Journal of Medicinal Chemistry

PERSPECTIVE

pubs.acs.org/jmc

Tripeptide Motifs in Biology: Targets for Peptidomimetic Design

Phuc Ung[†,‡] and David A. Winkler[*,†,‡]

[†]CSIRO Materials Science and Engineering, Bag 10, Clayton South MDC 3169, Australia

[‡]Monash Institute of Pharmaceutical Science, Parkville 3152, Australia

INTRODUCTION

The concept that nature tends to reuse motifs and structural components is commonly understood. In the case of proteins and peptides, common structural motifs such as helices, sheets, loops, etc. are well-known, and the concept that specific recognition events occur between amino acids in endogenous ligands and their native receptors forms a fundamental tenet of structural biology and drug design. Many of these amino acid recognition events involve relatively large numbers of interactions, but given the economy of nature, it is interesting to speculate what the smallest information-bearing motif may be.

Our reading of the literature has indicated that motifs as small as three contiguous amino acids play important roles in biology. While longer sequences are also undoubtedly important, we focus this review on tripeptide motifs to illustrate the concept of conserved contiguous motifs. Clearly not all 8000 possible tripeptides are likely to have biological importance, but our review suggests that a substantial number of them do play significant roles. As we will illustrate, endogenous tripeptides clearly have important signaling roles in biology, lending credence to the contention that motifs as small as three amino acids are indeed important and capable of valuable function. However, contiguous tripeptide sequence embedded in larger peptides and proteins also have useful signaling properties. The minimal useful length may be a product of the number of effective molecular interactions that is required between a ligand and protein receptor to have useful efficacy. Some evidence for optimality of a three-residue motif has been provided by the studies of Reynolds et al. and of Neduva and Russell. In the former work,[1] the authors proposed that 25 heavy atoms (HA) gave optimal ligand affinity or "maximal efficacy". Given that the average number of heavy (non-hydrogen) atoms in the natural amino acids is 8.3, three residues would on average contribute 25 heavy atoms. Figure 1 illustrates the distribution of heavy atoms across all 8000 possible tripeptides showing the peak of the distribution close to 25 heavy atoms.

Figure 2 shows how maximal ligand affinity varies as a function of ligand size (number of heavy atoms). The affinity curve peaks for 20 to 35 heavy atoms, and the ligand efficacy curve is roughly bell shaped, with efficacy falling for ligands with more than 40 heavy atoms (Figure 2).

Neduva and Russell[2] used informatic methods to study short linear motifs (usually shorter than 10 residues) likely to participate in protein interactions, localization, and posttranslational modifications in many biological processes.[2] They summarized the properties of previously determined linear peptide motifs between four and eight residues in length, which have two to four specified (i.e. non-"x", where x is any amino acid) positions, of which one to three are a single invariant amino acid (Figure 3). This analysis showed that motif length for known short motifs peaked at four residues. When the residues were specified, the peak was at three residues, and for invariant positions (a single specific residue in the motif), at one to two residues.

Hann et al.[3] also provide indirect evidence for efficacy of ligands having around 25 heavy atoms. Their work showed that in druglike libraries and the World Drug Index, the heavy atom ADEPT (a Daylight enumeration and profiling tool)[4] profile (histograms of calculated molecular properties) peaks at 25 heavy atoms. Leadlike libraries peak at slightly lower numbers of heavy atoms. As many drugs mimic biological signals, this over-representation of drugs with 25 heavy atoms is consistent with peptide motifs around three amino acids in length having biological relevance.

A relatively small number of papers have analyzed the knowledge base of proteins to show that tripeptide peptide motifs have nonrandom frequencies. Anishetty and co-workers have been the most active at investigating the frequency of tripeptides in the known protein sequence space. They employed informatic methods to investigate the distribution and structural flexibility of tripeptides and related these to protein stability.[5−7] Several researchers, notably Brooks et al., have studied the nonrandom distribution of peptide motifs in terms of evolution and selection pressure.[8] Even more relevant is the analysis by Gatto and Berg who analyzed the frequency of occurrence of C-terminal tripeptides in archaeal, bacterial, and eukaryotic genomes.[9] The sequence distribution in prokaryotes was essentially random. In contrast, eukaryotes contain large numbers of overrepresented sequences, some representing previously known targeting signals, but some have not been previously noted that represent novel functional sequences. Otaki et al. analyzed all 8000 possible tripeptide motifs and found that a substantial number of triplets (around 20%) are present more frequently than expected by chance and others are underrepresented.[10] The tripeptide frequency distribution was highly skewed in the positive direction, and only about 20% of triplet species occur completely randomly.

These reports have discussed the role of small peptide motifs in stabilizing proteins, in protein evolution, and in generating secondary structure, but none have explored the role of small motifs as biological signals that could be exploited in drug discovery. As biologists and medicinal chemists have only sparsely sampled the sequence space for tripeptides and biological modulators, it is likely that many more useful and important tripeptide motifs will be discovered. Surprisingly, no reviews of this type have been reported previously, although a paper that appeared as we were finalizing this review also discusses the peptide motifs in the

Received: October 8, 2010
Published: January 28, 2011

dx.doi.org/10.1021/jm1012984 | *J. Med. Chem.* 2011, 54, 1111–1125

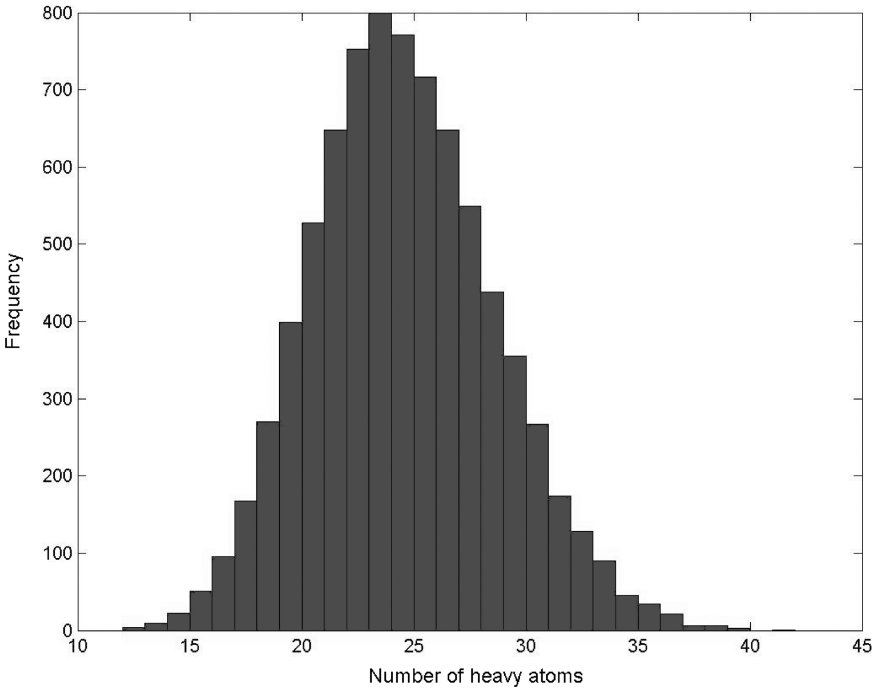

Figure 1. Distribution of heavy atoms in all 8000 possible tripeptides.

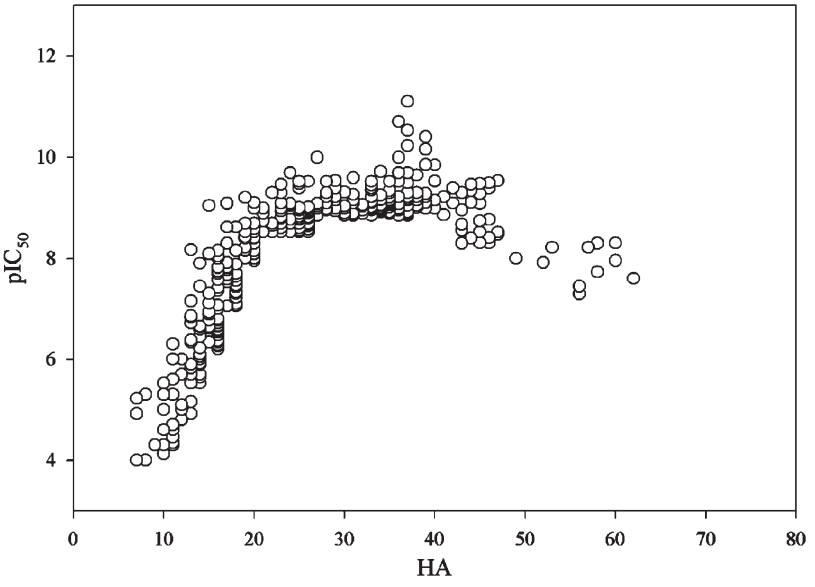

Figure 2. Ligand affinities versus size. The figure shows only the most potent ligands for a given ligand size. The change in affinity is clearly not linear with size. Reproduced from *Bioorganic & Medicinal Chemistry Letters* (http://www.sciencedirect.com/science/journal/0960894X), Vol. *17*, Reynolds, C. H.; Bembenek, S. D.; Tounge, B. A., The role of molecular size in ligand efficiency, pp 4258−4261, Copyright 2007, with permission from Elsevier.[1]

context of medical diagnosis and treatment, nanotechnology, and materials science.[11]

We conducted an extensive literature review to find examples of biologically relevant tripeptide motifs, either as endogenous peptides or as components of larger proteins. We were particularly interested in motifs that had not been mimicked by small molecules, in addition to those for which small molecule mimics had been reported. Extensively exploited tripeptides sequences, like the cell adhesion motif RGD, have been given a more cursory treatment because of the number of existing reviews in the literature.[12] Our review covers peptide motifs that occur across a wide range of biological systems and illustrates some of the small molecules used to mimic them. Many more important tripeptide motifs have yet to be discovered and exploited by peptide mimetic small molecule drugs. We propose that tripeptide motifs represent potentially important starting points for design of small molecule biological modulators. The existence of such a wide variety of tripeptide motifs in biology, together with the evidence of the preferred status of tripeptides in nature described above, suggests that three amino acids may represent an optimal if not minimal size for biological signaling.

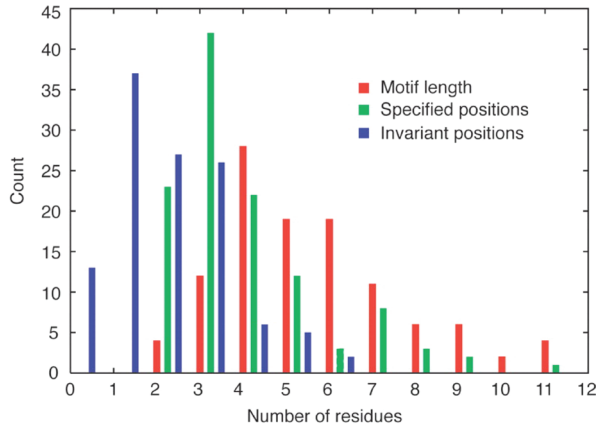

Figure 3. Distributions of length (red), number of specified, and invariant positions for 120 known linear motifs. Reproduced with permission from *Nucleic Acids Research*, Vol. *34*, Neduva, V.; Russell, R. B., DILIMOT: discovery of linear motifs in proteins, pp W350−W355, Copyright 2006, with permission from Oxford University Press.[2]

SUMMARY OF BIOLOGICALLY RELEVANT TRIPEPTIDE MOTIFS

The literature survey identified 16 important tripeptide motifs, some of which are well-known and others that have been less well studied but may have also been exploited by small molecule mimetics. Endogenous tripeptides include the following:

(1) ECG (glutathione), antioxidant, cofactor
(2) EHP, stimulates pituitary gland controlling thyroid-stimulating hormone secretion
(3) FEG, inhibition of anaphylaxis, anti-inflammatory, modulates cardiac leukocyte adhesion
(4) GHK, tissue remodeling and wound healing
(5) PLG, modulator of the dopamine D2 receptor

Tripeptide motifs in proteins include the following:

(6) DLF/SLF, inhibition of β protein of bacterial replisome, antimicrobial
(7) ELR, chemokine, growth factor binding motif
(8) GGQ, release factor, stop codon recognition
(9) GPE, neuroprotection
(10) HAV, cadherin motif, cell−cell interactions, and adhesion
(11) HGK, vitronectin inhibition
(12) HPQ, streptavidin binding motif
(13) KPV, anti-inflammatory properties
(14) LDV, vascular cell adhesion molecule 1 (VCAM-1)/fibronectin adhesion motif
(15) RGD, cell adhesion signal and modulation of thrombosis
(16) SKL, peroxisomal targeting

The following sections describe the role of each tripeptide motif and summarize attempts to design or discover small molecule mimics of the motif, where they exist.

