Peptidomimetics and Angiogenesis

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Abstract: Angiogenesis is the sprouting of new blood capillaries from surrounding preexisting blood vessels. This process is fundamental for embryonic development, wound healing and inflammation. In healthy adults angiogenesis is of minor importance. However, aberrant angiogenesis is essentially involved in disorders as diabetic retinopathy, rheumatoid arthritis and tumor growth, and blocking angiogenesis has emerged as a promising target for antagonizing these diseases. Therefore the development of new anti-angiogenic drugs is of great interest in academic and industrial research.

This review focuses on the employment of peptidomimetics in inhibiting pathologic angiogenesis. It will survey the individual aspects of angiogenesis where the usage of peptidomimetics is favored and will consider the current progresses on this field.

1. INTRODUCTION

Angiogenesis, or neovascularization, is the process in which new blood capillaries are formed from the surrounding pre-existing blood vessels. This process is of fundamental importance for embryogenesis, development, wound healing and inflammation. In healthy adults angiogenesis occurs normally only in the female reproductive tract. On the other side, aberrant neovascularization takes place in diabetic retinopathy, chronic inflammatory diseases and malignant processes [1]. Due to the observation that an adequate blood supply is necessary for tumor growth, Folkman postulated in 1971 that inhibitors of angiogenesis are of potential use in cancer therapy [2]. Subsequent investigations revealed that not only tumor growth but also tumor metastasis depends on angiogenesis [3,4]. In 1999 Richard Klausner, director of the U.S. National Cancer Institute, designated the development of antiangiogenic therapies for cancer as an issue of national priority. In the recent years many endogenous regulators of angiogenesis have been isolated and identified. Among the most important positive regulators are growth factors like vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), tumor necrosis factor α (TNF-α) and transforming growth factor β (TGF-β) [5]. An additional component controlling the progress of neovascularization on several stages is a protein derived from endothelial cells, SPARC (secreted protein, acidic, cystein-rich). The proteolytic degradation of SPARC generates fragments containing the sequence Gly-His-Lys which stimulates angiogenesis both *in vitro* and *in vivo* [6-8]. Two of the most potent negative regulators are endostatin [9] and angiostatin [10,11].

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Angiogenesis is a complex process which covers the following steps (scheme 1). First the endothelial cells (EC) and pericytes are activated by the angiogenic growth factors, the majority of which binds to receptor tyrosine kinases. Ligand binding triggers receptor dimerization and subsequent autophosphorylation of tyrosine residues [12]. The subsequent signalling cascade can be divided in two major pathways which are illustrated in scheme 2: The protein kinase b pathway (PKB or Akt) and the Ras-Raf-kinase pathway. The initial step of the first pathway is the activation of the PI3(phosphatidylinositol)-kinase, which yields in higher levels of the second messenger 3,4,5-phosphatidylinositoltrisphosphate (PIP3). Activation of PDK-1 by PIP3 leads to the phosphorylation of PKB which is a known mediator of cell survival [13]. The stimulation of endothelial NO-Synthase (eNOS) by PKB induces the synthesis of the second messenger NO which finally leads to the cellular response in this branch.

The Ras-Raf-pathway transduces the growth factor receptor signal via a phosphorylation cascade of certain kinases as the MAP kinase [14]. The MAP kinase in turn is responsible for the activation of the transcription factor Ets-1 [15,16]. In endothelial cells Ets-1 is crucial for the activation of numerous genes involved in cell cycle control and proliferation. Both pathways result in the expression of matrixmetalloproteinases (MMPs), urokinase type plasminogen activators (uPA) and integrins on the cellular surface.

To initiate the formation of new capillaries, endothelial cells of existing blood vessels have to degrade the underlying basement membrane and invade into the stroma of the neighboring tissue [17]. These processes of endothelial cell invasion require the activity of proteases like collagenases, plasminogen activator and MMPs. Then the endothelial cells migrate within the extracellular matrix towards the angiogenic stimulus. This migration is enabled by integrins and the continuous action of proteases. After the formation of a new capillary lumen and the appearance of pericytes around the new forming vessel, a new basal lamina

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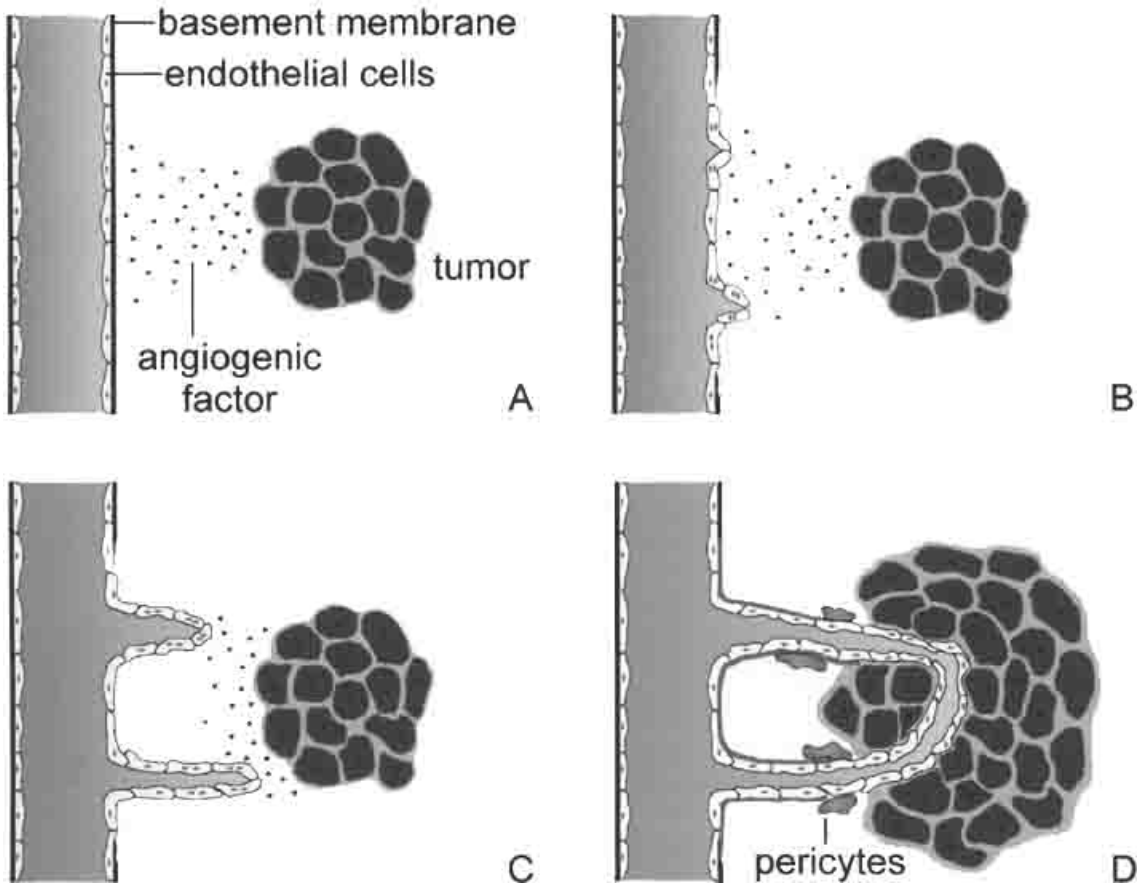


Scheme 1. The complex process of angiogenesis comprises the following steps: A) endothelial cell activation by growth factors and (B) subsequent degradation of the blood vessels' basement membrane by proteinases (collagenases, plasminogen activator), C) migration and proliferation of endothelial cells towards the angiogenic stimulus. Matrix metalloproteinases are recruited for extracellular matrix remodeling also during this step; D) formation of a new basement membrane around the immature blood vessels and merging of the ends of two outgrowing blood vessels (anastomosis). In general tumor vascularization also stimulates tumor growth.

