), 1449 (m), 1379 (m), 1331 (s), 1297 (m), 1261 (m), 1158 (s), 1089 (m), 1051 (m), 933 (w), 820 (m), 802 (m), 658 (m), 578 (m), 555 (m) cm^{-1}. Anal. Calcd For $C_{20}H_{32}ClN_7O_4S$: C, 47.85; H, 6.42; N, 19.53. Found C, 47.74; H, 6.54; N, 19.49.

*4.7.11. N-{4-[4-(3-{[Amino(imino)methyl]amino}propyl)-1H-1,2,3-triazol-1-yl]butyl}-N-[(4-methylphenyl)sulfonyl]glycine monohydrochloride (**23**).* Obtained from general procedure F starting from **15**. Pale yellow solid. ^{1}H NMR (300 MHz, D_2O) δ 7.87 (s, 1H), 7.52 (d, *J*=8.4 Hz, 2H), 7.23 (d, *J*=8.4 Hz, 2H), 4.26 (t, *J*=6.9 Hz, 2H), 3.87 (s, 2H), 3.11–3.04 (m, 4H), 2.71 (t, *J*=7.8 Hz, 2H), 2.24 (s, 3H), 1.85–1.79 (m, 2H), 1.72–1.65 (m, 2H), 1.35–1.28 (m, 2H). ^{13}C NMR (50 MHz, D_2O) δ 172.1, 155.8, 144.3, 133.6, 129.1, 126.1, 124.3, 50.6, 47.7, 47.4, 39.4, 26.1, 25.1, 23.1, 19.9. MS (ESI): *m*/*z* (%) 452.2 (100) (M^+). IR (KBr) 3397 (br s), 3192 (s), 2966 (m), 2926 (m), 2842 (w), 1743 (m), 1731 (m), 1659 (s), 1646 (s), 1623 (m), 1465 (m), 1444 (m), 1373 (m), 1329 (s), 1290 (m), 1258 (m), 1147 (s), 1083 (m), 1040 (m), 927 (w), 814 (m), 803 (m), 657 (m), 566 (m), 555 (m) cm^{-1}. Anal. Calcd For $C_{19}H_{30}ClN_7O_4S$: C, 46.76; H, 6.20; N, 20.09. Found C, 46.64; H, 6.29; N, 20.01.

4.8. Synthesis of alkynes

N,*N*′-Di-Boc-*N*″-(but-3-ynyl)-guanidine (**27**), *N*,*N*′-Di-Boc-*N*″-(pent-3-ynyl)-guanidine (**28**) were synthesized according to literature procedure.[37]

*4.8.1. Pent-4-ynyl-pyridin-2-yl-carbamic acid tert-butyl ester (**29**).* *N*-Boc-2-amino-pyridine[49] (0.5 g, 2.57 mmol) was dissolved in anhydrous DMF under N_2 atmosphere and cooled to 0 °C. To the vigorous stirred solution was slowly added NaH (0.129 g, 60% dispersion in mineral oil), paying attention to keep temperature below

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5 °C. The mixture was stirred under these conditions for 20 min, then (5-chloro-1-pentynyl)trimethylsilane (0.52 g, 530 μL, 2.96 mmol) was added dropwise, and stirring was continued at ≤5 °C for 30 min. The solution was allowed to reach room temperature and stirred for additional 1 h, then 1.33 mL of water was added. TBAF (1 M solution in THF, 5.15 mL, 5.15 mmol) was added, and the solution stirred overnight at rt. Finally, the mixture was diluted with water (6 mL) and extracted with diethyl ether (40 mL×2), the organic layer was washed with 0.1 M HCl, saturated $NaHCO_3$ and brine and dried over Na_2SO_4. The solvent was evaporated under vacuum. Purified via FCC P. E.–EtOAc 10:1 R_f=0.43. Colorless oil, 30% yield, 0.2 g. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.39–8.32 (m, 1H), 7.65–7.55 (m, 2H), 7.04–6.94 (m, 1H), 4.03 (t, *J*=7.1 Hz, 2H), 2.22 (td, *J*=7.4 Hz, 2.6 Hz, 2H), 1.94–1.80 (m, 3H), 1.51 (s, 9H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 147.6, 136.9, 119.9, 119.5, 83.9, 81.1, 68.3, 46, 28.3, 27.8, 16.2. MS (ESI): *m*/*z* (%) 260.9 (M+, 58%), 283.0 (M−Na, 100%). IR ($CHCl_3$) 3680 (m), 3595 (m), 3052 (s), 2985 (m), 2252 (m), 1607 (m), 1422 (m) cm^{-1}. Anal. Calcd for $C_{15}H_{20}N_2O_2$: C, 69.20; H, 7.74; N, 10.76. Found C, 69.31; H, 7.76; N, 10.73.