ENDOGENOUS PEPTIDES

ECG: Glutathione (GSH), Antioxidant. Glutathione (γ-L-glutamyl-L-cysteinylglycine) is an endogenous tripeptide that can exist in the reduced state GSH or the oxidized state GSSH, resulting in antioxidant activity. Unlike other tripeptides, glutathione has an unusual peptide linkage between the amine group of cysteine and the carboxyl group of the glutamate side chain. The presence of large amounts of reactive oxygen species (ROS) in the body leads to oxidative stress which has been linked to various neurodegenerative diseases such as Parkinson's disease.[13] GSH is a major antioxidant in the brain,[14] an essential cofactor for many enzymes, a nontoxic cysteine storage utility, a major redox buffer, and a neuromodulator/neurotransmitter in the central nervous system (CNS).[13] At present the precise mechanism for the transport of GSH from the blood through to the brain is not completely clear, as GSH is believed to not be blood−brain barrier permeable.[15,16] The existence of a GSH transporter was reported by Kannan et al.[17] Therapeutic use of exogenously administered GSH is difficult, as it is rapidly metabolized and eliminated when administered orally and intravenously.[13,18] A more viable approach involves targeting the endogenous mechanisms inducing GSH synthesis as suggested in the review by Aoyama et al.[13]

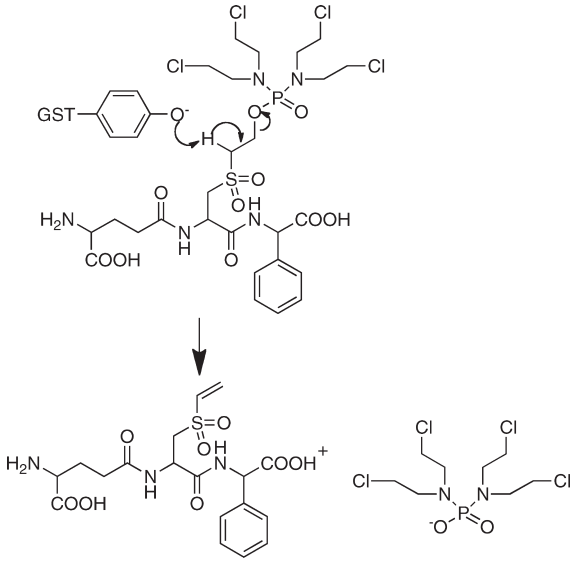

Figure 4. Mechanism by which TER286 liberates cyclophosphamide.

Overexpression of glutathione *S*-transferase (GST), particularly the P1-1 isozyme, is a characteristic of most tumor types.[19] GST plays a major role in the detoxification of certain electrophilic cytotoxins by conjugating them with the peptide scavenger GSH and thus has been suggested to lead to drug resistance.[20] However, this mechanism has been exploited in the generation of novel drug treatments for specific tumor cell types. For example, TER286 is nontoxic and inactive when intact but proton abstraction by tyrosine in the active site of GST results in the release of the sulfone−glutathione moiety and the active alkylating agent cyclophosphamide (Figure 4).[21]

EHP: Thyrotropin-Releasing Hormone (TRH), pyro-Glu-His-Pro-NH_2. TRH is an endogenous tripeptide with the sequence pyro-Glu-His-Pro-NH_2. It is the major stimulator of the pituitary gland controlling the secretion of thyroid-stimulating hormone (TSH, thyrotropin). TRH is localized throughout the brain, the CNS, the gastrointestinal tract, the pancreatic islets, and the reproductive system. There are two known thyrotropin-releasing hormone receptor (TRH-R) subtypes, both belonging to the G-protein-coupled receptor (GPCR) superfamilies TRH-R1 and TRH-R2. However, TRH-R1 is the most widely investigated subtype, as it is the only one present in humans.[22] The active conformation of the TRH tripeptide is unknown, and much effort has been put toward elucidating its binding conformation in TRH-R1. These studies include computer simulations,

dx.doi.org/10.1021/jm1012984 |*J. Med. Chem.* 2011, 54, 1111–1125

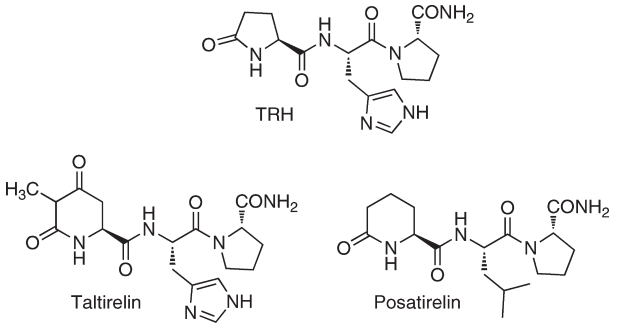

Figure 5. Structures of TRH and TRH analogues.

synthesis, and binding affinity measurement of constrained peptidomimetics to infer possible active conformations of the ligand.[23−26] The mode of binding and mechanisms of receptor activation have been thoroughly reviewed.[27] A large number of TRH analogues have been synthesized, with many of these being more potent than TRH itself.[28] Taltirelin (Figure 5) is an example of an analogue in which the N-terminal pGlu is replaced with a nonproteogenic amino acid. It is approximately 100 times more potent than TRH and has a much longer half-life. It is currently approved in Japan for the treatment of adult spinal muscular atrophy and is marketed under the trade name Ceredist.[29−31] Apart from modifications at the pGlu residue, TRH analogues with modified His, Pro-NH_2, and combinations thereof have all been explored.[28] Posatirelin (RGH-2202, Figure 5) is an example of a TRH analogue with simultaneous replacements at the pGlu and His residues. The resulting neutral molecule has enhanced CNS activity, being 5 times more potent than the parent peptide.[28] Studies have shown that posatirelin may improve cognitive and functional abilities in late onset Alzheimer's disease.[32]

TRH analogues, including peptide mutants and analogues, and small molecule mimetics also exhibit central nervous system activity.[33] They show substantial cholinergic and adrenergic responses. TRH shows cognitive, ergotrophic effects on consciousness and arousal, along with learning and memory improvements consistent with the role of acetylcholine.[34] Systemic TRH elicits motor and behavioral effects in many species, principally mediated by dopamine. Dopamine antagonists block TRH locomotor activation. TRH elevates dopamine in the cerebral cortex and increases tyrosine hydroxylase activity.[34]

FEG: Inhibitor of Intestinal Anaphylaxis. The tripeptide NH_3^+-Phe-Glu-Gly-COO- (FEG) is a potent inhibitor of intestinal anaphylaxis.[35,36] It also exhibits antihypotensive activity against anaphylactic shock[36] and anti-inflammatory activity[37,38] and regulates leukocyte adhesion in the heart.[39−42] Metwally et al. prepared a series of peptide analogues of FEG and performed 3D modeling in an attempt to elucidate structural features essential for its biological activity.[43] They identified five components of FEG that are required for its biological activity. These include aromaticity at the first residue, a carboxylic acid moiety in the second residue, restricted movement of the side chain in position 1, and free N- and C-termini.[43] Galeazzi et al. reported a synthetic protocol for the preparation of an FEG mimetic (Figure 6) based on a conformationally restricted EG dipeptide analogue.[44] On comparing the biological activity of the mimetic to the parent FEG peptide, Galeazzi et al. found a significant decrease in the ability of the mimetic to inhibit rat intestinal anaphylaxis.[44] Molecular dynamics simulations of the mimetic and

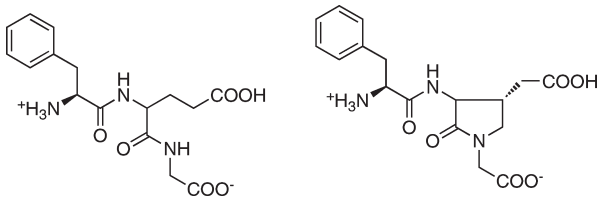

Figure 6. FEG (left) and mimetic (right).

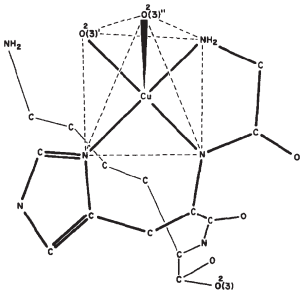

Figure 7. X-ray crystal structure of GHK coordination with a copper atom. Reproduced with permission from *Inorganica Chimica Acta* (http://www.sciencedirect.com/science/journal/00201693), Vol. *82*, Perkins, C. M.; Rose, N. J.; Weinstein, B.; Stenkamp, R. E.; Jensen, L. H.; Pickart, L., The structure of a copper complex of the growth factor glycyl-L-histidyl-L-lysine at 1.1 Å resolution, pp 93−99, Copyright 1984, with permission from Elsevier.[49]

the natural peptide showed that the conformational behavior and orientation of putative binding groups in the parent peptide was not preserved, thus explaining the decrease in activity.[44]

GHK/GHK-Cu Complex: Tissue-Remodeling Activity. The human tripeptide Gly-(L-His)-(L-Lys), GHK, has been strongly implicated in wound healing. However, the mechanism is rather complex and involves a plethora of underlying cellular processes. The tripeptide has a very high Cu^{2+} affinity, allowing it to form the complex GHK-Cu. It is generated after tissue injury by proteolytic degradation of proteins of the extracellular matrix. GHK/GHK-Cu is normally found in human plasma, saliva, and urine. Structural data for GHK-Cu in solution have been reported by Perkins et al. and in earlier publications.[45−49]

Both GHK and GHK-Cu also exhibit distinct biological actions, despite most of the literature reports relating to the copper complex (Figure 7). GHK/GHK-Cu exerts its tissue remodeling activity through a number of biochemical processes, including anti-inflammatory actions,[50] chemoattraction of healing cells,[51] enhanced nerve outgrowth,[52−54] increased stem cell proliferation and differentiation,[55] re-establishment of blood flow to damaged tissues (through angiogenesis, anticoagulation and vasodilation),[55−57] and simultaneous activation of the metalloproteinase and anti-protease production for removal of damaged proteins and protein synthesis to rebuild the extracellular matrix.[51,58] Further information may be found in the review by Pickard.[50]

Development of GHK/GHK-Cu into orally active drugs has not been a focus of researchers, as the many applications of this tripeptide involve topical use (e.g., wound healing, cosmetic remodeling) or intravenous or intramuscular injections (systemic wound healing). A novel use for GHK has recently been explored by Leblanc et al. where the high affinity for copper ions is exploited to generate a fluorescent chemosensor.[59] They realized that lysine did not contribute to the copper coordination, so

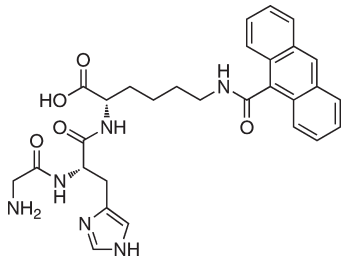

Figure 8. GHK-derivitized with anthracene for use as a chemosensor for copper ions.

they derivatized the lysyl side chain with 9-anthracenecarboxylic acid (a common fluorophore) (Figure 8). The basis for their chemosensor involves fluorescence quenching of the GHK-derived fluorophore upon binding to copper ions.

PLG: Dopaminergic Modulator. PLG (Pro-Leu-Gly-NH_2) is an endogenous tripeptide modulator of the dopamine D2 receptor in the central nervous system. It selectively enhances the responsiveness of the dopamine receptors to agonists by increasing the number of receptors in the high-affinity state and also by increasing the affinity of these receptors toward the agonist.[60,61] Its potential use in the treatment of diseases such as Parkinsonism or schizophrenia makes it an attractive drug candidate for further development. Structure−activity relationship studies on conformationally restricted PLG mimetics and X-ray analysis of PLG structure suggest that its bioactive conformation is a type II β-turn stabilized by intramolecular hydrogen bonding between the C-terminal amide NH and the proline carbonyl.[62−64] Because of the large number of peptide analogues[65−72] and peptidomimetics[63,64,73−80] of PLG that has been reported, we will not be reviewing the entire literature but will draw some key examples to illustrate the direction taken by various groups.

Johnson et al. prepared PLG analogues by exploiting a spiro bicyclic type II β-turn mimic previously suggested as the active conformation. They examined the effect of varying ring size in the highly rigid spiro bicyclic scaffold.[77] Figure 9 shows one such analogue incorporating a spiro tricyclic structure that enhanced the binding of [^{3}H]-2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN) to dopamine receptors compared to the parent peptide.[76,81]

Despite numerous reports suggesting the bioactive conformation of PLG to be a type II β-turn, Luthman et al. used a 2,3,4-substituted pyridine scaffold to build their PLG mimetic (Figure 9).[82] Conformational analysis of their pyridine-based tripeptide mimetic reveals that it cannot adopt the type II β-turn conformation. However, using a cell-based assay, Luthman et al. showed that their mimetic was more potent than PLG at enhancing the human D2 receptor activity of dopamine agonist *N*-propylapomorphine. At 10 nM, the maximum response using the mimetic was 146% compared to 115% for PLG. Luthman et al. synthesized the PLG mimetic via an eight-step synthetic procedure with 20% overall yield.[82]

■ TRIPEPTIDE MOTIFS IN PROTEINS

There are potentially a large number of highly conserved tripeptide motifs in proteins in nature, and much effort has undergone to identify these sequences and their biological function. For example, recently Neduva and Russell described a server, DILIMOT[2] for discovering short linear motifs within a set of proteins that share a common functional feature and that

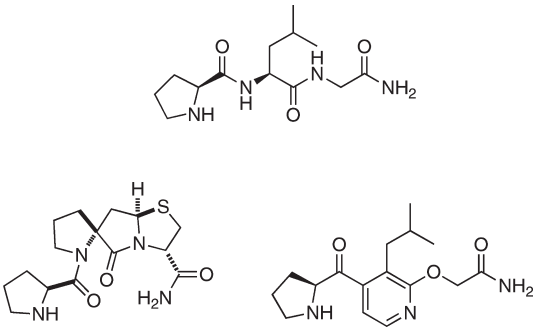

Figure 9. Structures of PLG and conformationally constrained analogues.

are over-represented. We summarize some tripeptide motifs that have been reported in the literature and describe their possible pharmacological role.