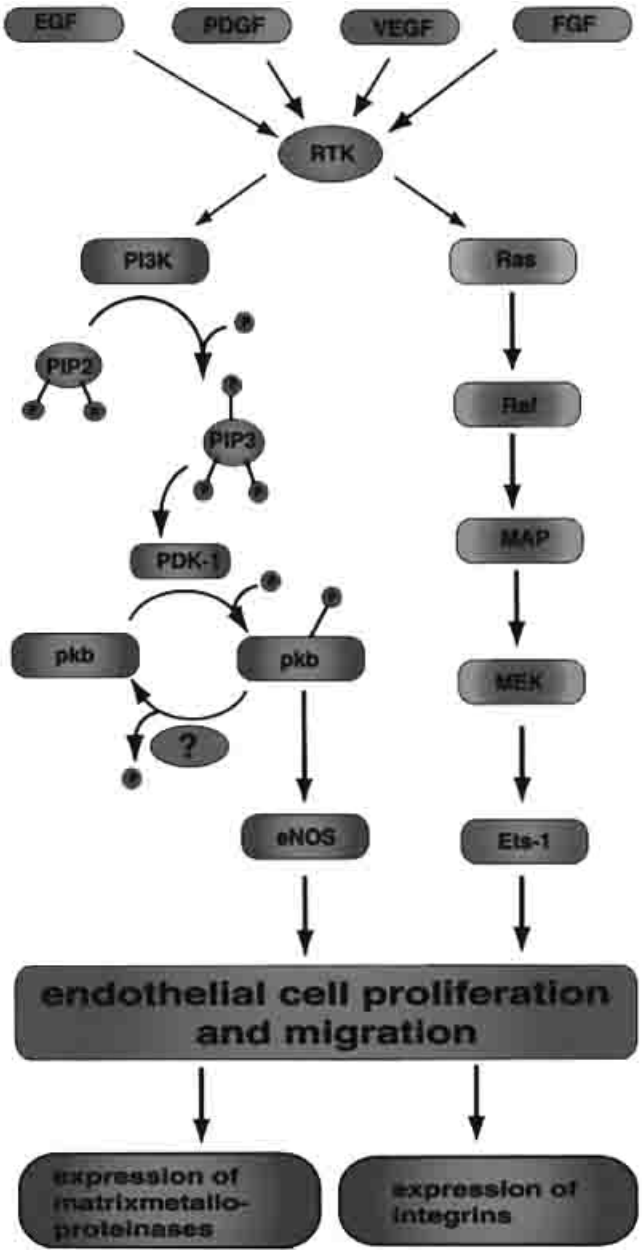
is developed. Finally, the anastomosis of the proximal ends from two new forming vessels enables the blood flow [18].

Each of the processes described above represent possible points of pharmacological intervention. This review will focus on the application of peptidomimetics [19] that target at different key players of the angiogenic process.

2.1 Peptidomimetics and Integrins

Integrins are one major class of cell surface glycoproteins which act as receptors for cell-cell and cell-matrix interaction [20,21]. These interactions are critical for important biological phenomena such as cell morphology, differentiation, organogenesis, angiogenesis and blood clotting. All integrins are heterodimers consisting of an α-subunit and a smaller β-subunit. Each of these subunits have a large extracellular portion, a single transmembrane domain and a short cytoplasmatic tail [22,23]. The integrin family includes at least 16 different α- and 8 β-subunits, which associate with each other to form more than 20 distinct integrins. The individual integrins are specific for different cell types, they have their own binding specificity and signaling properties. One member of this receptor class is the $\alpha_v\beta_3$-integrin (vitronectin receptor). The $\alpha_v\beta_3$-integrin mediates the adhesion of endothelial cells to the extracellular matrix by interaction with the tripeptid motif RGD (Arg-Gly-Asp). Binding of this ligand occurs in multivalent form and leads to a clustering of $\alpha_v\beta_3$-integrins [24]. This in turn initiates an intracellular signaling cascade which supports both, migration of endothelial cells and protection of the cells from apoptosis [25]. In contrast interaction of $\alpha_v\beta_3$-integrins with monovalent ligands or their analogs induces apoptosis in migrating endothelial cells and therefore prevents neovascularization [26]. New insights into integrin function and regulation have been provided recently by solving the crystal structure of the extracellular segment of the $\alpha_v\beta_3$-integrin [27]

The RGD sequence is a structural element of proteins belonging to the extracellular matrix, like vitronectin, fibronectin, osteopontin, thrombospondin and von Willebrandt factor. It also occurs in the blood protein fibrinogen, which is essential for blood coagulation. The RGD group serves not only as a ligand for the $\alpha_v\beta_3$ integrin, but also as ligand for additional integrins, e.g. $\alpha_5\beta_1$, $\alpha_v\beta_1$, $\alpha_v\beta_5$, $\alpha_v\beta_6$ as well as for the platelet integrin $\alpha_{IIb}\beta_3$ (fibrinogen-receptor). The specificity of the integrin-RGD interaction is generated by a combination of variations in the RGD conformation in different proteins and contributions of sequences near the RGD moiety [21]. Structure-activity



Scheme 2. Growth factor induced expression of MMPs and integrins. Growth factors bind on specific receptor tyrosine kinases (RTK) which transduce the signal through the plasma membrane by phosphorylation of distinct tyrosine residues (not shown). The different kinases PI3K and Ras are in turn activated by individual adaptor proteins leading to a branching of the transduction pathway at this point. PI3K-pathway: Activated PI3K phosphorylates the PIP2 to the second messenger PIP3. PIP3 stimulates PDK-1 which phosphorylates Akt/PKB. The phosphorylated pkb induces synthesis of NO by activation of eNOS. The increased levels of NO leading to the cellular response. Ras-Raf pathway: The signal is transduced by a cascade of kinase activation by phosphorylations (Raf, MEK, MAP). MAP phosphorylates the transcription factor Ets-1 which is than translocalized to the nucleus, resulting in transcription of certain genes.

relationships revealed that the replacement of glycine by alanine or aspartate by glutamate within linear RGD-containing peptides prevent recognition and binding of the resulting peptides by the integrins [28]. For a long time the search for RGD analogs was driven by the demand for potent, selective and orally available $\alpha_{IIb}\beta_3$-antagonists as inhibitors of platelet coagulation [29,30]. These anticoagulants constitute the first clinically valuable anti-integrins and represent an important contribution for the treatment of thromboembolic diseases. Encouragement obtained by the considerably successful results together with further insights into the importance of angiogenesis for tumor growth and metastasis prompted several groups in the pharmaceutical industry and the universities to search extensively for antagonists of the endothelial cell integrin $\alpha_v\beta_3$ in order to block angiogenesis.

