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Synthesis and initial pharmacology of dual-targeting ligands for putative complexes of integrin αVβ3 and PAR2†

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Unpublished data from our labs led us to hypothesize that activated protein C (aPC) may initiate an anti-inflammatory signal in endothelial cells by modulating both the integrin αVβ3 and protease-activated receptor 2 (PAR2), which may exist in close proximity on the cellular surface. To test this hypothesis and to probe the possible inflammation-related pathway, we designed and synthesized dual-targeting ligands composed of modified versions of two αVβ3 ligands and two agonists of PAR2. These novel ligands were connected *via* copper-catalyzed alkyne–azide cycloadditions with polyethylene glycol (PEG) spacers of variable length. Initial *in vitro* pharmacology with EA.hy926 and HUVEC endothelial cells indicated that these ligands are effective binders of αVβ3 and potent agonists of PAR2. These were also used in preliminary studies investigating their effects on PAR2 signaling in the presence of inflammatory agents, and represent the first examples of ligands targeting both PARs and integrins, though concurrent binding to αVβ3 and PAR2 has not yet been demonstrated.

Introduction

Protease-activated receptors (PARs) are an important family of G-protein coupled receptors (GPCRs) that possess their own agonists: a tethered ligand at the N-terminus that is activated upon cleavage by certain extracellular proteases.[1] The subtype PAR2 has been implicated in a range of inflammatory processes,[2] and PAR2 antagonists[3] have been investigated in cellular and animal models of inflammation, including examples related to colitis, arthritis, obesity, and pain,[4–8] as well as for cancer metastasis.[4] However, PAR2 ligands have yet to reach clinical stages, and it is unclear if they can be safely utilized without adversely affecting normal PAR2 signaling in a range of tissues. One approach could involve the use of biased PAR2 ligands that may more selectively inhibit pathological signaling.[5–8] The now well-established concept of biased GPCR signaling[9–12] suggests that the physiologic outcomes of PAR2 activation likewise depend on the cellular context (cell type), agonist/ligand-specific mode of activation, heterodimerization with other members of the PAR family,[13,14] and on the interaction of PARs with protease-specific co-receptors such as tissue factor or the endothelial cell protein C receptor.[15] A notable example of such cell- and context-specific PAR signaling outcomes has been described in animals subjected to experimental models of septic inflammation, where disease stage-specific protective effects of PAR1 signaling required transactivation of PAR2 on endothelial cells.[16] We reasoned that tissue and/or context-selectivity for PAR2 signal modulation could be facilitated by targeting putative heteromeric complexes of PAR2, especially with increasing evidence for the existence of complexes of PARs with other PARs or alternative receptors.[13,14] We hypothesize that these complexes can be targeted with heterobivalent ligands composed of scaffolds established to bind to the individual targets, connected by a suitable spacer.[17–19] We recently disclosed our first efforts in this area targeting putative PAR1/PAR2 heteromers.[20]

Recently, preliminary studies from our labs measuring the effects of several cytotoxins on both immune and endothelial cells suggested that the integrin receptor αVβ3 and PAR2 may play synergistic roles in mediating the anti-inflammatory action of activated protein C (aPC), a serine protease that is a central player in the coagulation cascade.[21] aPC acts as an

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† Electronic supplementary information (ESI) available: Additional assay data (Fig. S1 and S2); assay protocols; synthetic protocols; compound characterization data (^{1}H NMR, ^{13}C NMR, and LC-MS traces) (PDF). See DOI: 10.1039/d0md00098a

anticoagulant by downregulating the activation of thrombin, but it has also demonstrated a range of potent anti-inflammatory and cytoprotective effects, such that it was marketed for the treatment of sepsis prior to its withdrawal.[22] Its effects can be mediated by both PAR1 and PAR2, among numerous targets. Under the assumption that αVβ3 and PAR2 may both be activated by aPC and are both in close proximity on the cell surface, we reasoned that it could be possible to selectively recapitulate the anti-inflammatory effects of aPC by targeting both αVβ3 and PAR2 with a heterobivalent ligand (Fig. 1). Such ligands may also permit their delivery to specific cellular targets, such as αVβ3-expressing vascular endothelial cells. This manuscript describes the design and synthesis of the first examples of such ligands utilizing PAR2 agonists, along with our early efforts to study their effects in endothelial cells.

Materials and methods

The ESI† contains all assay protocols, synthetic protocols, and select characterization data (^{1}H NMR, ^{13}C NMR, and LC-MS traces).

Results and discussion

Design of bivalent ligands

In order to engage each of the target receptors with a bivalent ligand, it is critical to identify scaffolds that are suitably modifiable. Ideal scaffolds should possess structure–activity relationship (SAR) data and/or structural information that indicate where an "adapter" can be added for connection to a spacer to bridge the gap between the receptors. Several scaffolds have been identified in ligands targeting either αVβ3 or PAR2.