DLF/SLF: DNA Polymerase β Protein Recognition. The bacteria replisome offers a novel target for the development of new antibiotics to tackle the emergence of increasingly drug resistance strains. The β protein is an essential component of the replicative machinery of microbes, as it provides a platform for assembly of the other components of the DNA polymerase and the means by which the machine can transport along the DNA strand during the replication process. Wijffels et al.[83] reported a conserved pentapeptide motif QL[D/S]LF in DNA polymerase A (Pol A) proteins binding to the β protein that is conserved over many strains of bacteria including drug-resistant strains.[83,84] The consensus sequence was determined from the most frequently used amino acids in each position in the five amino acid motif. They showed that DLF exhibited moderate binding to the β protein and that cyclic versions of this motif were not active. Small molecule mimetics of the tripeptide motif (Figure 10) were designed using a conserved 3D conformation for DLF derived from the Protein Data Bank.[85] Subsequently, this group also reported an X-ray study of binding of the peptide motif, and small molecule mimetics to the recognition site of the β protein. These studies showed that the peptide motif and small molecule mimetics occupy overlapping binding sites on the β protein.[85]

ELR: CXC Binding Motif. The ELR (Glu-Leu-Arg) tripeptide motif is found near the N-terminus of a family of CXC chemokines and is believed to be partially responsible for its receptor binding. The CXC family can be classified into ELR$^+$ or ELR$^-$ depending on whether the ELR motif is present.[86] Examples of ELR$^+$ CXC chemokines include those that stimulate melanoma growth (CXCL1, CXCL2, and CXCL3), epithelial neutrophil activating protein (CXCL5), granulocyte chemotactic peptide 2 (CXCL6), neutrophilic activating protein (CXCL7), and interleukin 8 (CXCL8).[86] Of these, the most heavily exploited for pharmaceutical purposes have been the ELR$^+$ chemokines, CXC chemokine receptor 1 (CXCR1), and CXC chemokine receptor 2 (CXCR2).[87,88]

Over the years, numerous CXCR1 and CXCR2 targeting strategies and compounds have been developed. These have been described in an extensive series of papers by Mathison et al.[35,37,38,40−43,89] and Bizzarri.[90] These strategies include antibodies, competitive inhibitors, and noncompetitive allosteric inhibitors acting at a site downstream from receptor binding site. Many of these compounds have progressed to preclinical and clinical trials. For example, Anogen (Yes Biotech Laboratories) developed a topical cream for the treatment of psoriasis. The formulation comprises an anti-CXCL8 monoclonal

dx.doi.org/10.1021/jm1012984 |*J. Med. Chem.* 2011, 54, 1111–1125

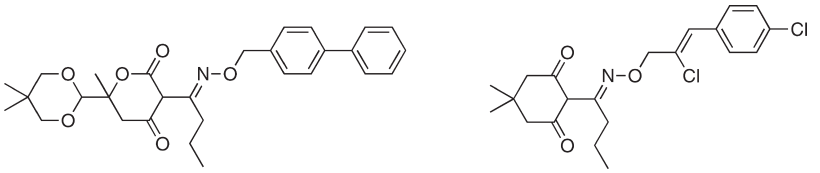

Figure 10. Small molecule DLF mimetics derived from a virtual screen of databases. These compounds inhibited the β protein with IC_{50} = 270 μM α/β and IC_{50} = 90 μM δ/β (left) and IC_{50} = 350 μM α/β and IC_{50} = 290 μM δ/β (right).

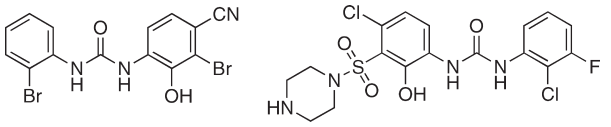

Figure 11. Examples of diarylureas CXCL8 antagonists.

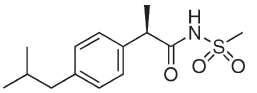

Figure 12. Reparixin.

antibody and is currently marketed as ABCream. The search for small molecule antagonists of CXCL8 is a highly active area of drug research. Programs at GlaxoSmithKline led to the discovery of the diarylurea scaffold,[91] the most widely investigated class of compounds for CXCL8 antagonism with extensive coverage in the patent literature.[92,93] Examples of active diarylureas are illustrated in Figure 11. These compounds are of particular interest, as they are being developed commercially and undergoing clinical studies. These compounds have a good selectivity profile when tested on a wide range of chemokine and non-chemokine receptors.[90]

Reparixin is a potent, selective, noncompetitive allosteric inhibitor of CXCL8 receptors. It was selected from a class of 2-arylphenylpropionic acid lead structures.[90] Bertini et al. investigated the mode of action of reparixin (Figure 12) and proposed that binding to the CXCL8 receptor locks it in an inactive conformation that prevents intracellular signal transduction.[94]

GPE: Neuroprotectant. Glycine−proline−glutamate (GPE) is a tripeptide sequence in the N-terminal domain of insulin-like growth factor 1 (IGF-1) currently under investigation for neuroprotective properties.[95] Following acute ischemic brain injury, administration of GPE has been shown to reduce both cortical damage and neuronal loss in the CA1-2 subregions of the hippocampus.[96−98]

Garcia-Lopez et al. used solid-phase synthesis to generate a library of GPE analogues.[99] They assessed the ability of the library to displace L-[^{3}H]glutamate from rat brain synaptic membranes and compared the results with the endogenous peptide GPE. Most compounds retained glutamate receptor binding affinity, while some had improved affinity. The compounds were then investigated for neuroprotection using cultured hippocampal neurons exposed to NMDA at 100 μM. Interestingly, all of the synthesized compounds showed lower neuroprotective potencies compared to the endogenous tripeptide GPE. The results suggest that the neuroprotective ability of these analogues after *N*-methyl-D-aspartic acid (NMDA) injury does not directly correlate with their glutamate receptor affinity.[99] Thus, several groups are investigating more closely the pharmacological activity of GPE via systematic modifications to the three residues.[99−105]

GGQ: Class 1 Release Factor Stop Codon Recognition. The GGQ (Gly-Gly-Gln) tripeptide motif is found in all class 1 release factors (RF) and is vital for their activity. Class 1 RFs are responsible for the recognition of stop codons in mRNA and promote peptidyl-tRNA cleavage on the ribosome.[106] In bacteria two class 1 RFs are responsible for the recognition of the three stop codons. In *Archaea* and *Eukarya* a single class 1 RF recognizes all three stop codons. Interestingly these RFs show no sequence similarity with bacteria RFs with the exception of the GGQ, which is conserved in all three kingdoms. Site directed mutagenesis on human eRF1 (a class 1 RF) shows that substitution of either glycyl residues results in complete loss of activity of the protein as a release factor toward all stop codons.[107]

HAV: Cadherin Antagonism, Cell-to-Cell Adhesion. The cadherins are calcium-dependent glycoprotein cell adhesion molecules found in several kinds of cell−cell contact. Cadherins play a central biological role, particularly in cell adhesion, morphogenesis, neurogenesis, and many other important functions. There are three main categories of cadherin, E-, N-, and P-cadherins, which interact directly and/or indirectly with a wide range of receptor tyrosine kinases, including the epidermal growth factor receptor, the c-Met receptor, the ephrin A2 receptor,[108] and the fibroblast growth factor receptor (FGFR).[109] The central role of cadherin in biology has been reviewed recently by Halbleib,[110] Gumbiner,[111] and Pokutta.[112]

A key recognition sequence, HAV (His-Ala-Val), in cadherin was first identified by Blaschuk et al.[113] This motif and an additional motif, INP reported by Williams' group, showed useful cadherin antagonist effects.[114] Later studies by Williams et al. showed that the amino acids immediately flanking the HAV sequence control the selectivity of the E-, N-, and P-cadherins.[115] An alanine scan of a short HAV-containing peptide that antagonized cadherin was conducted by Makagiansar et al.[116] and used to optimize the peptide sequence. The structures of most of these short linear peptides were not well-defined, as is common with peptides having lengths shorter than about 15 residues. However, an NMR and X-ray structure of one of these active antagonist peptides was reported by Lutz at al.[117] The peptide has an extended β sheet structure between residues Leu1 and Asp7, the same structural motif as that in the X-ray crystal structure of a similar sequence in haemagglutinin. The region from Asp7 to Gly10 was a β-turn. Cyclization of the active antagonist peptides also generated effective cadherin antagonists, and these had the advantage of having a more conformationally constrained presentation of the key HAV motif.

Cadherins cause dimerization of many tyrosine kinase receptors to exert agonist activity. Consequently, the short, linear peptide sequences only exhibited antagonist effects, as the peptides were too short to span the distance required to interact with the binding regions of two receptors. Williams et al. showed that dimerizing peptides containing the HAV motif converted the

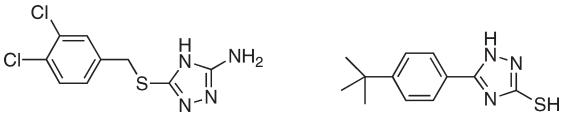

Figure 13. Lead HAV-mimetic compounds.[119]

antagonists into agonists. The *N*-Ac-CHAVDINGHAVDIC-NH_2 peptide stimulated neurite outgrowth from rat cerebellar granule cells in a dose-dependent manner, with a maximal 70% increase in neurite length found at 7.3 μM peptide.[108]

Given the conformational flexibility and instability of even cyclic peptides containing the cadherin recognition motif, it was clear that small molecule mimics of this sequence would be desirable. Burden-Gully et al. have very recently reported small molecule mimics that show moderate cadherin antagonist activities.[118] These leads were identified through their structural similarity to the N-cadherin specific cyclic peptide antagonist N-Ac-CHAVDC-NH_2 using a pharmacophore-based virtual screen of large available chemical databases. Database hit activity was improved by medicinal chemistry optimization techniques to yield N-cadherin antagonists with IC_{50} values of 5−30 μM as described in U.S. Patent 7,446,120 B2 (Figure 13). Burden-Gulley et al. also reported the conversion of small molecule antagonists to agonists.[119] The structures of the agonists were not disclosed, but presumably they were created by dimerizing the antagonist structures (as was done for the cyclic peptide antagonists) to generate first small molecule cadherin agonists.

HGK: Vitronectin-Mediated Metastasis Inhibitor. Kininogens are precursors of kinin, a member of the autocoid polypeptide family. Autocoids, notable examples of which include angiotensin and endothelins, are local hormones that exert a paracrine effect. There are two main types of kininogen: (1) a high molecular weight kininogen (HK) produced by the liver that mainly functions as a cofactor for blood coagulation and inflammation and (2) a low molecular weight kininogen produced by numerous tissues. Kinin-free HK is obtained after cleavage by kallikrein, factor XIIa, or plasmin, which releases kinin. Kawasaki et al. has reported that the tripeptide HGK in domain 5 of kinin-free HK, and peptide derivatives containing the amino acid sequence HGK, inhibited vitronectin-mediated metastasis of MDA-MB-231 cancer cells *in vitro* and B16-F10 lung metastasis in mice experiments.[120] To date, no small molecule mimics of HGK have been reported. Small molecule drugs based on this motif may play an important role in inhibition of metastasis.

HPQ: Streptavidin Recognition Motif. The HPQ (His-Pro-Gln) motif has been shown to have high affinity for streptavidin, a protein reagent commonly used as an affinity tag in a variety of biological applications and clinical diagnostics.[121] The streptavidin-binding HPQ motif has been incorporated into many recombinant proteins, allowing for their isolation through a streptavidin affinity column. The very high affinity streptavidin ligand, biotin, is used in streptavidin affinity columns to displace the HPQ incorporated proteins, allowing for their elution from the column.[122] The mechanism of binding of HPQ toward streptavidin has been probed through crystallography data and plasmon resonance binding measurements. The researchers found that in a set of HPQ-containing peptides, deprotonation of the His residue is required for high affinity binding to streptavidin both in the crystals and in solution.[123] The histidine side chain makes two hydrogen bonds ($N\delta1_{His}-N_{Gln}$ and $N\varepsilon2_{His}-O\gamma2_{Thr90}$) at pH ≥ 2.5.[124] These peptides, or small molecule mimics with high affinity, may find application in the targeting or endoradiotherapy of tumors.