One major problem in the design of $\alpha_v\beta_3$-antagonists is their selectivity with regard to other integrins. A suitable antagonist should have high affinity to the vitronectin receptor as well as favorable pharmacokinetic properties. It

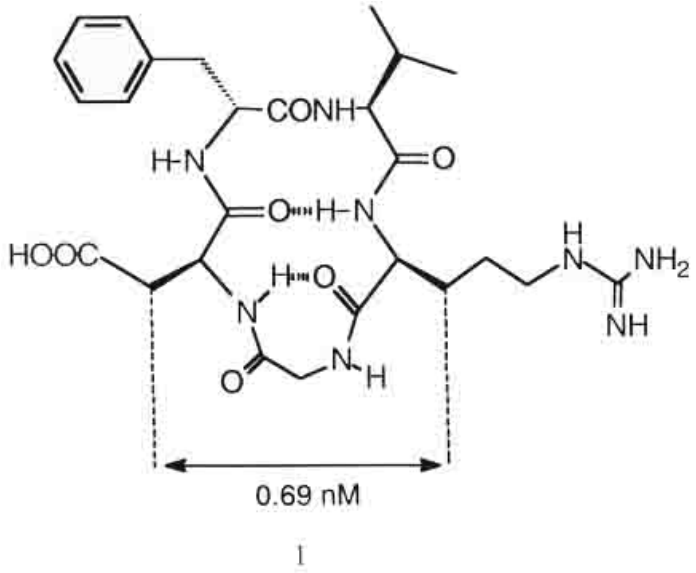


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Fig (1). Cyclopentapeptide c(RGDfV).

is desirable to develop potent, selective and orally available nonpeptidic ligands. The oral availability is of particular importance since it will be necessary in cancer therapy to block angiogenesis for a long period of time.

In order to elucidate the recogition and binding of the RGD motif by the $\alpha_v\beta_3$-integrin H. Kessler and co-workers incorporated this tripeptide sequence into different cyclopentapeptides. The affinities and selectivities of the individual cyclopeptides towards individual integrins were investigated, revealing the cyclopentapeptide cyclo(Arg-Gly-Asp-D-Phe-Val) (c(RGDfV)) **1** as a selective and potent inhibitor for the vitronectin receptor with an IC50 value of 50 nM [31,32].

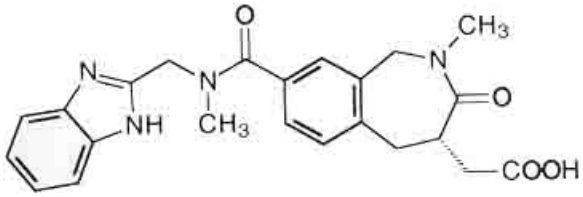
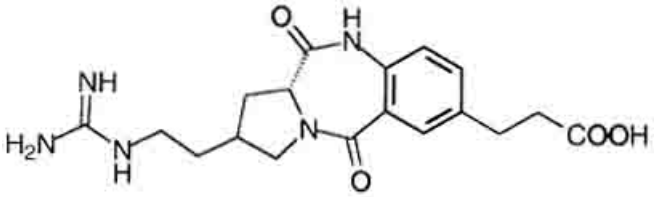
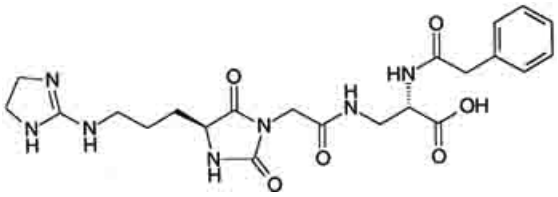
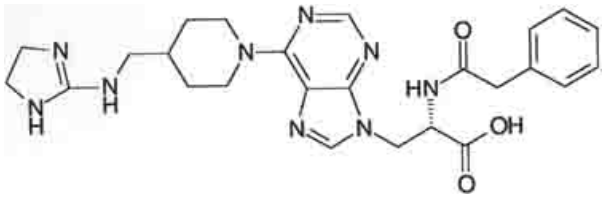
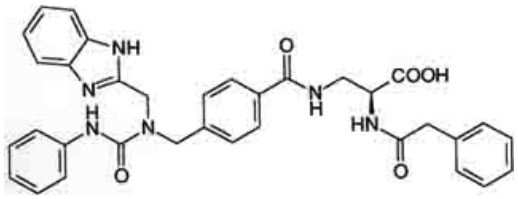
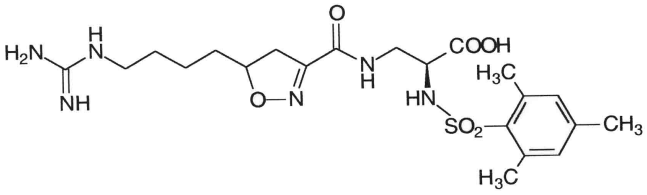
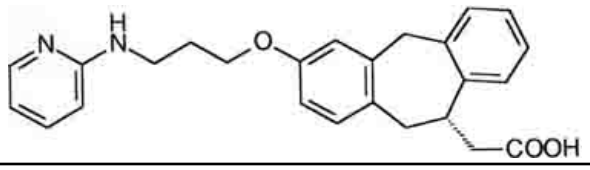
According to NMR-spectroscopic investigations in solution the cyclic peptide shows a βII´/γ-turn arrangement. As expected for a *D*-amino acid the Phe occupies the i+1 position of the βII´-turn and therefore determines the positioning of the remaining amino acids, Resulting in an antiparallel arrangement of the side chains of arginine and aspartate and the location of the glycine in the central position of the γ-turn. The distance between the β-carbon atoms of the Asp- and Arg- side chains within peptide **1** was determined to be 0.69 nm, that is considerably shorter than the distance that was thought to be optimal for recognition by the $\alpha_{IIb}\beta_3$-integrin (0.75 - 0.85 nm). To get further insight, all possible stereoisomers of peptide **1** and of its retro-sequence were synthesized (32 peptides) [33]. Interestingly the retro-inverso derivative c(-vFdGr-) showed a drastically reduced affinity towards the $\alpha_v\beta_3$-integrin. This is explained by a conformation different from that of peptide **1**. More importantly, another peptide, c(-VfdGr-), shows nearly no affinity to the vitronectin receptor albeit its identical side chain orientation compared with **1**. This indicates that not only the side chains but also the peptide backbone contributes to receptor binding by formation of at least one hydrogen bond. Furthermore assigning appropriate amino acid residues to the positions flanking the RGD motif are essential for high affinity. Therefore, a sterically demanding lipophilic group in position 4 (*D*-Phe) is required for the biological activity of cyclopeptide **1**. On the other hand the *L*-valine can be replaced by virtually any other amino acid.