Integrins are receptors that mediate cell adhesion, and their role in blood clotting, angiogenesis, bone remodeling, and immune responses has prompted the investigation of their ligands for the prevention or treatment of thrombosis, cancer, osteoporosis, and inflammatory diseases.[24–26] Many of the integrin ligands that have been developed mimic the conserved RGD binding domain of natural protein ligands such as fibronectin, fibrinogen, and vitronectin. We chose to focus on two such αVβ3 ligands developed by Kessler and coworkers,[27] the macrocyclic RGD-containing peptide cilengitide (**1**, Fig. 2 and 3),[28,29] and the β-amido acid **2** (Fig. 3).[30] Cilengitide is notable for being the first anti-angiogenic drug candidate (which progressed to phase 2 clinical trials for glioblastoma), and **2** is noteworthy for its high potency and excellent selectivity *versus* α5β1. We also chose cilengitide for the insight provided by its X-ray structure in complex with the extracellular segment of αVβ3.[23]

Inspection of the αVβ3–cilengitide structure (Fig. 2) showed that the valine residue of cilengitide is particularly solvent exposed, and we reasoned that this would be an ideal site for inclusion of the adapter required for spacer attachment (*vide infra*). Docking[31] of the peptidomimetic ligand **2** to αVβ3 (Fig. 3) showed the expected binding of the carboxylic acid of **2** to the manganese of the metal ion-dependent adhesion site (MIDAS), and the ligand overlaid nicely with the X-ray structure of cilengitide bound to the same site. The isopropoxy and methoxy groups of **2** are reasonably solvent exposed and could represent suitable adapter positions for bivalent ligands. Kiessling reported a similar strategy for the design of bifunctional molecules targeting αVβ3 and anti-α-galactosyl antibodies;[32] other bifunctional cyclic RGD ligands connected to various anticancer drugs[33] and labels[34–42] have also been reported.

The reported PAR2 agonists are variations of PAR2 tethered ligand peptides which are connected to the receptor at the C-termini. Therefore, it stands to reason that it should be possible to attach a spacer at the C-termini of these ligands. Fatty acid (palmitoyl)-based membrane anchors have been attached to PAR2 agonist peptides at sites proximal to the C-termini, and these have been reported by Boitano to be highly potent PAR2 agonists, in fact up to 200-fold higher

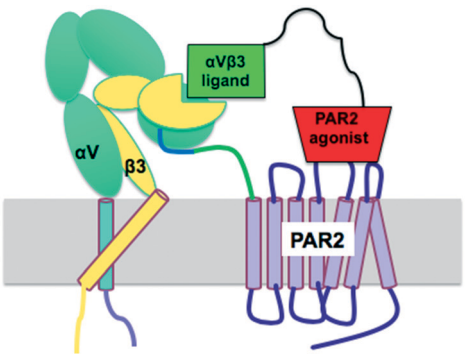


Fig. 1 Design of αVβ3–PAR2 bivalent ligands targeted to putative receptor complexes.

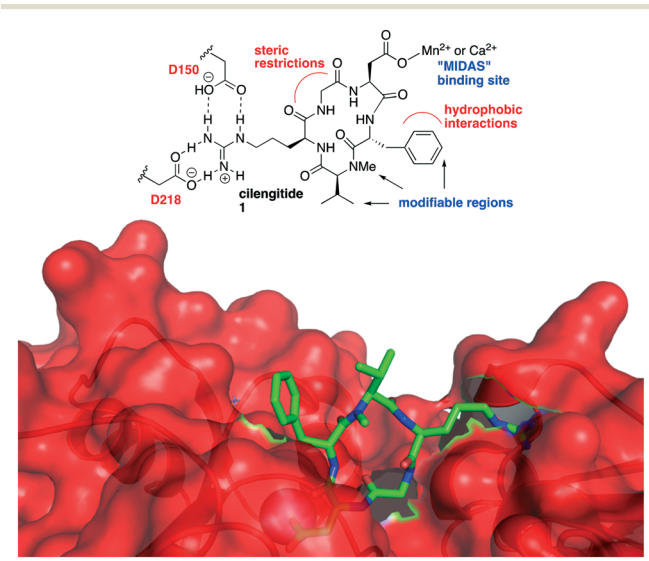


Fig. 2 X-ray structure of cilengitide bound to αVβ3.[23] Solvent accessible surface is colored red, rendered using MacPyMOL v.1.8.6.

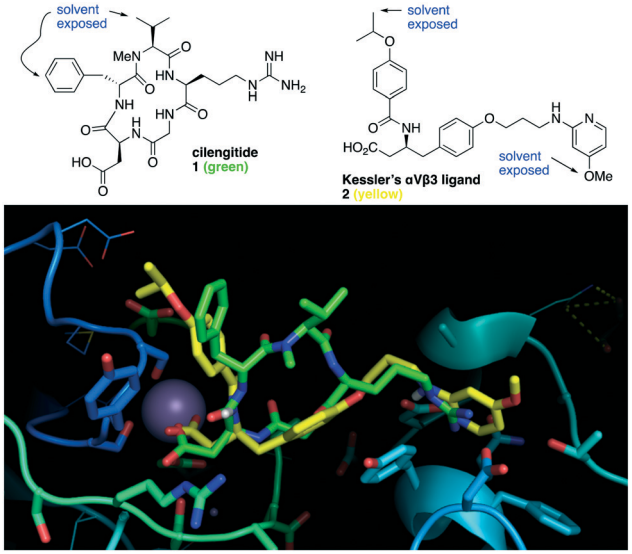
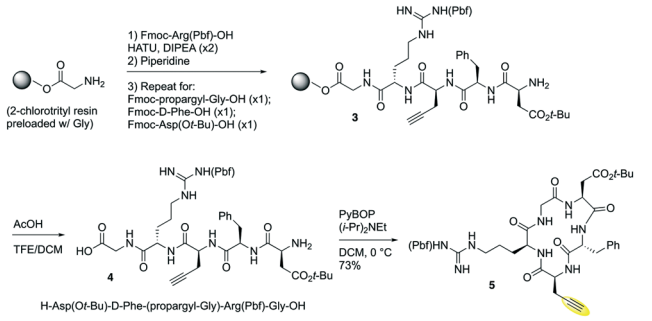