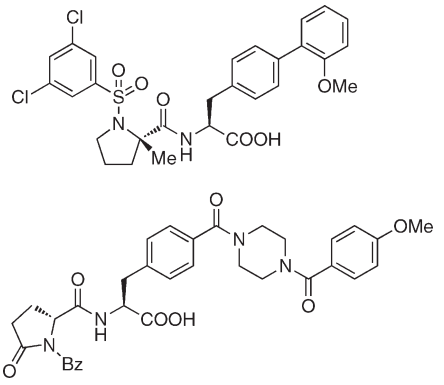

Figure 14. Biphenylalanine and 4-acylaminophenylalanine mimics of the LDV peptide motif.[135]

KPV: Antimicrobial, Anti-Inflammatory Tripeptide Motif. KPV (Lys-Pro-Val) is the C-terminal tripeptide sequence of α-melanocyte-stimulating hormone (α-MSH). The tripeptide, like its parent molecule, has been clearly demonstrated to exhibit anti-inflammatory activity in vitro and in vivo; however, the mechanism by which it exerts its pharmacological effects is poorly understood. The N-acetylated and C-amidated KPV tripeptide and several related stereoisomers and analogues have been shown to possess similar activity to α-MSH. These related tripeptides include the stereoisomers dKPV, KPdV, KdPV, and dKPdV and the structurally related peptide KdPT, whereby valine is replaced by the more polar threonine. Because of its size and pigmentation effects, use of α-MSH for treatment of human immune-mediated inflammatory diseases is compromised, making KPV or analogues a more attractive approach for therapy. These tripeptides are suitable for large-scale pharmaceutical production, although, being peptides, they have issues relating to pharmacokinetic profile, absorption, and metabolism. Peptides have been used successfully as drugs, but in general small molecule mimetics with improved pharmacokinetics properties are preferred. Extensive research into the anti-inflammatory effects of α-MSH related tripeptides, its analogues, and its stereoisomers in vitro and in vivo has been reported.[125] Recently, antimicrobial activity of α-MSH and KPV against *Staphylococcus aureus* and *Candida albicans* has also been established, making the therapeutic use of these peptides even more attractive in cases where infection and inflammation coexists.[126] An extensive review of α-MSH and related peptides has been published recently by Brzoska et al.[125] Very recently, Leoni et al. reported a small molecule agonist of the melanocortin MC1 receptor that inhibits leucocyte trafficking in inflamed vasculature; however, this does not appear to be a mimic of KPV. To date, no small molecule mimics of KPV or its related stereoisomers have been reported.

LDV: Fibronectin Adhesion Motif. Komoriya and co-workers[127] and Mould et al.[128] first reported the minimal essential sequence for major cell type specific adhesion. They studied one of the connecting segment domains of fibronectin and generated a series of peptides containing overlapping sequences taken from this domain. They identified the motif Leu-Asp-Val (LDV) as the essential tripeptide sequence responsible for recognition and adhesion and reported that the motif interacted with the integrin $\alpha4\beta1$. This stimulated interest in the properties of this peptide motif and in the design of small

dx.doi.org/10.1021/jm1012984 |*J. Med. Chem.* 2011, 54, 1111–1125

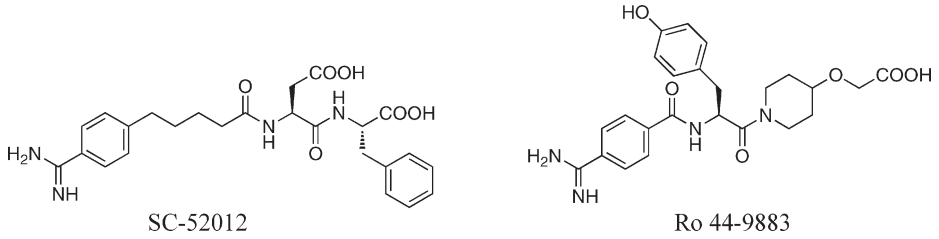

Figure 15. Small molecule RGD mimetics.

molecule drugs that mimic it. In a review by Akiyama et al.[129] fibronectin−integrin interactions were identified as important in tumor cell migration, invasion, metastasis, chemotaxis, and control of proliferation. Peptide and antibody inhibitors of cell adhesion were shown to be effective in inhibiting metastasis and for the study and control of cancer. The solution structure of a cyclic LDV peptide and structure−activity relationships were reported by Doyle and co-workers[130] and Viles et al.,[131] opening the way for design of small molecule antagonists of α4β1 integrins that recognize LDV. Jackson et al.[132] described the role of α4β1 integrins and cytokine inducible vascular cell adhesion molecule 1 (VCAM-1) at inflamed sites. Monoclonal antibodies generated against either α4β1 or VCAM-1 were shown to moderate inflammatory response in a variety of animal models. Jackson et al. described novel, highly potent, cyclic peptides that competitively inhibited α4β1 binding to VCAM-1, and fibronectin and lymphocyte migration in vivo. Yokosaki et al.[133] identified the tripeptide sequence IDG as homologous to the sequences LDV, IDA, and LDA in fibronectin and IDS in VCAM-1 and found that it too bound the α4β1/α9β1 subfamily of integrins. In 1999, Lin and co-workers[134] reported a series of potent (nanomolar) small molecule−peptide hybrid inhibitors of VCAM-1 binding and cell adhesion that inhibited allergic airway responses in animals.

Yang and Hagmann[135] reviewed the role of integrin antagonists in treatment of inflammatory diseases, and there has been a great deal of research activity in this area during the past decade. For example, 4-acylaminophenylalanine derivatives were reported to be potent VLA-4 antagonists.[136] The core structure was hypothesized to mimic the key pharmacophoric features of a cyclic LDV peptide. This hypothesis was supported in that the 4-acylaminophenylalanine analogue (Figure 14) was found to be equipotent with the cyclic peptide (44 nM).

Screening of a carboxylate-containing library led to the discovery of a series of sulfonylated dipeptides. The 4-biphenylalanine analogue (Figure 14) at 80 pM exhibited excellent activity in a VCAM-1 binding assay and also had favorable pharmacokinetic profile.[137,138] Many thousands of VCAM-1 antagonists that bind to the LDV site on integrins have been reported in the academic and patent literature in the past decade. A recent review by Tilley[139] summarized small-molecule α4 integrin antagonists from 2003 to 2008, some of which entered clinical trial for multiple sclerosis and other inflammatory conditions, and others show potential in cancer treatment.

RGD: Cell Adhesion Tripeptide Motif. Integrins are a large class of cell surface receptors responsible for cell adhesion and signaling bidirectionally across the membrane. They are involved in an array of biological processes such as thrombosis, inflammation, angiogenesis, and cancer. The discovery of the minimal peptide sequence RGD, which plays a prominent role in cell adhesion via integrin interaction, has led to a large increase in biomedical and biomaterials research on this motif.[140]

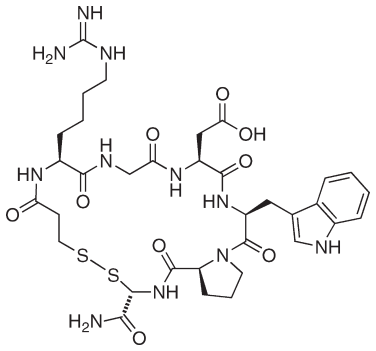

Figure 16. Cyclic heptapeptide eptifibatide.

RGD as Antithrombotic Agents. Thrombosis is a major cardiovascular disease resulting from aberrant platelet aggregation. The final step of this aggregation mechanism regardless of the primary stimulus involves the binding of fibrinogen to integrin $\alpha_{IIb}\beta_3$ (also known as the receptor glycoprotein IIb/IIIa). Its presence at high densities on platelets ensures that the aggregation step occurs rapidly. Many integrins can recognize specific short peptide sequences, with 8 of the 24 known integrins binding the RGD motif.

The therapeutic use of RGD peptidomimetics for the treatment of thrombosis was first reviewed in 1995 by Ojima et al.[141] Abciximab, formerly c7E3 Fab, is an example of a drug candidate targeting the $\alpha_{IIb}\beta_3$ receptor on platelet surfaces that is currently registered for clinical use.[141] Despite few RGD analogues being approved for clinical use, development of orally active RGD peptidomimetics have been significantly hindered because of low bioavailabilities. This is largely due to the metabolic lability of this class of compounds in the presence of proteases and peptidases and because of their high polarity and charge. Wang et al.[142] published a detailed review of peptidomimetic analogues of RGD and the strategies used to enhance their bioavailability.

Because of the significance of the $\alpha_{IIb}\beta_3$ integrin receptor and its potential therapeutic target, much effort has been put toward the elucidation of its structure. These include electron microscopy,[143] homology modeling,[144] NMR,[145] and X-ray crystallography[146] studies. Crystal structures of the extracellular domain were reported by Xiao et al.[146] Crystal structures were also reported for the ectodomain complexed with the drugs eptifibatide and tirofiban (Aggrastat).

In the design of RGD mimetics for drug optimization, the importance of an acidic C-terminus and a basic N-terminus has been established by comparison of several active analogues. This is illustrated by SC-52012 (Figure 15), which is currently under phase I clinical trials as an intravenous drug for antithrombosis.[147] Alig et al. also reported an RGD mimetic that was a thromboxane A_2 receptor antagonist,[148] however, with weak

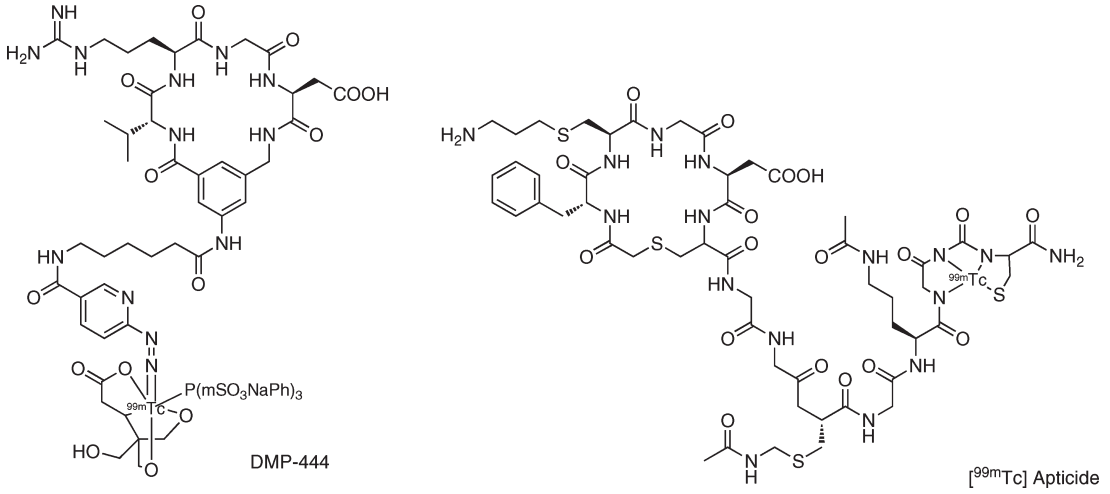

Figure 17. RGD-based radiolabeling agents.

affinity for the $\alpha_{IIb}\beta_3$ integrin. De-esterification and reintroduction of an acidic group at the C-terminus led to significant improvements in $\alpha_{IIb}\beta_3$ affinity and resulted in Ro 44-9883 (Figure 15), which is currently under phase II clinical trials.[148,149]

Disintegrins. A number of homologous peptides isolated from the *Viperidae* family of snakes were found to possess RGD tripeptide units. The peptides were named "disintegrins" because of their highly potent inhibition activity of integrin function. Most of the members of the disintegrin family display nonselective RGD-dependent inhibition of integrins. Barbourin and ussuristatin are the only two disintegrins that do not contain an RGD tripeptide sequence, and their activity arises from the conservative and closely related KGD motif.[150] Interestingly, barbourin shows high specificity for fibrinogen receptors.[150] Eptifibatide (Figure 16) is a cyclic heptapeptide mimic of barbourin that shows good selectivity for the $\alpha_{IIb}\beta_3$ integrin receptor and inhibits at the nanomolar level.[151]

RGD: Application for Selective Therapeutic Delivery and Imaging Agents. Exploitation of the RGD/integrin system allows the effective targeting of diagnostics and therapeutics. The expression of integrin ligands varies with cell and tissue type, with integrin subtypes dominating the surface of a particular cell. In particular, some tumor cells overexpress the $\alpha_V\beta_3$ integrin. Similarly the $\alpha_{IIb}\beta_3$ integrin is only observed on activated platelets. RGD-based radiolabels have been developed for the imaging of $\alpha_{IIb}\beta_3$ and $\alpha_V\beta_3$ integrin using ^{99m}Tc as the radionuclide.[12,152,153] Examples of such radioimaging agents include DMP444 and [^{99m}Tc]apticide (Figure 17), which can be used in imaging deep vein thrombosis.[12] Besides radioimaging, RGD-labeled compounds have also been developed for delivery of small molecule drugs, cytotoxins, liposomes, genes, and fluorescent tags.[12] Similarly, a large number of inorganic materials have been RGD functionalized to enhance their biocompatibility for research studies and medicinal applications. Various compounds have been developed for surface coatings, including RGD-containing proteins, peptides, and peptide mimetics.[12]

SKL: Peroxisomal Targeting Sequence. The carboxyl-terminal sequence Ser-Lys-Leu (SKL) has been reported to function as a topogenic peroxisome-targeting signal for the translocation of proteins into peroxisomes. The tripeptide sequence, also referred to as the peroxisomal targeting signal 1 (PTS1), was first described by Gould et al. in a study of firefly luciferase.[154] It is a highly conserved motif that can be found in most peroxisomal matrix proteins. Amino acid modifications to the tripeptide sequence has also been investigated in subsequent work by Gould et al. where they had found that conservative variants of the PTS such as (S/A/C)(K/R/H)(L/M) will suffice in directing proteins to the peroxisomes.[155]