4.9. Solid-phase integrin binding assay

[^{125}I]-Echistatin, labeled by the lactoperoxidase method to a specific activity of 2000 Ci/mmol, was purchased from GE Healthcare, and integrin $\alpha_v\beta_3$ from human placenta was purchased from Chemicon International Inc., Temecula, CA. Purified $\alpha_v\beta_3$ integrin was diluted in coating buffer (20 mM Tris (pH 7.4), 150 mM NaCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM $MnCl_2$) at concentrations of 500 or 1000 ng/mL. An aliquot of diluted integrin (100 μL/well) was added to a 96-well microtiter plate (Optiplate-96 HB, PerkinElmer Life Sciences, Boston, MA), and plates were incubated overnight at 4 °C. Plates were washed once with blocking/binding buffer (20 mM Tris (pH 7.4), 150 mM NaCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 1% BSA) and incubated at room temperature for additional 2 h. Plates were rinsed twice with same buffer, and then competition binding assays were performed with a constant concentration of [^{125}I]-echistatin (0.05 nM). Concentrations of tested compound were 1 μM and 10 μM. All assays were performed in triplicate in a final volume of 0.2 mL, each containing the following species: 0.05 mL of [^{125}I]-echistatin, 0.04 mL of tested compound, and 0.11 mL of blocking/binding buffer. Nonspecific binding was defined as [^{125}I]-echistatin bound in presence of an excess (1 μM) of unlabeled echistatin. After incubation at room temperature for 3 h, plates were washed three times with blocking/binding buffer and counted in a Top-Count NXT microplate scintillation counter (PerkinElmer Life Sciences, Boston, MA) using 200 μL/well of MicroScint-40 liquid scintillation (PerkinElmer Life Sciences, Boston, MA). Data are shown as means±SD from three independent experiments.

4.10. Docking calculations

Automated docking studies were carried out by Autodock 4.0.1 program[45] using the Lamarckian Genetic Algorithm (LGA) as a search engine. The AutoDockTools 1.4.5 (ADT) graphical interface[50] was used to prepare integrin and ligands PDBQT files. Coordinates of compound **21** were generated using Spartan (version 5.147), and then energy-minimized with the same program. The equilibrium geometry was calculated through the AM1 semi-empirical method. The coordinates of $\alpha_v\beta_3$ receptor were retrieved from the Protein Data Bank (PDB code: 1L5G), and ligand–protein complex was unmerged for achieving free receptor structure. Water molecules were removed. For protein receptor and compound **21**, all hydrogens were added, Gasteiger charges were computed, and non-polar hydrogens were merged. A charge value of +2.0 to each Mn atom of protein receptor was successively added. Three-dimensional energy scoring grids of 0.375 Å resolution and 40 Å×40 Å×40 Å dimensions were computed. The center of the grid was set to be coincident with mass center of ligands preliminary fitted on the X-ray structure of *c*[RGDf(Me)V] in the $\alpha_v\beta_3$ complex (1L5G). A total of 50 runs with a maximum of 2,500,000 energy evaluations were carried out for each ligand, using the default parameters for LGA. Cluster analysis was performed on docked results using a root-mean-square (rms) tolerance of 1.5 Å. Analysis of the binding mode, calculation of the binding energy, and prediction of the binding activity of docked conformations were carried out using Autodock plugin within PyMol software.[51]

4.11. Physico-chemical predictions

All physico-chemical parameters included in Table 2 were calculated from chemical structures of compounds **18**–**26**, **31**, and **32** with calculator plugins within the framework of MarvinSketch v6.2.2 (Chemaxon Ltd., http://www.chemaxon.com).

Acknowledgements

This work has received financial support from the Regione Toscana regional council through POR CRO FSE 2007-2013, UNIFI_FSE2012, and MIUR PRIN2010-2011 cod. 2010NRREPL funding actions.

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Please cite this article in press as: Fabbrizzi, P.; et al., Tetrahedron (2014), http://dx.doi.org/10.1016/j.tet.2014.06.125