Fig. 3 Docked structure of Kessler's αVβ3 ligand (yellow) overlaid on the X-ray structure of αVβ3–cilengitide (green). The manganese ion of the binding site is shown as a gray sphere. Docking was performed with FITTED[31] and results visualized using MacPyMOL v.1.8.6.

than the parent peptides.[43] Very recently and subsequent to our development of the ligands reported here, researchers at AstraZeneca reported a model for the binding of the PAR2 agonist peptide SLIGKV to PAR2,[44] using as a starting point the X-ray structure of a modified version of PAR2 complexed with the antagonist AZ8838.[45] These results all suggested that it should be feasible to attach a spacer to the C-termini of peptidic PAR2 agonists without losing activity to a punitive degree.

Synthesis of αVβ3 ligands

We proceeded to synthesize an analog of cilengitide with the valine residue replaced by propargyl glycine. We omitted the proximal *N*-methyl group for synthetic convenience, which is not strictly required for decent binding to αVβ3, but improves selectivity and presumably stability.[29] The propargyl group, which we expect to be solvent-exposed in the ligand-bound complex, permits convenient, late stage connection to azide-containing spacers using copper-catalyzed alkyne–azide



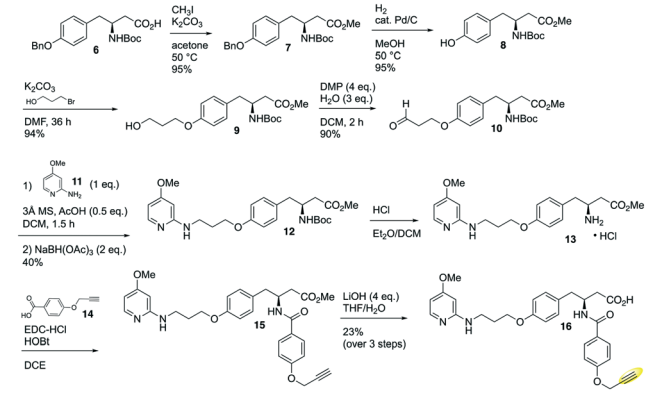
Scheme 1 Synthesis of alkynyl-cilengitide analog **5**.

cycloadditions (CuAAC) (Scheme 1).[46,47] This was prepared starting with a solid-phase peptide synthesis (SPPS) using an automated microwave peptide synthesizer, though Yamada and Shimizu report an alternative protocol optimized for cyclic RGD peptides.[48] A chlorotrityl resin, preloaded with glycine, was coupled sequentially with the Fmoc-protected amino acids Pbf-arginine,[49] propargyl-glycine, D-phenylalanine, and *t*-Bu-aspartate, using COMU® as the coupling reagent.[50] The peptide was cleaved from the resin using acetic acid in 2,2,2-trifluoroethanol/DCM, leaving the Pbf and *t*-Bu protecting groups intact. The resulting linear peptide **4** was cyclized with PyBOP at 0 °C to yield the protected macrocycle **5** with alkyne adapter ready for conjugation.

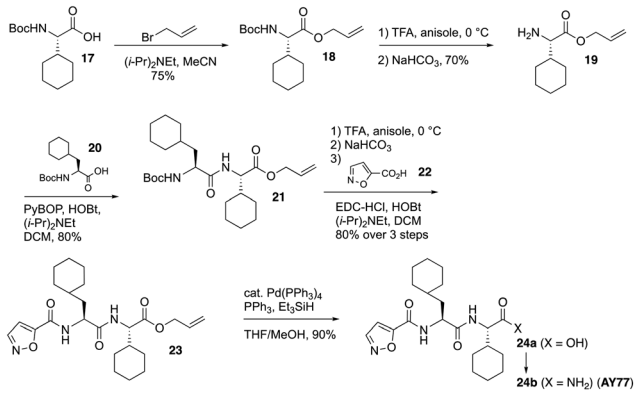
Additionally, we elected to replace the isoproxy group of the peptidomimetic αVβ3 ligand **2** with a suitable alkyne for connection to spacers (Scheme 2). The commercially available protected β-amino acid **6** was converted to methyl ester **7**, then the phenol was deprotected *via* hydrogenolysis to give phenol **8**. Following Kessler's reported synthesis of **2**,[30] this was alkylated with 3-bromo-1-pentanol to give alcohol **9**, which was oxidized with Dess–Martin periodinane (DMP) (providing aldehyde **10**) and subjected to a reductive amination reaction with 2-amino-4-methoxypyridine (**11**) to give amine **12**. Removal of the Boc group with HCl, followed by amide coupling with the benzoic acid **14** yielded amide **15**, which was hydrolyzed to yield the desired acid **16**, possessing a pendant alkyne.