In the cytosol, the peroxisomal targeting signal 1 receptor PEX5 is responsible for the binding of PTS1 and delivery of the protein to the organelle surface where other components of the import machinery aid its translocation. The interaction between PTS1 and PEX5 occurs through a series of tetratricopeptide repeats (TPRs) found within the receptor C-terminal domain. Crystallographic data portraying a PTS1-containing peptide interacting with seven predicted TPR motifs in human PEX5 have been reported.[156]

To our knowledge, exploitation of the SKL motif has not been a focus for drug optimization, either as peptidomimetics or as peptide drugs. Extensive mutagenesis studies elucidating the peroxisome−protein translocation mechanisms and requirements can be found in the literature, and this has been recently reviewed by Brocard and Hartig.[157]

■ CONCLUSION AND PERSPECTIVE

The number of examples of biologically important tripeptide motifs described in this review is small relative to the entire tripeptide sequence space. However, we postulate that this may be a function of sparse exploration of sequence space rather than low natural occurrence of biologically relevant tripeptide motifs. The informatic studies summarized in the Introduction show that tripeptide motifs are often over-represented in nature, and they also have the correct physical size to function as efficient ligands for protein targets. Clearly the examples presented here have formed the nucleus for a substantial body of research and in some cases have generated intellectual property, clinical trial candidates, useful reagents, and drugs. It would be fair to say that these active tripeptides and their mimics have been discovered either randomly, through an understanding of how the motif interacts with a biological target, or by combination of both. We contend that if three contiguous amino acids constitute a useful and minimal biological recognition signal, this may form a useful

new paradigm for discovering peptides and small organic molecule mimics that are useful modulators of biological function. Similarly, it is reasonable that a small library of all 8000 possible tripeptides could be generated and used for screening against multiple targets and, even if capping of the termini was also considered, the number of peptides is quite accessible given current library synthesis technologies. Some studies have reported synthesis of partial or complete libraries of tripeptides or tripeptide conjugates for biological or nonbiological screening. Methods for generating such libraries and applications to generate leads were reviewed comprehensively by Lam and coworkers over a decade ago[158] and more recently for peptide and small molecule libraries by Boger et al.[159]

Sequence versus Structure. Although nature may use tripeptide motifs and minimal signals, in many cases the sequence is not sufficient. Although libraries containing all 8000 possible tripeptides can be generated, the 3D properties of the motifs are important. Short peptides are well-known to have poorly defined structures in solution, so conformational constraint has been used in many cases (e.g., cyclic RGD and HAV peptides discussed above) to reduce the flexibility of the peptides and induce some kind of structure. A future extension of this hypothesis involves answering whether the three-dimensional structures of tripeptides may also be conserved and reused by nature as well as tripeptide sequences. We are conducting work in this area based on the conservation of the 3D structure of the DLF motif described above.[85] In this case, the 3D structure of the tripeptide motif is surprisingly well conserved when structures in the Protein Data Bank (http://www.rcsb.org) are queried. Novel ways of analyzing small peptide motifs, their 3D structures, and design of small molecule mimics should provide productive ways of probing chemical biology and discovering new drug leads.

While the small number of examples in this review does not prove that tripeptide motifs are the minimal useful signal exploited by nature, it does provide tantalizing evidence for our hypothesis. Subsequent work will be required to determine how broadly applicable this new paradigm is.

AUTHOR INFORMATION

Corresponding Author

*Phone: +61 3 9545 2477. Fax: +61 3 9545 2446. E-mail: dave.winkler@csiro.au.

BIOGRAPHIES

Phuc Ung is a Ph.D. candidate at the Monash Institute of Pharmaceutical Sciences (MIPS), Monash University, Melbourne, Australia. He graduated from Monash University in 2007 to obtain his Bachelor's degree in Medicinal Chemistry. After working extensively to develop new fluorophores using "click chemistry", he received his Honours degree under the supervision of Dr. Bim Graham. Thereafter, he joined the group of Professor Dave Winkler at CSIRO Molecular and Health Technologies. He is currently working with Dr. Bim Graham and Dr. James D. Swarbrick and is interested in the development of novel lanthanide binding tags and novel application of lanthanides in protein NMR spectroscopy and cellular imaging of GPCRs.

Dave A. Winkler is a Senior Principal Research Scientist with CSIRO and an Adjunct Professor at Monash University. His research interests have mainly involved molecular design and complex systems. Recently, he has moved into regenerative medicine where he collaborates with international stem cell biologists and tissue engineers. He employs computational methods developed for small molecule research and uses them in these complex biological systems. He was awarded traveling fellowships to Kyoto and Oxford and a Newton Turner Fellowship in 2009. He is a past Board Chairman of the Royal Australian Chemical Institute and past President of Asian Federation for Medicinal Chemistry. He also represents Australia on the committee organizing of Pacifichem. He has published 150 scientific papers and patents.

ACKNOWLEDGMENT

Financial assistance from a CSIRO vacation scholarship is gratefully acknowledged. Thanks are extended to Drs. Paul Savage and Andrew Riches for very helpful suggestions on improving the manuscript.

ABBREVIATIONS USED

α-MSH, melanocyte-stimulating hormone; ADEPT, A Daylight enumeration and profiling tool; ADTN, 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene; HGFR, hepatocyte growth factor receptor; CNS, central nervous system; FGFR, fibroblast growth factor receptor; GPCR, G-protein-coupled receptor; GSH, glutathione; GST, glutathione *S*-transferase; HA, heavy atom; HK, high molecular weight kininogen; IGF-1, insulin-like growth factor 1; MC, melanocortin; NMDA, *N*-methyl-D-aspartic acid; PEX5, peroxisomal biogenesis factor 5; pIC_{50}, $-\log IC_{50}$; Pol A, DNA polymerase A; PTS1, peroxisomal targeting signal 1; RF, release factor; ROS, reactive oxygen species; TPR, tetratricopeptide repeat; TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone, thyrotropin; VCAM-1, vascular cell adhesion molecule 1; VLA-4, very late antigen 4, integrin $\alpha 4\beta 1$

REFERENCES

(1) Reynolds, C. H.; Bembenek, S. D.; Tounge, B. A. The role of molecular size in ligand efficiency. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4258–4261.

(2) Neduva, V.; Russell, R. B. DILIMOT: discovery of linear motifs in proteins. *Nucleic Acids Res.* **2006**, *34*, W350–W355.

(3) Hann, M. M.; Leach, A. R.; Harper, G. Molecular complexity and its impact on the probability of finding leads for drug discovery. *J. Chem. Inf. Comput. Sci.* **2001**, *41*, 856–864.

(4) Leach, A. R.; Bradshaw, J.; Green, D. V. S.; Hann, M. M.; Delany, J. J. Implementation of a system for reagent selection and library enumeration, profiling, and design. *J. Chem. Inf. Comput. Sci.* **1999**, *39*, 1161–1172.

(5) Anishetty, S.; Anishetty, R.; Pennathur, G. Understanding mutations and protein stability through tripeptides. *FEBS Lett.* **2006**, *580*, 2071–2080.

(6) Anishetty, S.; Pennathur, G. Distribution of tripeptides in MHC binding peptides. *Protein Pept. Lett.* **2007**, *14*, 552–556.

(7) Anishetty, S.; Pennathur, G.; Anishetty, R. Tripeptide analysis of protein structures. *BMC Struct. Biol.* **2002**, *2*, 9.

(8) Brooks, D. J.; Fresco, J. R.; Lesk, A. M.; Singh, M. Evolution of amino acid frequencies in proteins over deep time: inferred order of introduction of amino acids into the genetic code. *Mol. Biol. Evol.* **2002**, *19*, 1645–1655.

(9) Gatto, G. J.; Berg, J. M. Nonrandom tripeptide sequence distributions at protein carboxyl termini. *Genome Res.* **2003**, *13*, 617–623.

(10) Otaki, J. M.; Gotoh, T.; Yamamoto, H. Potential implications of availability of short amino acid sequences in proteins: an old and new approach to protein decoding and design. *Biotechnol. Annu. Rev.* **2008**, *14*, 109–41.

(11) Shiba, K. Natural and artificial peptide motifs: their origins and the application of motif-programming. *Chem. Soc. Rev.* **2010**, *39*, 117–126.

(12) Meyer, A.; Auernheimer, J.; Modlinger, A.; Kessler, H.; Targeting, R. G. D. Recognizing integrins: drug development, biomaterial research, tumor imaging and targeting. *Curr. Pharm. Des.* **2006**, *12*, 2723–2747.

(13) Aoyama, K.; Watabe, M.; Nakaki, T. Regulation of neuronal glutathione synthesis. *J. Pharmacol. Sci.* **2008**, *108*, 227–238.

(14) Dringen, R. Metabolism and functions of glutathione in brain. *Prog. Neurobiol.* **2000**, *62*, 649–671.

(15) Jain, A.; Martensson, J.; Stole, E.; Auld, P. A.; Meister, A. Glutathione deficiency leads to mitochondrial damage in brain. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 1913–1917.

(16) Zeevalk, G. D.; Manzino, L.; Sonsalla, P. K.; Bernard, L. P. Characterization of intracellular elevation of glutathione (GSH) with glutathione monoethyl ester and GSH in brain and neuronal cultures: relevance to Parkinson's disease. *Exp. Neurol.* **2007**, *203*, 512–520.

(17) Kannan, R.; Yi, J. R.; Tang, D.; Li, Y.; Zlokovic, B. V.; Kaplowitz, N. Evidence for the existence of a sodium-dependent glutathione (GSH) transporter. Expression of bovine brain capillary mRNA and size fractions in *Xenopus laevis* oocytes and dissociation from gamma-glutamyltranspeptidase and facilitative GSH transporters. *J. Biol. Chem.* **1996**, *271*, 9754–9758.

(18) Cornford, E. M.; Braun, L. D.; Crane, P. D.; Oldendorf, W. H. Blood−brain barrier restriction of peptides and the low uptake of enkephalins. *Endocrinology* **1978**, *103*, 1297–1303.

(19) Montali, J. A.; Wheatley, J. B.; Schmidt, D. E., Jr. Comparison of GST levels in predicting the efficacy of a novel alkylating agent. *Cell. Pharmacol.* **1996**, *2*, 241–247.

(20) Wang, R. W.; Newton, D. J.; Huskey, S. E. W.; McKeever, B. M.; Pickett, C. B.; Lu, A. Y. H. Site directed mutagenesis of glutathione *S*-transferase YaYa. *J. Biol. Chem.* **1987**, *267*, 19866–19871.

(21) Morgan, A. S.; Sanderson, P. E.; Borch, R. F.; Tew, K. D.; Niitsu, Y.; Takayama, T.; Von Hoff, D. D.; Izbicka, E.; Mangold, G.; Paul, C.; Broberg, U.; Mannervik, B.; Henner, W. D.; Kauvar, L. M. Tumor efficacy and bone marrow-sparing properties of TER286, a cytotoxin activated by glutathione *S*-transferase. *Cancer Res.* **1998**, *58*, 2568–2575.

(22) Sun, Y.; Lu, X.; Gershengorn, M. C. Thyrotropin-releasing hormone receptors, similarities and differences. *J. Mol. Endocrinol.* **2003**, *30*, 87–89.

(23) Buesen, D. D.; Shands, E. F. B. Systematic search strategies in conformational analysis. *Drug Discovery Today* **1996**, *1*, 429–437.

(24) Dammkoehler, R. A.; Karasek, S. F.; Shands, E. F. B.; Marshall, G. R. Constrained search of conformational hyperspace. *J. Comput.-Aided Mol. Des.* **1989**, *3*, 3–21.

(25) Dammkoehler, R. A.; Karasek, S. F.; Shands, E. F. B.; Marshall, G. R. Sampling conformational hyperspace: techniques for improving completeness. *J. Comput.-Aided Mol. Des.* **1995**, *9*, 491–499.

(26) Tong, Y.; Olczak, J.; Zabrocki, J.; Gershengorn, M. C.; Marshall, G. R.; Moeller, K. D. Constrained peptidomimetics for TRH: cis-peptide bond analogs. *Tetrahedron* **2000**, *56*, 9791–9800.

(27) Engel, S.; Gershengorn, M. C. Thyrotropin-releasing hormone and its receptors: a hypothesis for binding and receptor activation. *Pharmacol. Ther.* **2007**, *113*, 410–419.

(28) Monga, V.; Meena, C. L.; Kaur, N.; Jain, R. Chemistry and biology of thyrotropin-releasing hormone (TRH) and its analogs. *Curr. Med. Chem.* **2008**, *15*, 2718–2733.

(29) Mason, G. A.; Rezvani, A. H.; Grady, D. R.; Garbutt, J. C. The subchronic effects of the TRH analog TA-0910 and bromocriptine on alcohol preference in alcohol-preferring rats: development of tolerance and cross-tolerance. *Alcoholism* **1994**, *18*, 1196–1201.

(30) Prokai, L. Central nervous system effects of thyrotropin-releasing hormone and its analogues: opportunities and perspectives for drug discovery and development. *Prog. Drug Res.* **2002**, *59*, 134–169.