Evidence that cyclopeptide **1** is suitable as blocker of angiogenesis was presented by Brooks *et al.* [26]. The authors implanted human melanoma tissue on the chorioallantoic membrane (CAM) of 10 days old chick embryos. After 24 hours a single dose of peptide **1** (300 mg/100 ml) was administered intravenously which led to interruption of tumor neovascularization. A control peptide cyclo-(Arg-Ala-Asp-*D*-Phe-Val) had no influence on growth of new blood vessels. Comparable results were achieved with monoclonal antibodies against $\alpha_v\beta_3$-integrin. In this case, a regression of different tumors implanted on CAM was observed. It is important to note that this treatment had no influence on already existing vessels of the CAM. These studies showed for the first time that $\alpha_v\beta_3$-antagonists are potentially beneficial in the therapy of malignant diseases and other disorders characterized by excessive angiogenesis. More importantly, in subsequent studies Hammes *et al.* [34]. showed that in a mouse model of hypoxia-induced retinal neovascularization twice daily administration of 1 to 20 mg peptide **1** per kilogram of body weight reduced capillary proliferation in a dose-dependent manner without obvious side-effects.

Following the Kessler approach further $\alpha_v\beta_3$-antagonists with a RGD-sequence which has been incorporated in a cyclic peptide backbone have been published by academic and industrial research groups (Figure **2**) [35-39].

Additionally, also the linear peptides or related structures as aza or azacarba peptides [40] with high affinity and selectivity for the vitronectin receptor have been rationally developed [41].

Table 1. Activity of RGD Peptidomimetics Against Integrin $\alpha_v\beta_3$ and Integrin $\alpha_{IIb}\beta_3$

compound	IC_{50} $\alpha_v\beta_3$ [nM]	IC_{50} $\alpha_{IIb}\beta_3$ [nM]	Ref.
	2	30000	42
	4000	-	43
	20	> 10000	44
	50	4000	45
	2,8	1300	46
	0.7	-	48
	4	9000	49

More recently a number of non-peptide $\alpha_v\beta_3$-antagonists have been described. These compounds are all composed of a central scaffold which acts as a spacer bearing the appendages carrying the carboxylate and the guanidino group (or mimetics thereof) in an appropriate distance and orientation. As central scaffolds among others bicyclic and tricyclic benzodiazepines [42,43], hydantoines [44], purines [45], substituted benzoic acids [46,47] isoxazolines [48] and dibenzocycloheptanes [49] have been used successfully with good to excellent selectivity towards the $\alpha_v\beta_3$- over the $\alpha_{IIb}\beta_3$-integrin (Table 1).

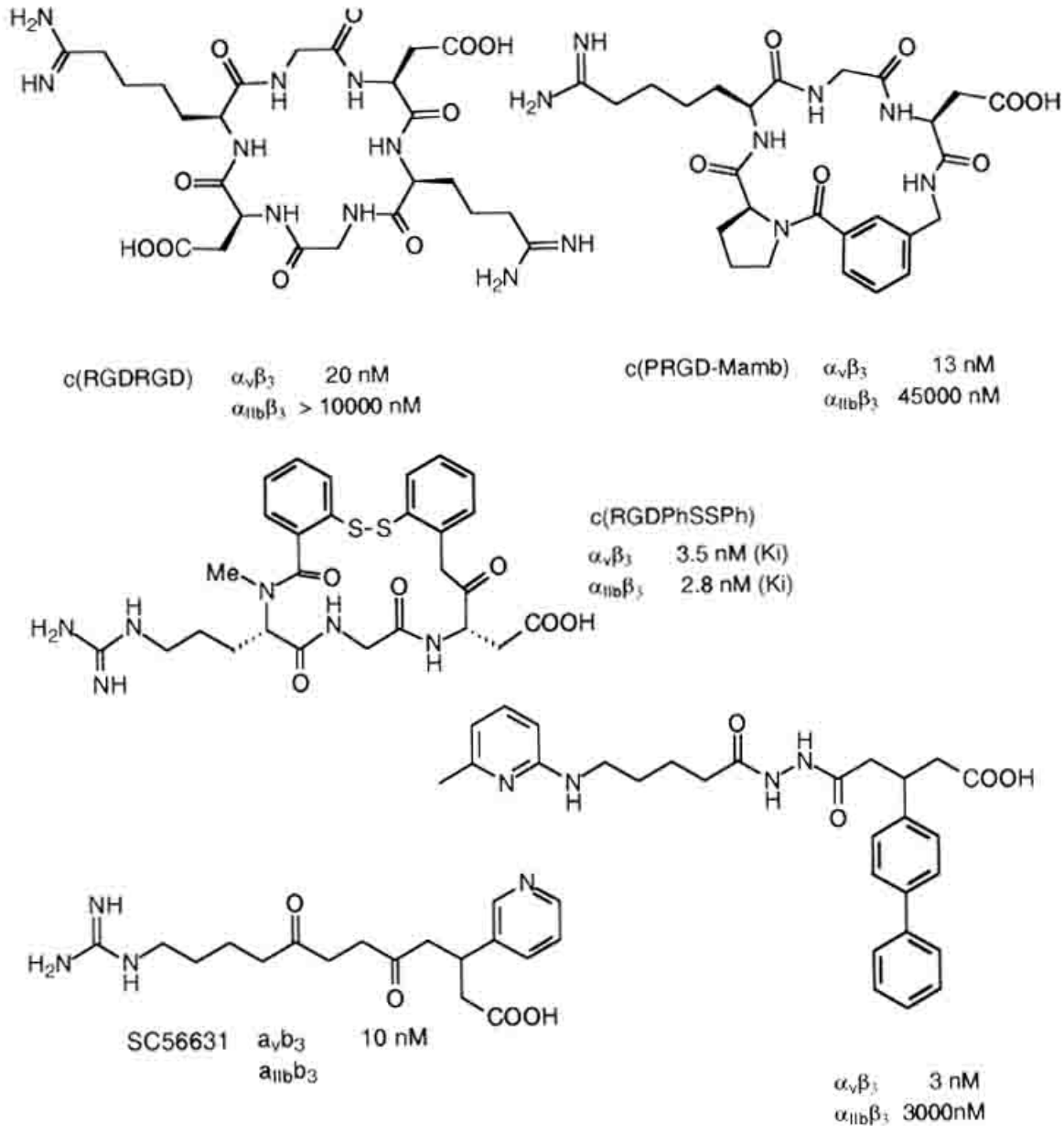
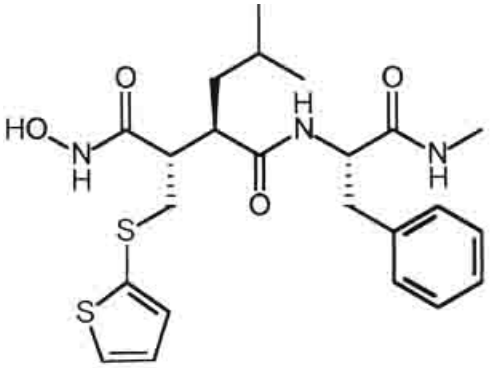


Fig. (2). Selected cyclic and linear peptidic antagonists of integrin $\alpha v\beta_3$.