Synthesis of PAR2 ligands

We selected several reported PAR2 peptide agonists of varying lengths to be included in our set of ligands. It should be noted that ligands with variable PAR2 signal biases have been reported; for example, Hollenberg reported peptides that could activate intracellular calcium mobilization without activating mitogen-activated protein kinase (MAPK) in human embryonic kidney (HEK) cells, and peptides that activated both.[5] More recently, Fairlie reported peptides with varying biases for PAR2-driven calcium mobilization and ERK1/2 phosphorylation.[7]



Scheme 2 Synthesis of alkynyl-modified Kessler ligand **15**.



Scheme 3 Synthesis of the acid derivative of the PAR2 agonist AY77.

AY77 is a dipeptide with C-terminal amide reported by Fairlie to be a potent PAR2 agonist for calcium mobilization (EC_{50} = 33 nM in human PAR2-expressing CHO cells).[51] We selected it as our smallest PAR2 agonist that may be suitable for conjugation, and with its relatively simple structure, larger quantities would be easily accessible. We synthesized it as the C-terminal acid *via* solution phase (Scheme 3), rather than with the solid phase protocol reported for the preparation of the C-terminal amide. The acid moiety of *N*-Boc cyclohexylglycine **17** was protected *via* allylation, then *N*-deprotected and coupled with cyclohexylalanine **20** to generate the protected dipeptide **21**. The Boc group was removed and the amine was coupled with isoxazole-acid **22**, then the allyl group was removed with catalytic palladium to yield the desired acid **24a**.

Longer PAR2 agonist peptides were prepared *via* SPPS analogously to **5**, using chlorotrityl resin, automated microwave reactor, and cleaving the peptides from the resin with protected side chains intact (Fig. 4). In this manner, the protected and C-terminal acid version (**25b**) of the potent and

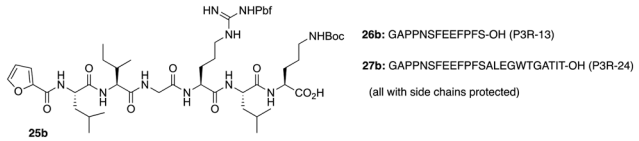
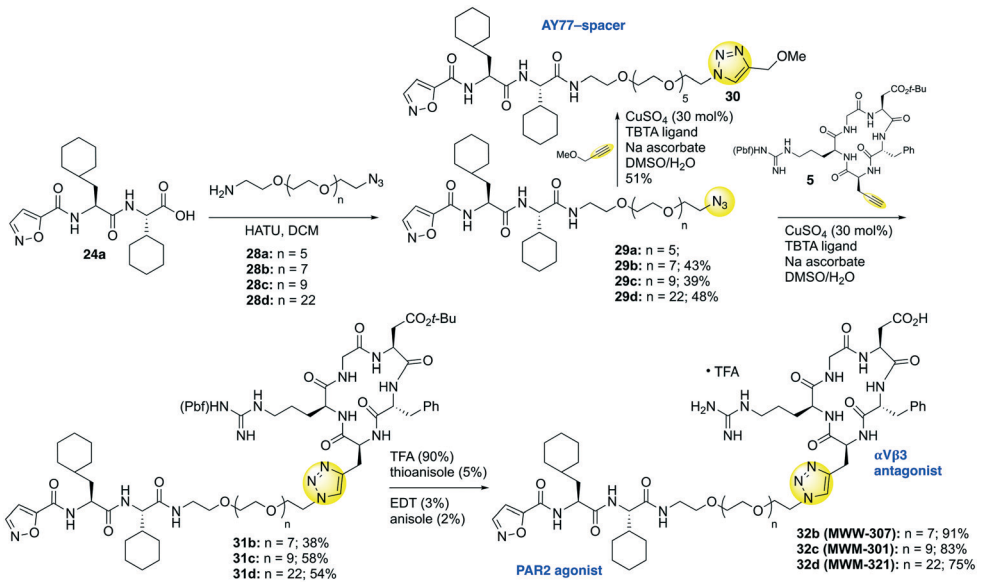


Fig. 4 PAR2 peptide agonists synthesized *via* SPPS. All side chains were kept protected prior to conjugation steps.