(31) Yamamura, M.; Kinoshita, K.; Nakagawa, H.; Tanaka, T.; Maeda, K.; Ishida, R. Pharmacological study of TA-0910, a new thyrotropin-releasing hormone(TRH) analog. (I). Effects on the central nervous system by oral administration. *Jpn. J. Pharmacol.* **1990**, *53*, 451–461.

(32) Parnetti, L.; Ambrosoli, L.; Abate, G.; Azzini, C.; Balestreri, R.; Bartorelli, L.; Bordin, A.; Crepaldi, G.; Cristianini, G.; Cucinotta, D.; Cuzzupoli, M.; Decandia, O.; Fabris, F.; Maggioni, M.; Scarpa, R.; Villardita, C.; Girardello, R.; Poli, A.; Senin, U. Posatirelin for the Treatment of late-onset Alzheimer's-disease: a double-blind multicenter study vs citicoline and ascorbic-acid. *Acta Neurol. Scand.* **1995**, *92*, 135–140.

(33) Nutt, R. F.; Holly, F. W.; Homnick, C.; Hirschmann, R.; Veber, D. F.; Arison, B. H. Synthesis of thyrotropin-releasing-hormone analogs with selective central nervous-system effects. *J. Med. Chem.* **1981**, *24*, 692–698.

(34) Nillni, E. A.; Sevarino, K. A. The biology of pro-thyrotropin-releasing hormone-derived peptides. *Endocr. Rev.* **1999**, *20*, 599–648.

(35) Mathison, R.; Davison, J. S.; Moore, G. Submandibular gland peptide-T (Peptide-T): modulation of endotoxic and anaphylactic shock. *Drug Dev. Res.* **1997**, *42*, 164–71.

(36) Mathison, R.; Lo, P.; Moore, G.; Scott, B.; Davison, J. S. Attenuation of intestinal and cardiovascular anaphylaxis by the salivary gland tripeptide FEG and its D-isomeric analog feG. *Peptides* **1998**, *19*, 1037–1042.

(37) Dery, R. E.; Ulanova, M.; Puttagunta, L.; Stenton, G. R.; James, D.; Merani, S.; Mathison, R.; Davison, J.; Befus, A. D. Frontline: inhibition of allergen-induced pulmonary inflammation by the tripeptide feG: a mimetic of a neuro-endocrine pathway. *Eur. J. Immunol.* **2004**, *34*, 3315–3325.

(38) Turesin, F.; Sadr, A.; Davison, J. S.; Mathison, R. The tripeptide feG ameliorates systemic inflammatory responses to rat intestinal anaphylaxis. *BMC Physiol.* **2002**, *2*, 13.

(39) Bao, F.; John, S. M.; Chen, Y.; Mathison, R. D.; Weaver, L. C. The tripeptide phenylalanine-(D) glutamate-(D) glycine modulates leukocyte infiltration and oxidative damage in rat injured spinal cord. *Neuroscience* **2006**, *140*, 1011–1022.

(40) Mathison, R.; Befus, A. D.; Davison, J. S.; Woodman, R. C. Modulation of neutrophil function by the tripeptide feG. *BMC Immunol.* **2003**, *4*, 3.

(41) Mathison, R.; Woodman, R.; Davison, J. S. Regulation of leukocyte adhesion to heart by the tripeptides feG and feG(NH2). *Can. J. Physiol. Pharmacol.* **2001**, *79*, 785–792.

(42) Mathison, R. D.; Davison, J. S. The tripeptide feG regulates the production of intracellular reactive oxygen species by neutrophils. *J. Inflammation* **2006**, *3*, 9.

(43) Metwally, E.; Pires, J. M.; Moore, G. J.; Befus, D. A.; Davison, J. S.; Mathison, R. Submandibular gland tripeptide FEG (Phe-Glu-Gly) and analogues: keys to structure determination. *Peptides* **2002**, *23*, 193–199.

(44) Galeazzi, R.; Martelli, G.; Marcucci, E.; Mobbili, G.; Natali, D.; Orena, M.; Rinaldi, S. A new conformationally restricted mimetic of dipeptide EG synthesis of an analogue of FEG. *Eur. J. Org. Chem.* **2007**, *26*, 4402–4407.

(45) Freedman, J. H.; Pickart, L.; Weinstein, B.; B., M. W.; Peisach, J. Structure of the glycyl-L-histidyl-L-lysine-copper(II) complex in solution. *Biochemistry* **1982**, *21*, 4540–4544.

(46) Kwa, E.; Bor-Sheng, L.; Rose, N.; B., W.; Pickart, L. PMR studies of copper(II) and zinc(II) interaction with glycyl-L-histidyl-L-lysine and related peptides. *Peptides* **1983**, *8*, 805–808.

(47) Lau, S. J.; Sarkar, B. The interaction of copper(II) and glycyl-L-histidyl-L-lysine, a growth-modulating tripeptide from plasma. *Biochem. J.* **1981**, *199*, 649–656.

(48) Laussac, J. P.; R., H.; Sarkar, B. N.m.r. and e.p.r. investigation of the interaction of copper(II) and glycyl-L-histidyl-L-lysine, a growth-modulating tripeptide from plasma. *Biochem. J.* **1983**, *209*, 533–539.

(49) Perkins, C. M.; Rose, N. J.; Weinstein, B.; Stenkamp, R. E.; Jensen, L. H.; Pickart, L. The structure of a copper complex of the growth factor glycyl-L-histidyl-L-lysine at 1.1 Å resolution. *Inorg. Chim. Acta* **1984**, *82*, 93–99.

(50) Pickart, L. The human tri-peptide GHK and tissue remodeling. *J. Biomater. Sci., Polym. Ed.* **2008**, *19*, 969–988.

(51) Pickart, L. The human tri-peptide GHK and tissue remodeling. *J. Biomater. Sci., Polym. Ed.* **2008**, *19*, 969–988.

(52) Ahmed, M. R.; Basha, S. H.; Gopinath, D.; R., M.; Jayakumar, R. Initial upregulation of growth factors and inflammatory mediators

during nerve regeneration in the presence of cell adhesive peptide-incorporated collagen tubes. *J. Peripher. Nerv. Syst.* **2005**, *10*, 17–30.

(53) Lindner, G.; Grosse, G.; W., H.; Henklein, P. The effect of a synthetic tripeptide nervous tissue cultured in vitro. *Z. Mikrosk. Anat. Forsch.* **1979**, *93*, 820–828.

(54) Sensenbrenner, M.; Jaros, G. G.; Moonen, G.; Mandel, P. Effects of synthetic tripeptide on the differentiation of dissociated cerebral hemisphere nerve cells in culture. *Neurobiology* **1975**, *5*, 207–213.

(55) Kang, Y. A.; Choi, H. R.; Na, J. I.; Huh, C. H.; Kim, M. J.; Youn, S. W.; Kim, K. H.; Park, K. C. Copper-GHK increases integrin expression and p63 positivity by keratinocytes. *Arch. Dermatol. Res.* **2009**, *301*, 301–306.

(56) Lane, T. F.; Iruelaarispe, M. L.; Johnson, R. S.; Sage, E. H. Sparc is a source of copper-binding peptides that stimulate angiogenesis. *J. Cell Biol.* **1994**, *125*, 929–943.

(57) Maquart, F. X.; Bellon, G.; Chaqour, B.; Wegrowski, J.; Patt, L. M.; Trachy, R. E.; Monboisse, J. C.; Chastang, F.; Birembaut, P.; Gillery, P.; Borel, J. P. In-vivo stimulation of connective-tissue accumulation by the tripeptide copper complex glycyl-L-histidyl-L-lysine-Cu^{2+} in rat experimental wounds. *J. Clin. Invest* **1993**, *92*, 2368–2376.

(58) Simeon, A.; Emonard, H.; Hornebeck, W.; Maquart, F. X. The tripeptide−copper complex glycyl-L-histidyl-L-lysine-Cu^{2+} stimulates matrix metalloproteinase-2 expression by fibroblast cultures. *Life Sci.* **2000**, *67*, 2257–2265.

(59) Zheng, Y. J.; Huo, Q.; Kele, P.; Andreopoulos, F. M.; Pham, S. M.; Leblanc, R. M. A new fluorescent chemosensor for copper ions based on tripeptide glycyl-histidyl-lysine (GHK). *Org. Lett.* **2001**, *3*, 3277–3280.

(60) Saitton, S.; Del Tredici, A. L.; Saxin, M.; Stenstrom, T.; Kihlberg, J.; Luthman, K. Synthesis and evaluation of novel pyridine based PLG tripeptidomimetics. *Org. Biomol. Chem.* **2008**, *6*, 1647–1654.

(61) Mishra, R. K.; Srivastava, L. K.; Johnson, R. L. Modulation of high-affinity CNS dopamine-D2 receptor by L-Pro-L-Leu-glycinamide (Plg) analog 3(*R*)-(*N*-L-prolylamino)-2-oxo-1-pyrrolidineacetamide. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **1990**, *14*, 821–827.

(62) Reed, L. L.; Johnson, P. L. Solid state conformation of the C-terminal tripeptide of oxytocin, L-Pro-L-Leu-Gly-$NH_2 \cdot 0.5H_2O$. *J. Am. Chem. Soc.* **1973**, *95*, 7523–7524.

(63) Sreenivasan, U.; Mishra, R. K.; Johnson, R. L. Synthesis and dopamine receptor modulating activity of lactam conformationally constrained analogues of Pro-Leu-Gly-NH_2. *J. Med. Chem.* **1993**, *36*, 256–263.

(64) Subasinghe, N. L.; Bontems, R. J.; McIntee, E.; Mishra, R. K.; Johnson, R. L. Bicyclic thiazolidine lactam peptidomimetics of the dopamine-receptor modulating peptide Pro-Leu-Gly-NH_2. *J. Med. Chem.* **1993**, *36*, 2356–2361.

(65) Baures, P. W.; Pradhan, A.; Ojala, W. H.; Gleason, W. B.; Mishra, R. K.; Johnson, R. L. Synthesis and dopamine receptor modulating activity of unsubstituted and substituted triproline analogues of L-prolyl-L-leucyl-glycinamide (PLG). *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2349–2352.

(66) Evans, M. C.; Pradhan, A.; Venkatraman, S.; Ojala, W. H.; Gleason, W. B.; Mishra, R. K.; Johnson, R. L. Synthesis and dopamine receptor modulating activity of novel peptidomimetics of L-prolyl-L-leucyl-glycinamide featuring alpha,alpha-disubstituted amino acids. *J. Med. Chem.* **1999**, *42*, 1441–1447.

(67) Johnson, R. L.; Bontems, R. J.; Yang, K. E.; Mishra, R. K. Synthesis and biological evaluation of analogues of Pro-Leu-Gly-NH_2 modified at the leucyl residue. *J. Med. Chem.* **1990**, *33*, 1828–1832.

(68) Johnson, R. L.; Rajakumar, G.; Mishra, R. K. Dopamine receptor modulation by pro-leu-gly-NH_2 analogs possessing cyclic amino-acid-residues at the C-terminal position. *J. Med. Chem.* **1986**, *29*, 2100–2104.

(69) Johnson, R. L.; Rajakumar, G.; Yu, K. L.; Mishra, R. K. Synthesis of pro-leu-gly-NH_2 analogs modified at the prolyl residue and evaluation of their effects on the receptor-binding activity of the central dopamine receptor agonist, ATDN. *J. Med. Chem.* **1986**, *29*, 2104–2107.

(70) Palomo, C.; Aizpurua, J. M.; Benito, A.; Miranda, J. I.; Fratila, R. M.; Matute, C.; Domercq, M.; Gago, F.; Martin-Santamaria, S.; Linden, A. Development of a new family of conformationally restricted peptides as potent nucleators of beta-turns. Design, synthesis, structure, and biological evaluation of beta-lactam peptide analogue of melanostatin. *J. Am. Chem. Soc.* **2003**, *125*, 16243–16260.

(71) Rajakumar, G.; Naas, F.; Johnson, R. I.; Chiu, S.; Yu, K. L.; Mishra, R. K. Down-regulation of halperidol-induced striatal dopamine receptor supersensitivity by active analogs of L-prolyl-L-leucyl-glycinamide (PLG). *Peptides* **1987**, *8*, 855–861.

(72) Thomas, C.; Ohnmacht, U.; Niger, M.; Gmeiner, P. Beta analogues of PLG (L-prolyl-L-leucyl-glycinamide): ex-chiral pool syntheses and dopamine D-2 receptor modulating effects. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2885–2890.

(73) Baures, P. W.; Ojala, W. H.; Costain, W. J.; Ott, M. C.; Pradhan, A.; Gleason, W. B.; Mishra, R. K.; Johnson, R. L. Design, synthesis, and dopamine receptor modulating activity of diketopiperazine peptidomimetics of L-prolyl-L-leucylglycinamide. *J. Med. Chem.* **1997**, *40*, 3594–3600.

(74) Dolbeare, K.; Pontoriero, G. F.; Gupta, S. K.; Mishra, R. K.; Johnson, R. L. Iso-lactam and reduced amide analogues of the peptidomimetic dopamine receptor modulator 3(*R*)-(2(*S*)pyrrolidinylcarbonyl) amino-2-oxo-1-pyrrolidineacetamide. *Bioorg. Med. Chem.* **2003**, *11*, 4103–4112.