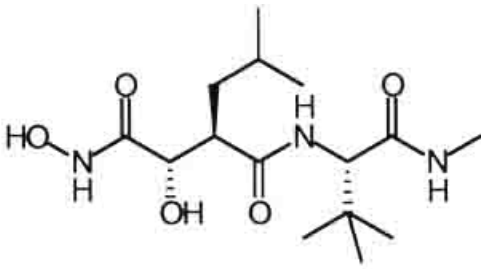
All structures share a common feature. They carry a hydrophobic residue, preferentially a benzyl moiety, which is located next to the carboxylate or incorporated into the central scaffold, suggesting the existence of a hydrophobic pocket. Furthermore, the basic guanidino group has been substituted by different mimetics which show enhanced activities and positively influence pharmacokinetic aspects as increased oral availability [49].

Benzimidazoles, aminopyridines, aminopyrimidines and the corresponding saturated heterocyles turned out to be suited for this purpose. The additional spatial requirements caused by this substitution indicate that the inhibitors (and the natural ligand) are bound to the vitronectin receptor in a "side-on" and not in an "end-on" manner.

Fortunately, many of these novel compounds demonstrate their activity not only in isolated receptor and cellular assays but also *in vivo*. For example, SCH221153 inhibited bFGF induced angiogenesis in the CAM assay and the growth of tumor xenographts in SCID mice [46]. The RGD analogue SC68448 demonstrated significant activity against Leydig cell tumor growth [47]. Currently a N-methylated derivative of c(RGDfV) **1** EMD121974 is in clinical trials (phase I/II) for the treatment of HIV related Kaposi's sarcoma and for progressive or recurrent anaplastic gliomas (protocol IDs JHOC-NABTT-9911, NABTT-9911, AMC-023) [50].



Batimastat **2**



Marimastat **3**

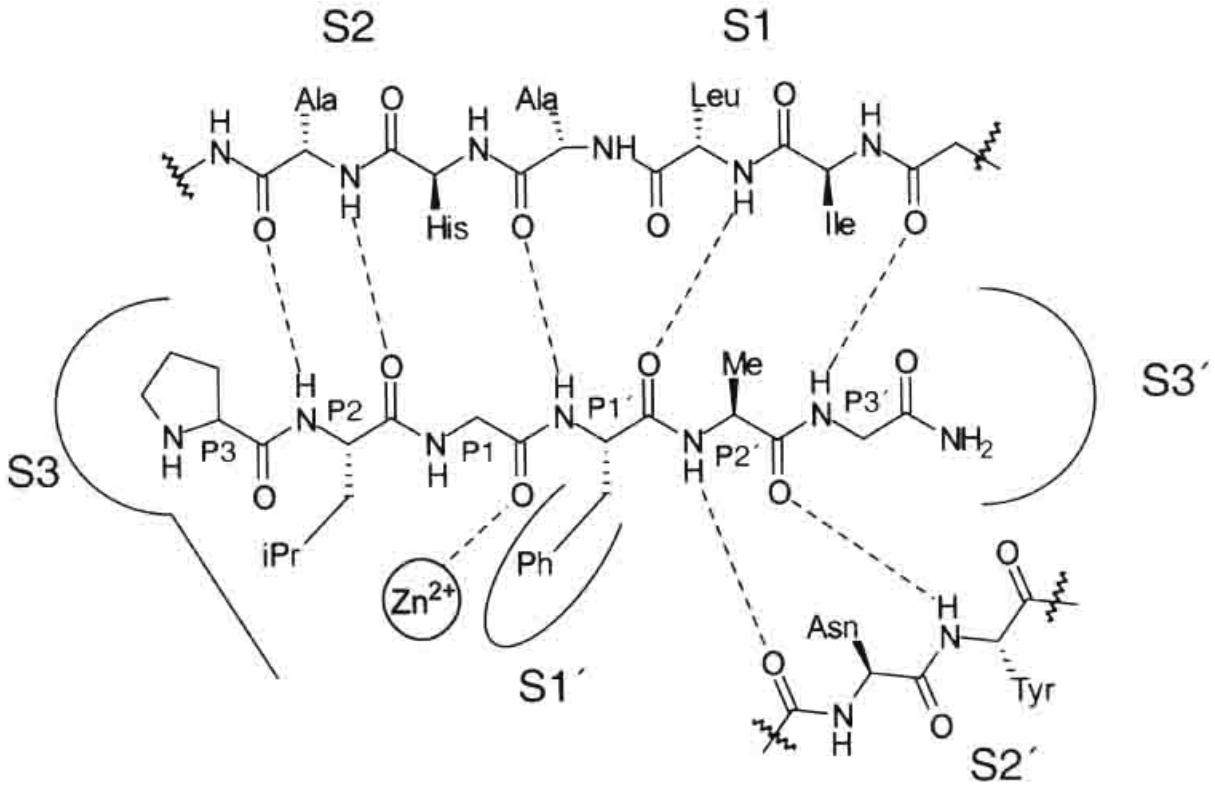
Fig (3). Structures of Batimastat **2** and Marimastat **3**.

2.2 Peptidomimetics and Matrixmetalloproteinases (MMPs)

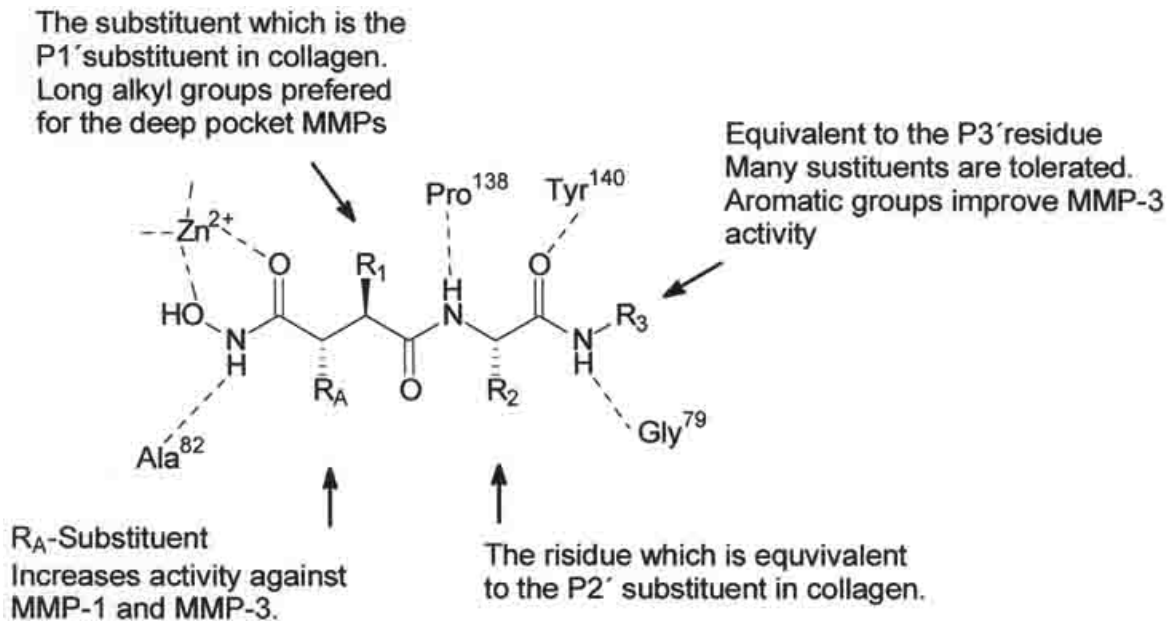
The MMPs are a family of zinc-dependent neutral endopeptidases that are capable of degrading essentially all components of the extracellular matrix (like fibrillar and nonfibrillar collagen, gelatin, myelin, laminin, fibronectins and elastin). The human MMP-family consists of at least 20 members which can be divided into five classes according to their primary structure and substrate specificity: collagenases (MMP-1, MMP-8 and MMP-13), gelatinases (MMP-2 and MMP-9), stromelysins (MMP-3, MMP-7, MMP-10, MMP-11 and MMP-12), membrane type (MT)-MMPs (MT1-MMP, MT2-MMP, MT3- MMP and MT4- MMP, Mt5-MMP) and nonclassified MMPs [51,52].