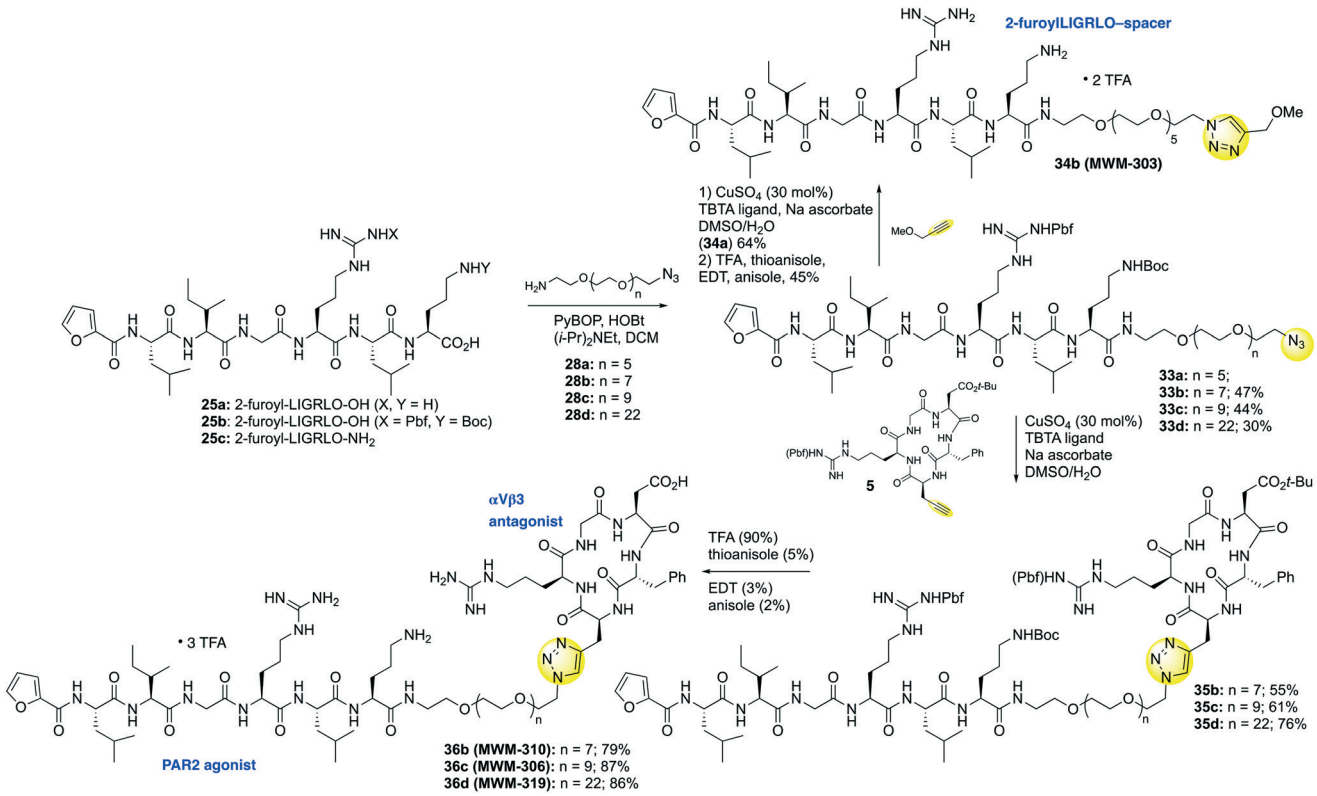
widely used PAR2 agonist hexapeptide reported by McGuire (termed 2-furoyl-LIGRLO-NH_2) was synthesized.[52] Finally, the protected 13-mer **26** (termed P3R-13) and 24-mer **27** (termed P3R-24) were synthesized, which correspond to the N-terminal 13 and 24 residue tethered peptides of aPC-cleaved PAR3. These peptides were reported by Mosnier to promote barrier protection in endothelium,[53] and prior work from Hansen and coworkers suggested that related PAR3 tethered ligands could activate either PAR1 or PAR2 in Jurkat T-cells.[54]

Assembly of dual-targeting ligands

Synthesis of the dual-targeting ligands and their respective monovalent spacer-linked controls proceeded according to the protocols outlined in Schemes 4 to 6. The carboxylic acid (**24a**) corresponding to AY77 (**24b**) was coupled using HATU to commercially available amino-PEG–azide spacers (**28a–d**) of four widely different lengths (termed informally as short, medium, long, and extra-long). These spacers are 24, 30, 36, and 75 atoms long; as a comparison, optimal bivalent ligands for GPCR heteromers have been reported by Portoghese to have 16 to 22 atom spacers for mu opioid/cholecystokinin 2 receptor heteromers,[55] and by Gmeiner to have 88 atom spacers for bivalent ligands bridging dopamine 2/neurotensin S_1 receptor heteromers.[56] The resulting amides **29a–d** were subjected to CuAAC reaction with the protected alkynyl-



Scheme 4 Assembly of dual-targeting ligands derived from the cilengitide analog and AY77.



Scheme 5 Assembly of dual-targeting ligands derived from the cilengitide analog and 2-furoyl-LIGRLO-NH_2.

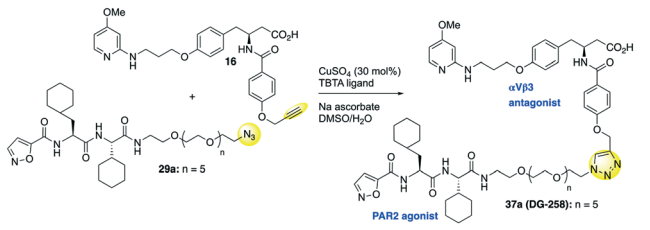
cilengitide analog **5**, generating the triazoles **31b–d**, respectively. Improved results were obtained when using the ligand TBTA reported by Fokin.[57] Global deprotection with a cocktail of TFA and carbocation scavengers generated the dual-targeting ligands **32b–d**. Alternatively, the azide **29a** was subjected to CuAAC with methyl propargyl ether to generate the control compound **30**, composed of the PAR2 agonist with a triazole-capped spacer. All final compounds and intermediates in Schemes 4 to 6 were purified *via* preparative HPLC.