(75) Dolbeare, K.; Pontoriero, G. F.; Gupta, S. K.; Mishra, R. K.; Johnson, R. L. Synthesis and dopamine receptor modulating activity of 3-substituted gamma-lactam peptidomimetics of L-prolyl-L-leucyl-glycinamide. *J. Med. Chem.* **2003**, *46*, 727–733.

(76) Genin, M. J.; Mishra, R. K.; Johnson, R. L. Dopamine receptor modulation by a highly rigid spiro bicyclic peptidomimetic of Pro-Leu-Gly-NH_2. *J. Med. Chem.* **1993**, *36*, 3481–3483.

(77) Khalil, E. M.; Ojala, W. H.; Pradhan, A.; Nair, V. D.; Gleason, W. B.; Mishra, R. K.; Johnson, R. L. Design, synthesis, and dopamine receptor modulating activity of spiro bicyclic peptidomimetics of L-prolyl-L-leucyl-glycinamide. *J. Med. Chem.* **1999**, *42*, 628–637.

(78) Khalil, E. M.; Pradhan, A.; Ojala, W. H.; Gleason, W. B.; Mishra, R. K.; Johnson, R. L. Synthesis and dopamine receptor modulating activity of substituted bicyclic thiazolidine lactam peptidomimetics of L-prolyl-L-leucyl-glycinamide. *J. Med. Chem.* **1999**, *42*, 2977–2987.

(79) Weber, K.; Ohnmacht, U.; Gmeiner, P. Enantiopure 4-and 5-aminopiperidin-2-ones: regiocontrolled synthesis and conformational characterization as bioactive beta-turn mimetics. *J. Org. Chem.* **2000**, *65*, 7406–7416.

(80) Yu, K. L.; Rajakumar, G.; Srivastava, L. K.; Mishra, R. K.; Johnson, R. L. Dopamine receptor modulation by conformationally constrained analogues of Pro-Leu-Gly-NH_2. *J. Med. Chem.* **1988**, *31*, 1430–1436.

(81) Genin, M. J.; Johnson, R. L. Design, synthesis, and conformational analysis of a novel spiro-bicyclic system as a type II .beta.-turn peptidomimetic. *J. Am. Chem. Soc.* **1992**, *114*, 8778–8783.

(82) Saitton, S.; Del Tredici, A. L.; Mohell, N.; Vollinga, R. C.; Bostrom, D.; Kihlberg, J.; Luthman, K. Design, synthesis and evaluation of a PLG tripeptidomimetic based on a pyridine scaffold. *J. Med. Chem.* **2004**, *47*, 6595–6602.

(83) Wijffels, G.; Dalrymple, B. P.; Prosselkov, P.; Kongsuwan, K.; Epa, V. C.; Lilley, P. E.; Jergic, S.; Buchardt, J.; Brown, S. E.; Alewood, P. F.; Jennings, P. A.; Dixon, N. E. Inhibition of protein interactions with the β2 sliding clamp of *Escherichia coli* DNA polymerase III by peptides from beta(2)-binding proteins. *Biochemistry* **2004**, *43*, 5661–5671.

(84) Dalrymple, B. P.; Kongsuwan, K.; Wijffels, G.; Dixon, N. E.; Jennings, P. A. A universal protein−protein interaction motif in the eubacterial DNA replication and repair systems. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 11627–11632.

(85) Alewood, P.; Briscoe, S.; Buchardt, J.; Christensen, C.; Dalrymple, B.; Dixon, N. E.; Epa, V. C.; Hofmann, A.; Jennings, P.; Oakley, A.; Polley, M.; Wijffels, G.; Winkler, D. A. Bacterial replisome β clamp inhibitors as potential broad-spectrum antimicrobials. Unpublished results.

(86) Murphy, P. M.; Baggiolini, M.; Charo, I. F.; Hebert, C. A.; Horuk, R.; Matsushima, K. International union of pharmacology: XXII. Nomenclature for chemokine receptors. *Pharmacol. Rev.* **2000**, *52*, 145–176.

(87) Holmes, W. E.; Lee, J.; Kuang, W. J.; Rice, G. C.; Wood, W. I. Structure and functional expression of a human interleukin-8 receptor. *Science* **1991**, *253*, 1278–1280.

(88) Murphy, P. M.; Tiffany, H. L. Cloning of complementary DNA encoding a functional human interleukin-8 receptor. *Science* **1991**, *253*, 1280–1283.

(89) Mathison, R. Modulation of neutrophil function by hormones. *Curr. Immunol. Rev.* **2006**, *2*, 247–259.

(90) Bizzarri, C.; Beccari, A. R.; Bertini, R.; Cavicchia, M. R.; Giorgini, S.; Allegretti, M. ELR+ CXC chemokines and their receptors (CXC chemokine receptor 1 and CXC chemokine receptor 2) as new therapeutic targets. *Pharmacol. Ther.* **2006**, *112*, 139–149.

(91) Widdowson, K. L.; Elliott, J. D.; Veber, D. F.; Nie, H.; Rutledge, M. C.; McCleland, B. W.; Xiang, J. N.; Jurewicz, A. J.; Hertzberg, R. P.; Foley, J. J.; Griswold, D. E.; Martin, L.; Lee, J. M.; White, J. R.; Sarau, H. M. Evaluation of potent and selective small-molecule antagonists for the CXCR2 chemokine receptor. *J. Med. Chem.* **2004**, *47*, 1319–1321.

(92) Bertini, R.; Bizzari, C.; Sabbatini, V.; Porzio, S.; Caselli, G.; Allegretti, M. *N*-(2-Aryl-propionyl)-sulfonamides and Pharmaceutical Preparations Containing Them. WO 2000024710, 2000.

(93) Widdowson, K. L. IL-8 Receptor Antagonist. WO 1997049-286, 1997.

(94) Bertini, R.; Allegretti, M.; Bizzarri, C.; Moriconi, A.; Locati, M.; Zampella, G.; Cervellera, M. N.; Di Cioccio, V.; Cesta, M. C.; Galliera, E.; Martinez, F. O.; Di Bitondo, R.; Troiani, G.; Sabbatini, V.; D'Anniballe, G.; Anacardio, R.; Cutrin, J. C.; Cavalieri, B.; Mainiero, F.; Strippoli, R.; Villa, P.; Di Girolamo, M.; Martin, F.; Gentile, M.; Santoni, A.; Corda, D.; Poli, G.; Mantovani, A.; Ghezzi, P.; Colotta, F. Noncompetitive allosteric inhibitors of the inflammatory chemokine receptors CXCR1 and CXCR2: prevention of reperfusion injury. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 11791–11796.

(95) Batchelor, D. C.; Lin, H.; Wen, J. Y.; Keven, C.; Van Zijl, P. L.; Breier, B. H.; Gluckman, P. D.; Thomas, G. B. Pharmacokinetics of glycine-proline-glutamate, the N-terminal tripeptide of insulin-like growth factor-1, in rats. *Anal. Biochem.* **2003**, *323*, 156–163.

(96) Guan, J.; Waldvogel, H. J.; Faull, R. L.; Gluckman, P. D.; Williams, C. E. The effects of the N-terminal tripeptide of insulin-like growth factor-1, glycine-proline-glutamate in different regions following hypoxic-ischemic brain injury in adult rats. *Neuroscience* **1999**, 649–659.

(97) Saura, J.; Curatolo, L.; Williams, C. E.; Gatti, S.; Benatti, L.; Peeters, C.; Guan, J.; Dragunow, M.; Post, C.; Faull, R. L.; Gluckman, P. D.; Skinner, S. J. Neuroprotective effects of Gly-Pro-Glu, the N-terminal tripeptide of IGF-1, in the hippocampus in vitro. *NeuroReport* **1999**, *10*, 161–164.

(98) Sizonenko, S. V.; Sirimanne, E. S.; Williams, C. E.; Gluckman, P. D. Neuroprotective effects of the N-terminal tripeptide of IGF-1, glycine-proline-glutamate, in the immature rat brain after hypoxic−ischemic injury. *Brain Res.* **2001**, *922*, 42–50.

(99) De Diego, S. A. A.; Gutierrez-Rodriguez, M.; de Vega, M. J. P.; Casabona, D.; Cativiela, C.; Gonzalez-Muniz, R.; Herranz, R.; Cenarruzabeitia, E.; Frechilla, D.; Del Rio, J.; Jimeno, M. L.; Garcia-Lopez, M. T. New Gly-Pro-Glu (GPE) analogues: expedite solid-phase synthesis and biological activity. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1392–1396.

(100) Alonso De Diego, S. A.; Gutierrez-Rodriguez, M.; Perez de Vega, M. J.; Gonzalez-Muniz, R.; Herranz, R.; Martin-Martinez, M.; Cenarruzabeitia, E.; Frechilla, D.; Del Rio, J.; Jimeno, M. L.; Garcia-Lopez, M. T. The neuroprotective activity of GPE tripeptide analogues does not correlate with glutamate receptor binding affinity. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3396–3400.

(101) Alonso DeDiego, S. A.; Munoz, P.; Gonzalez-Muniz, R.; Herranz, R.; Martin-Martinez, M.; Cenarruzabeitia, E.; D., F.; Del Rio, J.; Jimeno, M. L.; Garcia-Lopez, M. T. Analogues of the neuroprotective tripeptide Gly-Pro-Glu (GPE): synthesis and structure−activity relationships. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2279–2283.

(102) Brimble, M. A.; Trotter, N. S.; Harris, P. W. R.; Sieg, F. Synthesis and pharmacological evaluation of side chain modified glutamic acid analogues of the neuroprotective agent glycyl-L-prolyl-L-glutamic acid (GPE). *Bioorg. Med. Chem.* **2005**, *13*, 519–532.

(103) Harris, P. W. R.; Brimble, M. A.; Muir, V. J.; Lai, M. Y. H.; S., T. N.; Callis, D. J. Synthesis of proline-modified analogues of the neuroprotective agent glycyl-L-prolyl-L-glutamic acid (GPE). *Tetrahed.* **2005**, *61*, 10018–10035.

(104) Lai, M. Y. H.; Brimble, M. A.; Callis, D. J.; Harris, P. W. R.; Levi, M. S.; Sieg, F. Synthesis and pharmacological evaluation of glycine-modified analogues of the neuroprotective agent glycyl-L-prolyl-L-glutamic acid (GPE). *Bioorg. Med. Chem.* **2005**, *13*, 533–548.

(105) Trotter, N. S.; Brimble, M. A.; Harris, P. W.; Callis, D. J.; Sieg, F. Synthesis and neuroprotective activity of analogues of glycyl-L-prolyl-L-glutamic acid (GPE) modified at the α-carboxylic acid. *Bioorg. Med. Chem.* **2005**, *13*, 501–517.

(106) Kisselev, L.; Ehrenberg, M.; Frolova, L. Termination of translation: interplay of mRNA, rRNAs and release factors? *EMBO J.* **2003**, *22*, 175–182.

(107) Frolova, L. Y.; Tsivkovskii, R. Y.; Sivolobova, G. F.; Oparina, N. Y.; Serpinsky, O. I.; Blinov, V. M.; Tatkov, S. I.; Kisselev, L. L. Mutations in the highly conserved GGQ motif of class 1 polypeptide release factors abolish ability of human eRF1 to trigger peptidyl-tRNA hydrolysis. *RNA* **1999**, *5*, 1014–1020.

(108) Williams, G.; Williams, E. J.; Doherty, P. Dimeric versions of two short N-cadherin binding motifs (HAVDI and INPISG) function as N-cadherin agonists. *J. Biol. Chem.* **2002**, *277*, 4361–4367.

(109) Williams, E. J.; Furness, J.; Walsh, F. S.; Doherty, P. Activation of the Fgf receptor underlies neurite outgrowth stimulated by L1, N-CAM, and N-cadherin. *Neuron* **1994**, *13*, 583–594.

(110) Halbleib, J. M.; Nelson, W. J. Cadherins in development: cell adhesion, sorting, and tissue morphogenesis. *Genes Dev.* **2006**, *20*, 3199–3214.

(111) Gumbiner, B. M. Regulation of cadherin-mediated adhesion in morphogenesis. *Nat. Rev. Mol. Cell Biol.* **2005**, *6*, 622–634.

(112) Pokutta, S.; Weis, W. I. Structure and mechanism of cadherins and catenins in cell−cell contacts. *Annu. Rev. Cell Dev. Biol.* **2007**, *23*, 237–261.

(113) Blaschuk, O. W.; Sullivan, R.; David, S.; Pouliot, Y. Identification of a cadherin cell-adhesion recognition sequence. *Dev. Biol.* **1990**, *139*, 227–229.

(114) Williams, E. J.; Williams, G.; Gour, B.; Blaschuk, O.; Doherty, P. INP, a novel N-cadherin antagonist targeted to the amino acids that flank the HAV motif. *Mol. Cell. Neurosci.* **2000**, *15*, 456–464.

(115) Williams, E.; Williams, G.; Gour, B. J.; Blaschuk, O. W.; Doherty, P. A novel family of cyclic peptide antagonists suggests that N-cadherin specificity is determined by amino acids that flank the HAV motif. *J. Biol. Chem.* **2000**, *275*, 4007–4012.

(116) Makagiansar, I. T.; Avery, M.; Hu, Y.; Audus, K. L.; Siahaan, T. J. Improving the selectivity of HAV-peptides in modulating E-cadherin−E-cadherin interactions in the intercellular junction of MDCK cell monolayers. *Pharm. Res.* **2001**, *18*, 446–453.