The MMPs are composed of a signal peptide, a propeptide domain, a catalytic domain with a highly conserved zinc-binding site and a haemopexin-like domain (with exception of MMP-7) that is linked to the catalytic domain by a hinge region [53]. In addition, the MMP-2 and MMP-9 contain fibronectin type II inserts within the catalytic domain which can be bound by the integrin $\alpha_v\beta_3$ [54].

Like most proteases the MMPs are secreted as proenzymes that are proteolytically activated in the extracellular matrix. In the proenzymes the access to the catalytic pocket is blocked by a covalent interaction between a cystein residue of the propeptide chain and the zinc atom in



Scheme 3. Schematic illustration of the MMP-active site.



Scheme 4. Succinyl hydroxamat - MMP interactions. The residues R_A and R_3 point at the pockets formed by the enzyme. (Numeration follows that of MMP-1).

the catalytic site. Cleavage of the propeptide chain results in the activation of the enzyme by unblocking the catalytic pocket.

In the next step one MMP is activated by another via the proteolytical cleavage of the propeptide region building up a complex and only partially deciphered network of proteases.

The proteolytic activity of the MMPs is inhibited by nonspecific endogenous inhibitors, like α2-macroglobulin and α1-anti-protease, and also by the specific tissue inhibitors of metalloproteinases (TIMPs) [55]. The TIMP-family consists of four structurally related proteins (TIMP-1, -2, -3 and –4). These proteins control the activity of MMPs by inhibiting both, the active form of the MMPs and their activation process. The inhibition is achieved by forming a noncovalent stoichiometric complex with the active zinc-binding site of MMPs and pro-MMPs [56].

Gelatinase A and B play an important role in angiogenesis. Latent gelatinase A and B are secreted by endothelial cells and the recent *in vitro* and *in vivo* studies have implicated gelatinases in angiogenesis. In a Matrigel-assay the addition of recombinant gelatinase A increased the formation of tubular networks, whereas it was decreased by the addition of a neutralising antibody or TIMP-2 [57].

The first peptide derivative MMP-inhibitors were designed on the basis of the substrate structure and the sequence of the active site. In the last years X-ray and NMR-investigations of the MMP inhibitor complexes have opened up the possibility for the rational design of non-peptide MMP-inhibitors. Also quite a lot of SAR-studies have been conducted, so today the requirements of an inhibitor are well known and there is the possibility to develop new selective or broad-spectrum MMP-inhibitors [53-59]. The most prominent and well-studied inhibitors for MMPs are the peptidomimetic compounds batimastat **2** and marimastat **3**.

The active sites of the different MMPs contain a highly conserved sequence motif (**H**EXX**H**XX*G*XX**H**) which coordinates the zinc(II) ion and besides comprises of a likewise conserved glutamate residue which acts as a proton acceptor during the proteolysis. Apart from this, four pockets and four residues can form hydrogen bonds to amide groups on the right side of the catalytic centre (see scheme 3). Since the left side of the catalytic centre shows only subtle differences between the individual MMPs, this region is not suitable for individual interactions and therefore of minor interest for the development of selective MMPI.

All inhibitors share a zinc binding group (which can be a carboxylic acid, a sulfhydryl or a hydroxamic acid), functional groups which can build hydrogen bonds to the enzyme backbone and some side chains that are able to interact effectively with the pockets.

Meanwhile it has been shown, that the hydroxamic acid is the most potent zinc binding group, due to its bidentate ligand nature with its oxygen at an optimal distance from the active site zinc(II) ion [60,61]. Many carboxylic acid derivatives have also been tested, since the carboxylate is a precursor to the hydroxamic acid, and some have revealed to be effective MMP inhibitors. Compound Bay 12-9566 **4** is the most prominent representative of this inhibitor class and is in phase II of the clinical trials for cancer (Figure **4**) [62].

Substituents in α-position to the zinc binding group can be varied in order to increase the activity against MMP-1 and MMP-3; a simultaneous truncation of the residues at R2 and R3 leads to a selectivity for MMP-1, MMP-8 and MMP-13 over other MMPs [63]. This position can also be used to introduce polar groups to enhance the oral availability, which was realized in the case of marimastat [64].

The major determinant for activity and selectivity is the substituent R1 (Scheme 4). It has been shown that the pocket

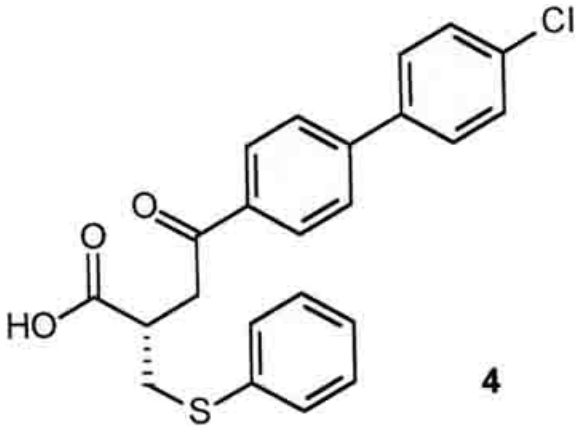


Fig (4). Structure of synthetic MMP inhibitor Bay 12-9566.

S1 at this position is very small for MMP-1 and MMP-7, so using long alkyl or phenylalkyl residues for R1 provides selectivity over these two MMPs. However, this condition is not absolute, since the obstructing Arg-214 (MMP-1 numbering) can undergo conformational changes in the case of individual substituents. (As for a phenolic residue in MMP-1) [65]

Further SAR-studies revealed, that charged or polar groups are not tolerated at this position [53,61].

For the R2 residue a wide range of substituents are tolerated, but it was shown, that bulky alkylic groups are very well suited for the oral bioavailability [66]. The bulky group shields the adjacent amide bonds, which reduces their hydration. This results in a lower desolvation barrier which must be passed by the absorption to a lipophil environment like cell membranes [67]. The residue can also be cyclised with Rα or R3.

In the R3 position a wide range of substituents is accepted, although aromatic residues improved the activity against MMP-3 [68].

The amide backbone is more sensitive, since *N*-methyl derivatives or reverse amides have a significant lower activity. Only certain amide isosteres are tolerated in the second amide position [69-71].