In a similar fashion, the protected McGuire peptide **25b** was used to generate cilengitide analog-conjugated dual-targeting ligands **36b–d**, as well as the spacer-linked control compound **34b** (Scheme 5). We were unsuccessful in generating in pure form several ligands derived from the longer peptides P3R13 (**26**) and P3R24 (**27**), as the protected versions of these suffered from extremely low solubility after coupling reactions, and the products were not successfully isolated after normal- or reverse-phase chromatography.

Finally, the alkynylated analog of Kessler's peptidomimetic (**16**) was subjected to CuAAC with several PAR2 agonists, including AY77 (Scheme 6). Compound **37a** is the first dual-targeting ligand we prepared using **16**, with our early pharmacology data reported here.

Integrin binding assays

To confirm that our modifications to the integrin ligands were well tolerated, we performed two preliminary binding assays. First, the binding of Alexa Fluor 647-labeled fibronectin to HEK293FT cells transfected with αVβ3 was measured in the presence of several dual-targeting ligands (Fig. 5A). Cells expressing αVβ3 were also treated with an antibody to β3 integrin (AlexaFluor 488-labeled AP3), which was used to sort cells *via* flow cytometer, prior to fluorescence measurement. Both of the cilengitide-based ligands **32d** (MWM-321) and **36d** (MWM-319) substantially inhibited fibronectin (Fn) binding at a concentration of 5 μM, at a similar level to the established integrin binder cilengitide (cRGD). Though concentration–response curves have not yet been generated, these early results confirm that the attachment of the spacer to the cilengitide analog does not appear to adversely impact its binding to αVβ3. Since our focus is on endothelial cells, we repeated this experiment with vitronectin coated plates and HUVEC, since HUVEC also express fibronectin-binding α5β1. Cell binding was qualitatively inhibited by **32d**, **36d**, and **37a** (DG-258) (Fig. 5B).



Scheme 6 Assembly of dual-targeting ligands derived from Kessler's ligand and AY77.

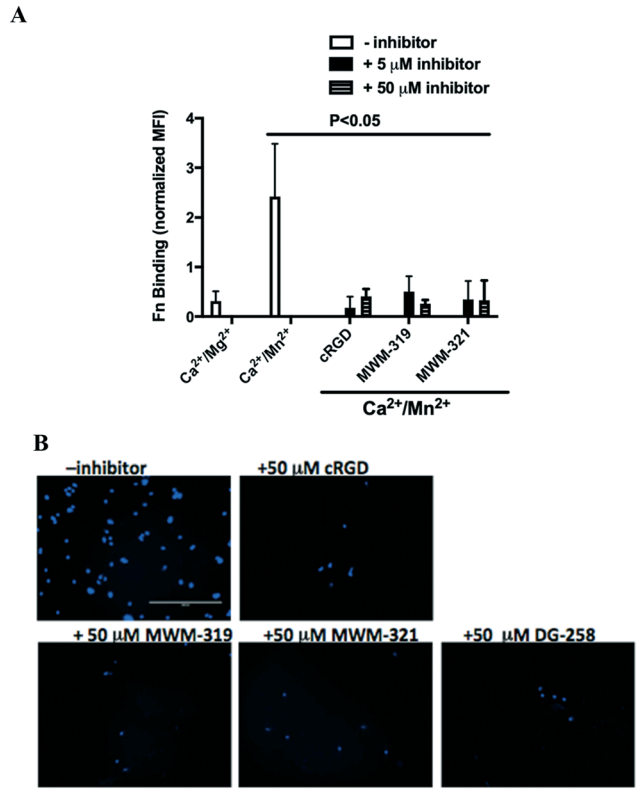


Fig. 5 A) Inhibition of AlexaFluor 647-labeled fibronectin (Fn) binding to αVβ3-containing HEK293 cells was measured in the presence of the ligands cilengitide (cRGD), **33d** (MWM-321) and **36d** (MWM-319). The binding was measured in buffer containing 1 mM $CaCl_2$ plus 1 mM $MgCl_2$ (Ca^{2+}/Mg^{2+}) or 0.2 mM $CaCl_2$ plus 2 mM $MnCl_2$ (Ca^{2+}/Mn^{2+}). Data are mean ± SEM (n = 4). Two-tailed t-test was used to compare the Ca^{2+}/Mn^{2+} condition with inhibitors and without inhibitors. MFI = mean fluorescence intensity. B) Binding of HUVEC (stained with DAPI, 5 μg mL^{-1}) to vitronectin-coated plates, and inhibition of binding by 50 μM cRGD, MWM-319, MWM-321, and DG-258. See ESI† for details. Scale bar = 400 μm.