(117) Lutz, K. L.; Jois, S. D. S.; Siahaan, T. J. Secondary structure of the HAV peptide which regulates cadherin-cadherin interaction. *J. Biomol. Struct. Dyn.* **1995**, *13*, 447–455.

(118) Burden-Gulley, S. M.; Gates, T. J.; Craig, S. E. L.; Lou, S. F.; Oblander, S. A.; Howell, S.; Gupta, M.; Brady-Kalnay, S. M. Novel peptide mimetic small molecules of the HAV motif in N-cadherin inhibit N-cadherin-mediated neurite outgrowth and cell adhesion. *Peptides* **2009**, *30*, 2380–2387.

(119) Burden-Gulley, S. M.; Gates, T. J.; Craig, S. E. L.; Gupta, M.; Brady-Kalnay, S. M. Stimulation of N-cadherin-dependent neurite outgrowth by small molecule peptide mimetic agonists of the N-cadherin HAV motif. *Peptides* **2010**, *31*, 842–849.

(120) Kawasaki, M.; Maeda, T.; Hanasawa, K.; I., O.; T., T. Effect of His-Gly-Lys motif derived from domain 5 of high molecular weight kininogen on suppression of cancer metastasis both in vitro and in vivo. *J. Biol. Chem.* **2003**, *278*, 49301–49307.

(121) Giebel, L. B.; Cass, R. T.; Milligan, D. L.; Young, D. C.; Arze, R.; Johnson, C. R. Screening of cyclic peptide phage libraries identifies ligands that bind streptavidin with high affinities. *Biochemistry* **1995**, *34*, 15430–15435.

(122) Skerra, A.; Schmidt, T. G. M. Use of the strep-tag and streptavidin for detection and purification of recombinant proteins. *Methods Enzymol.* **2000**, *326*, 271–304.

(123) Katz, B. A.; Cass, R. T. Crystals of complexes of streptavidin with peptide ligands containing the HPQ sequence the pK_a of the peptide histidine is less than 3.0. *J. Biol. Chem.* **1997**, *272*, 13220–13228.

(124) Katz, B. A. Streptavidin-binding and -dimerizing ligands discovered by phage display, topochemistry, and structure-based design. *Biomol. Eng.* **1999**, *16*, 57–65.

(125) Brzoska, T.; Luger, T. A.; Maaser, C.; Abels, C.; Bohm, M. Melanocyte-stimulating hormone and related tripeptides: biochemistry, antiinflammatory and protective effects in vitro and in vivo, and future perspectives for the treatment of immune-mediated inflammatory diseases. *Endocr. Rev.* **2008**, *29*, 581–602.

(126) Cutuli, M.; Cristiani, S.; Lipton, J. M.; Catania, A. J. Antimicrobial effects of alpha-MSH peptides. *J. Leukocyte Biol.* **2000**, *67*, 233–239.

(127) Komoriya, A.; Green, L. J.; Mervic, M.; Yamada, S. S.; Yamada, K. M.; Humphries, M. J. The minimal essential sequence for a major cell type-specific adhesion site (Cs1) within the alternatively spliced type-III connecting segment domain of fibronectin is leucine-aspartic acid-valine. *J. Biol. Chem.* **1991**, *266*, 15075–15079.

(128) Mould, A. P.; Komoriya, A.; Yamada, K. M.; Humphries, M. J. The CS5 peptide is a 2nd site in the IIIC S region of fibronectin recognized by the integrin $\alpha4\beta_1$. Inhibition of alpha-4-beta-1 function by RGD peptide homologs. *J. Biol. Chem.* **1991**, *266*, 3579–3585.

(129) Akiyama, S. K.; Olden, K.; Yamada, K. M. Fibronectin and integrins in invasion and metastasis. *Cancer Metastasis Rev.* **1995**, *14*, 173–189.

(130) Doyle, P. M.; Harris, J. C.; Moody, C. M.; Sadler, P. J.; Sims, M.; Thornton, J. M.; Uppenbrink, J.; Viles, J. H. Solution structure of a biologically active cyclic LDV peptide analogue containing a type II′ beta-turn mimetic. *Int. J. Pept. Protein Res.* **1996**, *47*, 427–436.

(131) Viles, J. H.; Mitchell, J. B. O.; Gough, S. L.; Doyle, P. M.; Harris, C. J.; Sadler, P. J.; Thornton, J. M. Multiple solution conformations of the integrin-binding cyclic pentapeptide cyclo(-Ser-D-Leu-Asp-Val-Pro-) analysis of the (phi,psi) space available to cyclic pentapeptides. *Eur. J. Biochem.* **1996**, *242*, 352–362.

(132) Jackson, D. Y.; Quan, C.; Artis, D. R.; Rawson, T.; Blackburn, B.; Struble, M.; Fitzgerald, G.; Chan, K.; Mullins, S.; Burnier, J. P.; Fairbrother, W. J.; Clark, K.; Berisini, M.; Chui, H.; Renz, M.; Jones, S.; Fong, S. Potent alpha 4 beta 1 peptide antagonists as potential anti-inflammatory agents. *J. Med. Chem.* **1997**, *40*, 3359–3368.

(133) Yokosaki, Y.; Matsuura, N.; Higashiyama, S.; Murakami, I.; Obara, M.; Yamakido, M.; Shigeto, N.; Chen, J.; Sheppard, D. Identification of the ligand binding site for the integrin $\alpha9\beta_1$ in the third fibronectin type III repeat of tenascin-C. *J. Biol. Chem.* **1998**, *273*, 11423–11428.

(134) Lin, K. C.; Ateeq, H. S.; Hsiung, S. H.; Chong, L. T.; Zimmerman, C. N.; Castro, A.; Lee, W. C.; Hammond, C. E.; Kalkunte, S.; Chen, L. L.; Pepinsky, R. B.; Leone, D. R.; Sprague, A. G.; Abraham, W. M.; Gill, A.; Lobb, R. R.; Adams, S. P. Selective, tight-binding inhibitors of integrin $\alpha4\beta_1$ that inhibit allergic airway responses. *J. Med. Chem.* **1999**, *42*, 920–934.

(135) Yang, G. X.; Hagmann, W. K. VLA-4 antagonists: potent inhibitors of lymphocyte migration. *Med. Res. Rev.* **2003**, *23*, 369–392.

(136) Chen, L.; Tilley, J.; Trilles, R. V.; Yun, W. Y.; Fry, D.; Cook, C.; Rowan, K.; Schwinge, V.; Campbell, R. *N*-Acyl-L-phenylalanine derivatives as potent VLA-4 antagonists that mimic a cyclic peptide conformation. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 137–140.

(137) Hagmann, W. K. The discovery and potential of N-sulfonylated dipeptide VLA-4 antagonists. *Curr. Top. Med. Chem.* **2004**, *4*, 1461–1471.

(138) Hagmann, W. K.; Durette, P. L.; Lanza, T.; Kevin, N. J.; de Laszlo, S. E.; Kopka, H. E.; Young, D.; Magriotis, P. A.; Li, B.; Lin, L. S.; Yang, G.; Kamenecka, T.; Chang, L. L.; Wilson, J.; MacCoss, M.; Mills, S. G.; Van Riper, G.; McCauley, E.; Egger, L. A.; Kidambi, U.; Lyons, K.; Vincent, S.; Stearns, R.; Colletti, A.; Teffera, J.; Tong, S.; Fenyk-Melody, J.; Owens, K.; Levorse, D.; Kim, P.; Schmidt, J. A.; Mumford, R. A. The discovery of sulfonylated dipeptides as potent VLA-4 antagonists. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2709–2713.

(139) Tilley, J. W. Very late antigen-4 integrin antagonists. *Expert Opin. Ther. Pat.* **2008**, *18*, 841–859.

(140) Pierschbacher, M. D.; Ruoslahti, E. Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule. *Nature* **1984**, *309*, 30–33.

(141) Ojima, I.; Chakravarty, S.; Dong, Q. Antithrombotic agents: from RGD to peptide mimetics. *Bioorg. Med. Chem.* **1995**, *3*, 337–360.

(142) Wang, W.; Borchardt, R. T.; Wang, B. Orally active peptidomimetic RGD analogs that are glycoprotein IIb/IIIa antagonists. *Curr. Med. Chem.* **2000**, *7*, 437–453.

(143) Adair, B. D.; Yeager, M. Three-dimensional model of the human platelet integrin $\alpha IIb\beta_3$ based on electron cryomicroscopy and X-ray crystallography. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 14059–14064.

(144) Feuston, B. P.; Culberson, J. C.; Hartman, G. D. Molecular model of the $\alpha IIb\beta_3$ integrin. *J. Med. Chem.* **2003**, *46*, 5316–5325.

(145) Claasen, B.; Axmann, M.; Meinecke, R.; Meyer, B. Direct observation of ligand binding to membrane proteins in living cells by a saturation transfer double difference (STDD) NMR spectroscopy method shows a significantly higher affinity of integrin $\alpha IIb\beta_3$ in native platelets than in liposomes. *J. Am. Chem. Soc.* **2005**, *127*, 916–919.

(146) Xiao, T.; Takagi, J.; Coller, B. S.; Wang, J.-H.; Springer, T. A. Structural basis for allostery in integrins and binding to fibrinogen-mimetic therapeutics. *Nature* **2004**, *432*, 59–67.

(147) Zablocki, J. A.; Miyano, M.; Garland, R. B.; Pireh, D.; Schretzman, L.; Rao, S. N.; Lindmark, R. J.; Panzerknodle, S. G.; Nicholson, N. S.; Taite, B. B.; Salyers, A. K.; King, L. W.; Campion, J. G.; Feigen, L. P. Potent in vitro and in vivo inhibitors of platelet aggregation based upon the Arg-Gly-Asp-Phe sequence of fibrinogen. A proposal on the nature of the binding interaction between the Arg-guanidine of RGDX mimetics and the platelet GP IIb-IIIa receptor. *J. Med. Chem.* **1993**, *36*, 1811–1819.

(148) Alig, L.; Edenhofer, A.; Hadvary, P.; Hurzeler, M.; Knopp, D.; Muller, M.; Steiner, B.; Trzeciak, A.; Weller, T. Low molecular weight, non-peptide fibrinogen receptor antagonists. *J. Med. Chem.* **1992**, *35*, 4393–4407.

(149) Jones, C. R.; Ambros, R. J.; Rapold, H. J.; Steiner, B.; Weller, T.; van Heiningen, P.; Crijins, H. J. M. J.; Jonkman, J. H. G.; Hadvary, P. A novel non peptide GPIIb/IIIa antagonist in man. *Thromb. Haemostasis* **1993**, *69*, 560.

(150) Scarborough, R. M.; Rose, J. W.; Hsu, M. A.; Phillips, D. R.; Fried, V. A.; Campbell, A. M.; Nannizzi, L.; Charo, I. F. Barbourin. A GPIIb-IIIa-specific integrin antagonist from the venom of Sistrurus m. barbouri. *J. Biol. Chem.* **1991**, *266*, 9359–9362.

(151) Scarborough, R. M.; Naughton, M. A.; Teng, W.; Rose, J. W.; Phillips, D. R.; Nannizzi, L.; Arfsten, A.; Campbell, A. M.; Charo, I. F. Design of potent and specific integrin antagonists. Peptide antagonists with high specificity for glycoprotein IIb-IIIa. *J. Biol. Chem.* **1993**, *268*, 1066–1073.

(152) Blum, J. E.; Handmaker, H. Small peptide radiopharmaceuticals in the imaging of acute thrombus. *Curr. Pharm. Des.* **2002**, *8*, 1815–26.

(153) Haubner, R.; Wester, H. J. Radiolabeled tracers for imaging of tumor angiogenesis and evaluation of anti-angiogenic therapies. *Curr. Pharm. Des.* **2004**, *10*, 1439–55.

(154) Gould, S. J.; Keller, G.; Subramani, S. Identification of a peroxisomal targeting signal at the carboxy terminus of firefly luciferase. *J. Cell Biol.* **1987**, *105*, 2923–2931.

(155) Gould, S. J.; Keller, G.; Hosken, N.; Wilkinson, J.; Subramani, S. A conserved tripeptide sorts proteins to peroxisomes. *J. Cell Biol.* **1989**, *108*, 1657–1664.

(156) Gatto, G. J. J.; Geisbrecht, B. V.; Gould, S. J.; Berg, J. M. Peroxisomal targeting signal-1 recognition by the TPR domains of human PEX5. *Nat. Struct. Biol.* **2000**, *7*, 1091–1095.

(157) Brocard, C.; Hartig, A. Peroxisome targeting signal 1: Is it really a simple tripeptide? *Biochim. Biophys. Acta, Mol. Cell Res.* **2006**, *1763*, 1565–1573.

(158) Lam, K. S.; Lebl, M.; Krchnak, V. The "one-bead-one-compound" combinatorial library method. *Chem. Rev.* **1997**, *97*, 411–448.

(159) Boger, D. L.; Desharnais, J.; Capps, K. Solution-phase combinatorial libraries: modulating cellular signaling by targeting protein-protein or protein−DNA interactions. *Angew. Chem., Int. Ed.* **2003**, *42*, 4138–4176.