In recent years many synthetic peptidomimetic and non-peptidomimetic inhibitors of the MMPs have been discovered and evaluated in pre-clinical and clinical studies. Some of them are in the phase II/III of clinical studies as anticancer drugs.

Batimastat **2** was the first MMPI that was tested clinically. It is a succinyl hydroxamic acid derivative which mimics the collagen structure. Due to its extreme poor solubility in water it was applicated intraperitoneally or intrapleurally. Batimastat is a potent but nonselective MMPI, with IC_{50} values in the low nanomolar range. (less than 10 ng/mL for MMP-1, -2, -3, -7, -9). While this agent inhibited tumor growth and metastasis in animal models, the clinical trials were stopped due to the lack of orally availability [72-74].

Marimastat **3**, an succinyl hydroxamic acid derivative which is in contrast to batimastat orally available, was tested in several clinical trials [75]. It is also a broadspectrum MMPI which has IC_{50} values in the low nanomolar range for several MMPs (less than 5 ng/mL for MMP-1, -2, -7, -9). Preclinical studies revealed the reduction of the number and size of metastases of lung and breast cancer in treated versus control animals [76]. Preclinical toxicological studies with high doses (100 to 500 mg/kg/day) of marimastat showed many side-effects like gastrointestinal toxicity, weight loss as well as hemorrhage, fibrosis, inflammation and necrosis at periarticular ankle and knee tissues [77]. Furthermore several phase I-II studies of lung, colon, ovaries, prostate, pancreas and other tumors have shown that marimastat can be administered in doses of 2-100 mg twice a day, the principal toxic effect in these studies was the appearance of a dose-limiting inflammatory polyarthritis, which was reversible. This side-effect is known as the musculoskeletal syndrome (MSS). The side-effects of marimastat have been linked to its nonselective character. So the current view is directed to the development of selective inhibitors that target only those enzymes which play a role in the given disease. Some selective peptidomimetic and non-peptidomimetic MMPI have been synthesised and evaluated [63-79].

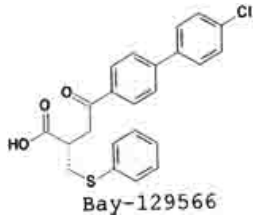
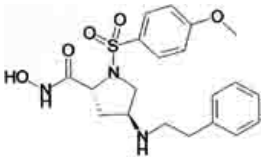
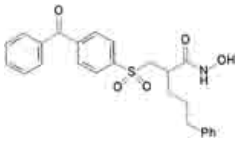
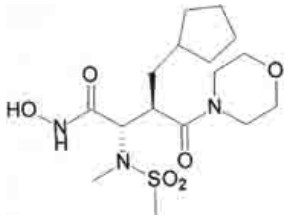
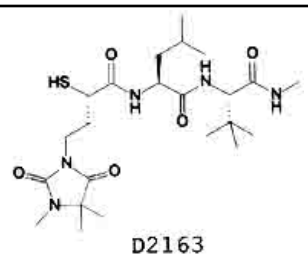
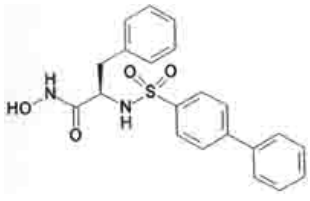
These MMPI are depicted in Table 2. However, only Bay-129566, a highly selective deep pocket non-peptidomimetic carboxylic acid based inhibitor for gelatinase A, has been clinically evaluated so far. Bay-129566 has no signs of the MSS-sideeffects [80]. Despite of that it was withdrawn from clinical trials.

Another promising starting point for the inhibition of angiogenesis is the interaction between MMP-2 and integrin $\alpha_v\beta_3$ which leads to the colocalization on the surface of stimulated endothelial cells [54] (scheme5).

This interaction is a requisite step in the cellular utilization of MMP-2 on the surface of invasive endothelial cells [81]. MMP-2 binds with a short 193 residue C-terminal fragment called PEX to the integrin. Adding this fragment leads to the inhibition of the binding of MMP-2 to the integrin $\alpha_v\beta_3$, resulting in the disruption of angiogenesis and tumor growth in the chick chorioallantonic membrane assay

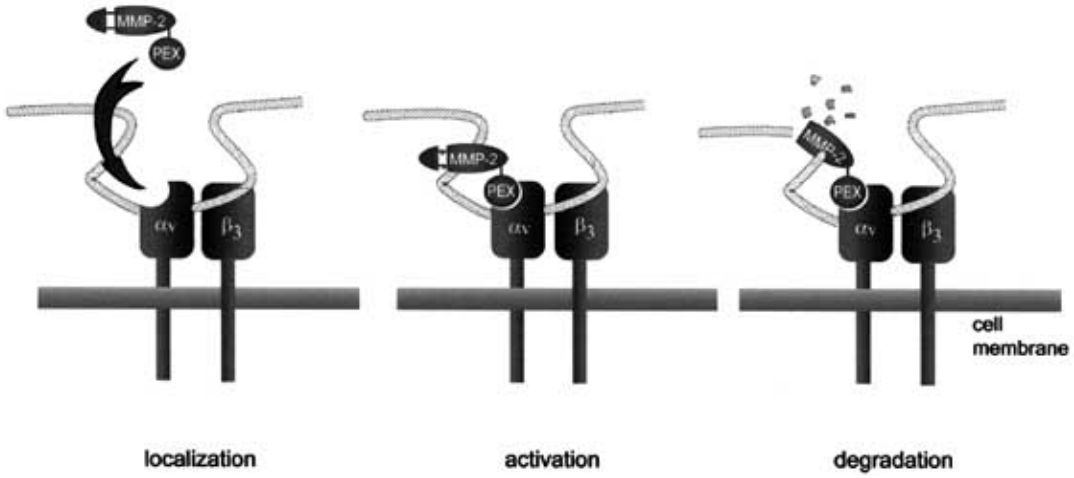
(CAM-assay). Although the exact binding mode remains unknown, it has been established that the integrin does not interact via the known RGD-sequence which it normally binds (e.g. in vitronectin). Recently Silletti *et al.*

Table 2. Selective MMP-Inhibitors

Compuond	IC_{50}(nm)						
	MMP-1	MMP-2	MMP-3	MMP-7	MMP-9	MMP-13	Lit.
 Bay-129566	>5000	11	143	-	301	1470	53
	560	1	57	1400	-	1	78
	2000	4	100	-	15	-	79
	5	400	800	-	-	20	63
 D2163	25	41	157	-	25	4	53
	970	12	>1000	800	16	-	53

2.3 Peptidomimetics and Urokinase-type Plasminogen Activators (uPA)

Another protease which is involved in the proteolytic modulation of the extracellular matrix is plasmin. This broad-spectrum serine protease of tryptic specificity is expressed in an inactive form as plasminogen. The activation of plasminogen is performed by two serine proteases, urokinase-type plasminogen activator (uPA) and tissue PA (tPA), which have a very restricted substrate specificity