Calcium mobilization assays

Next, we studied the ability of our dual-targeting ligands to activate PAR2 by measuring Gq-driven calcium mobilization in adherent endothelial cells, according to our reported plate reader protocol using the calcium sensing dye Fluo-4/AM.[58]

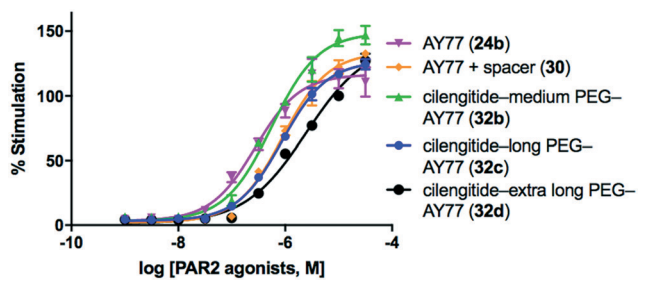


Fig. 6 Stimulation of calcium mobilization in EA.hy926 cells by cilengitide analog–AY77 PAR2 agonists. Error bars indicate SEM ($n \geq 3$). % stimulation values were normalized to the response with 100 μM SLIGKV-NH_2 (100%).

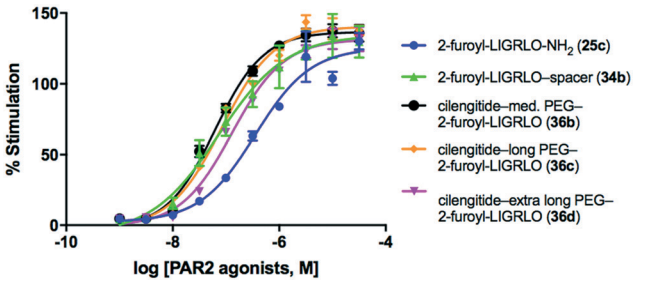


Fig. 7 Stimulation of calcium mobilization in EA.hy926 cells by cilengitide analog–McGuire peptide PAR2 agonists. Error bars indicate SEM ($n \geq 3$). % stimulation values were normalized to the response with 100 μM SLIGKV-NH_2 (100%).

The release of calcium from the endoplasmic reticulum in response to Gq signaling leads to diverse effects in different cell types, and this calcium mobilization is easily quantified using calcium binding dyes that can be absorbed into cells. Such assays are widely used to characterized GPCR activation, in this case *via* Gq. All curves were fitted using data from $n \geq 3$ measurements on a single 96 well plate, with the error bars indicating the standard error of the mean (SEM). The cilengitide analog–AY77-based compounds from Scheme 4 were studied first in EA.hy926 cells (Fig. 6). The control compound composed of AY77 with attached PEG spacer (**30**) had only moderately decreased potency (EC_{50} = 0.89 μM) relative to AY77 (**24b**, EC_{50} = 0.27 μM), with similar efficacy. The ligand with the medium length spacer (**32b**) provided the best results with comparable potency to AY77 (EC_{50} = 0.56 μM), with significantly increased efficacy. The ligands with the long (EC_{50} = 0.88 μM) and extra-long (EC_{50} = 2.5 μM) spacers possessed lower potencies in this assay.

Concentration–response curves generated with the dual-targeting ligands containing the McGuire peptide (2-furoyl-LIGRLO-NH_2) are shown in Fig. 7. Interestingly, all of the spacer-linked agonists were more potent than the parent compound **25c** (EC_{50} = 0.35 μM). The potencies were similar for the spacer-linked control **34b** (EC_{50} = 0.073 μM) and the medium, long, and extra-long ligands **36b**, **36c**, and **36d** (EC_{50} = 0.062, 0.084, and 0.13 μM, respectively). Again, the dual-targeting ligand with the medium length PEG spacer (28 atoms, including one nitrogen at each end) was optimal. We speculate that the longer 2-furoyl-LIGRLO-NH_2 likely has its

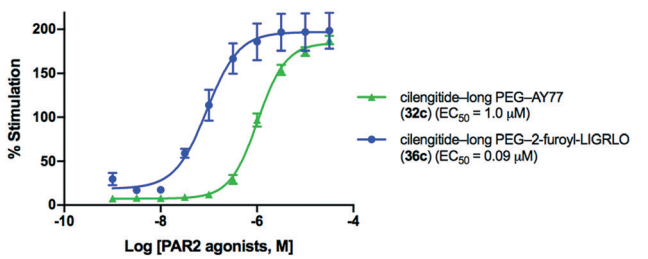


Fig. 8 Stimulation of calcium mobilization in HUVEC by dual-targeting PAR2 agonists **32c** and **36c**. % stimulation values were normalized to the response with 100 μM SLIGKV-NH_2 (100%).

C-terminus closer to the mouth of the receptor than AY77, and is thus better able to tolerate the addition of the PEG-based spacer.

Several dual-targeting ligands were also tested in cultured human umbilical vein endothelial cells (HUVEC) (Fig. 8). The results were very similar compared to the EA.hy926 cells, with the ligand derived from the McGuire peptide (**36c**, EC_{50} = 0.09 μM) approximately 10-fold more potent than the analogous ligand with AY77 (**32c**, EC_{50} = 1.0 μM).

We did not observe any significant increase in potency for the dual-targeting ligands over the monovalent controls, which would support the existence of αVβ3–PAR2 complexes, or at least the two receptors residing within the span of the longest ligands (possessing 72 atom spacers). Nonetheless, we performed competition studies to test for bivalency by adding a competing monovalent ligand. If a dual-targeting ligand shows increased binding affinity and activity due to concurrent interactions with two receptors (*e.g.* within a heteromeric complex), we would expect binding and activity to decrease upon addition of a competing monovalent ligand. In our studies, the activities of our dual-targeting ligands at PAR2 were measured (*via* calcium mobilization) in the presence of the αVβ3 ligand cilengitide (Fig. 9). No decrease in potency of the dual-targeting ligand **32d** (composed of the cilengitide analog and AY77, joined by the extra-long PEG spacer) was observed in EA.hy926 cells upon addition of 10 μM cilengitide, although a decrease in efficacy was observed at the highest concentrations.

We reasoned that putative integrin–PAR complexes may only emerge under conditions of cellular stress, and evidence suggests that PAR2 plays an important role in the inflammatory responses to lipopolysaccharide (LPS),[59] the bacterial cell membrane component.[60] Concentration–response curves with EA.hy926 cells were measured with **32d** after a 2 h pretreatment with 10 μg mL^{-1} LPS. No significant differences in potency were noted, with or without the addition of cilengitide (Fig. 9). Similar results were obtained with the dual-targeting ligand derived from the McGuire peptide (**36d**, Fig. S1†).

We also did not observe evidence of bivalency or increased potency after pretreatment with LPS using the dual-targeting ligand derived from Kessler's peptidomimetic αVβ3 inhibitor and the PAR2 agonist AY77 (**37a**, DG-258). In these cases, there was no statistical difference in potency (EC_{50} = 1.2 μM for **37a** alone) with or without the addition of 10 μM cilengitide, and with or without pretreatment for 2 h with 10 μg mL^{-1} LPS (Fig. S2†).

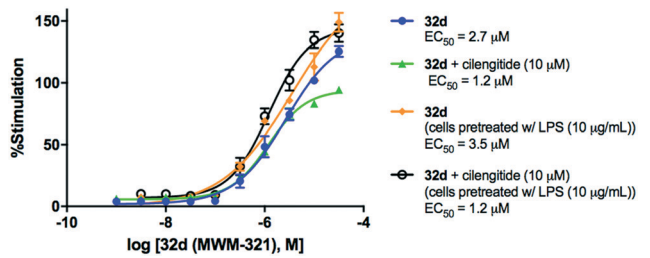


Fig. 9 Stimulation of calcium mobilization in EA.hy926 cells pretreated with LPS by dual-targeting PAR2 agonist **32d** (MWM-321), with and without cilengitide as a competing ligand. Cells were pretreated with 10 μg mL^{-1} LPS for 2 h prior to addition of ligand(s). % stimulation values were normalized to the response with 100 μM SLIGKV-NH_2 (100%).

Conclusions

We have reported a toolbox of bifunctional molecules designed to target both integrins and PARs. Consistent with the results of Kiessling[32] and others, known αVβ3 ligands could be modified with the attachment of spacers without precluding integrin binding. Similarly, inspired by the prior results of Boitano,[43] PAR2 agonists could be successfully modified by attachment of spacers without decreases in potency for the activation of calcium mobilization in endothelial cells. In preliminary experiments, we did not observe obvious changes in concentration–responses in cultured endothelial cells that would be diagnostic of the presence of a population of putative αVβ3–PAR2 complexes that could be selectively activated by our dual-targeting ligands. However, investigations in cellular models of inflammation are pending with these and related dual-targeting ligands in various cell types. These compounds have the potential to characterize and modify signaling unique to specific cell surface receptor complexes, and these or related ligands may have diagnostic and therapeutic applications in the future, including integrin-mediated delivery of PAR2 ligands to specific tissues or cell types.

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Notes

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Abbreviations

Boc	*tert*-Butoxycarbonyl
cRGD	Cilengitide
COMU	(1-Cyano-2-ethoxy-2-oxoethylidenaminooxy) dimethylamino-morpholino-carbenium hexafluorophosphate
CuAAc	Copper-catalyzed alkyne/azide cycloadditions
DAPI	4′,6-Diamidino-2-phenylindole
DCE	1,2-Dichloroethane

DCM	Dichloromethane
DIEA	*N*,*N*-Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DPPF	1,1′-Bis(diphenylphosphino)ferrocene
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
FDA	U.S. Food and Drug Administration
GPCR	G-protein coupled receptor
Fmoc	Fluorenylmethyloxycarbonyl
HATU	Hexafluorophosphate azabenzotriazole tetramethyl uronium
HOBt	1-Hydroxybenzotriazole
HUVEC	Human umbilical vein endothelial cells
IC_{50}	Half-maximal inhibitory concentration
iCa^{2+}	Intracellular calcium mobilization
MIDAS	Metal ion-dependent adhesion site
PAR	Protease-activated receptor
Pbf	2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl
PyBOP	(Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
SEM	Standard error of the mean
SPPS	Solid phase peptide synthesis
TBTA	Tris(benzyltriazolylmethyl)amine

Author contributions

Conceived the project: C. D., H. W. Designed compounds: C. D. Designed synthetic routes: C. D., M. W. M., D. M. G. Synthesized and characterized compounds: M. W. M., D. M. G., T. H., R. R. Cultured cells: I. H., M. W. M., D. M. G., R. R. Performed assays: M. W. M., R. R., Z. W. Analyzed data: M. W. M., C. D., Z. W., J. Z., H. W. Wrote the manuscript: M. W. M., C. D. Prepared ESI:† M. W. M., D. M. G., R. R., C. D., Z. W., J. Z.

Conflicts of interest

There are no conflicts of interest to declare.

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