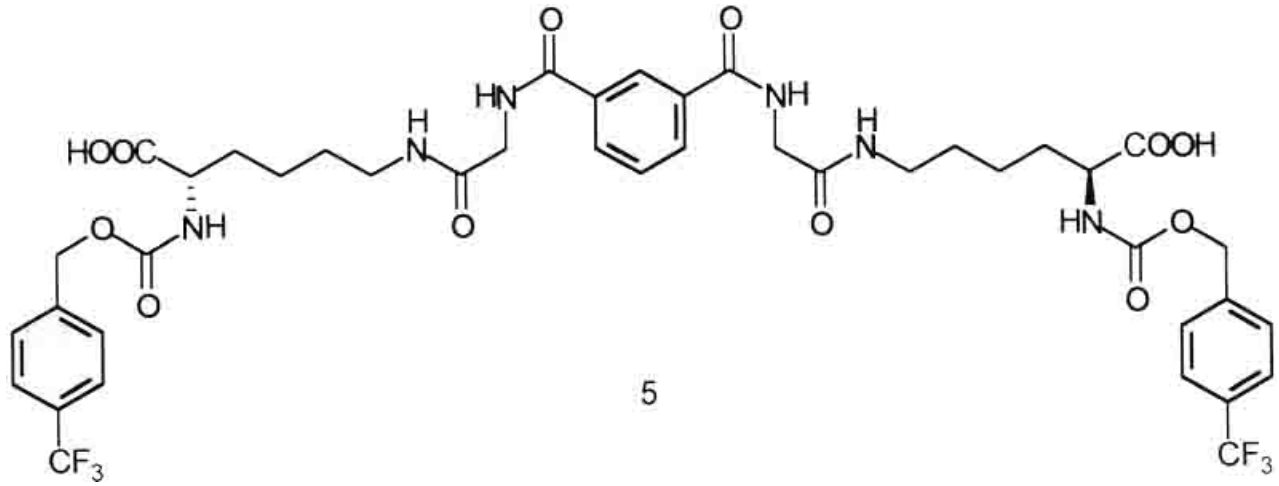


Scheme 5. Pro-MMP2 is localized to integrin $\alpha_v\beta_3$ via its binding domain PEX (a). In turn the MMP2 is activated by cleavage of the pro domain (b). The active protease degrades extracellular matrix components bound to the vitronectin receptor (c) and thereby enables cell migration.

(only plasminogen, fibronectin and hepatocyte growth factor)[83] and are present on a variety of cells including endothelial cells. uPA is a three-domain protein comprising an N-terminal epidermal growth factor (EGF)-like domain, a kringle domain and a C-terminal serine protease domain [84]. It is located to the cell surface by binding to a specific glycosylphosphatidyl-inositol (GPI)-anchored receptor, the uPA-receptor (uPAR), via the EGF-like domain at the N-terminus. The binding of uPA or pro-uPA to uPAR on the plasma membrane leads to two separate events which are independent of each other. First, it accelerates plasminogen activation and the following proteolytic cascade [85]. The other event is a transmembrane signal transduction which leads to the stimulation of cell differentiation and motility of several cell types, most notably endothelial cells, epithelial cells and leukocytes [86]. Therefore, it is possible to block the uPA system in two distinct ways. First, by inhibition of the protease activity or alternatively by blocking the translocation to the cell surface via inhibition of the binding to the uPAR [87].

The serine proteases select their substrates by using specific pockets S1-S4 and S1´-S4´ formed by the enzyme which bind the corresponding peptides of the substrate P4-P1, left to scissible bond, and P1´-P4´ on the other side. The trypsine-like serine proteases bind specifically an arginine or lysine residue in the subside S1. The other pockets facilitate the substrate specificity of the protease. So the inhibitor should have a positively charged moiety that binds to the conserved Asp189 in the pocket S1 [88]. As well as for the MMPs a lot of peptidomimetic and non-peptidomimetic inhibitors of the protease function have been described which have IC_{50} values in the low nanomolar range and show good selectivities for uPA. However, none of them has reached the clinical trials so far (Table 3) [89-92].

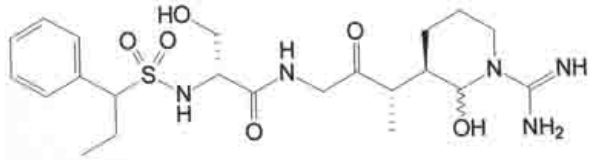
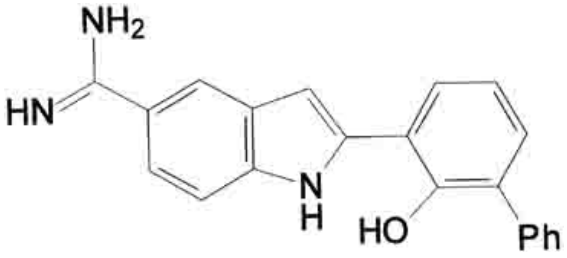
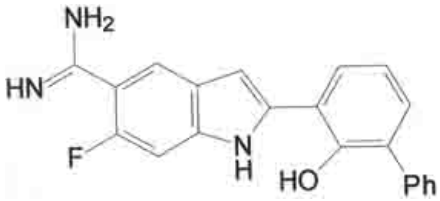
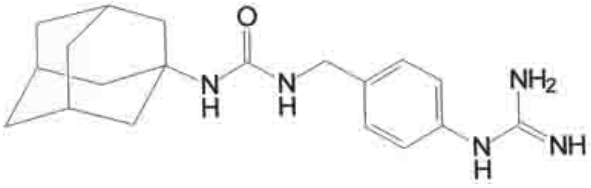
The other possibility to block the uPA activity is by binding to its receptor. The uPAR has three structural homologous, independently folded domains which are all involved in the formation of the uPA binding site [93]. The N-terminal domain I of uPAR contains the major determinants for the binding. For uPA a defined continuous peptide sequence at the N-terminal region has been identified as the uPAR-binding site. Several experiments have shown,



5

Fig (5). Structure of PEX-binding inhibitor **5**.

Table 3. Inhibitors of Urokinase-type Plasminogen Activators

Compound	uPA	K_i (nM)	tPA	Lit.
		Plasmin		
	3.1	367	>2500	89
	86	32100	1700	90
	8	100	35	91
	20	350	3600	88
	2400	$>10^6$	$>10^6$	92

that the minimal binding region of uPA was located at uPA_{19-31} and that Cys19, Lys23, Tyr24, Phe25, Ile28, Trp30 and Cys31 are crucial for the binding affinity [94]. Furthermore, the region between Thr18 and Asn32 forms a flexible, seven residue Ω-Loop which is forced in a ring like structure [95]. Recently a cyclic peptide (cyclo^{19,31}[D-Cys^{19}]-uPA_{19-31}) with an IC_{50} value in the nanomolar range which replaces uPAR-bound uPA and inhibits the uPA-mediated plasminogen activation and fibrin degradation in ovarian cancer cells, has been described [96]. Furthermore, a non-competitive, allosteric antagonist of pro-uPA/uPAR-interaction (Å6-peptide) derived from the non-receptor binding region of uPA($uPA_{136-143}$) was also recently identified. It inhibits the breast cancer cell invasion (IC_{50} 5-25 μM), endothelial cell migration (IC_{50} 25-50 μM) and exhibits any cytotoxic or anti-proliferative activity *in vitro*. It was also shown that it blocks growth and metastasis of rat breast cancer and human breast cancer *in vivo* [97].

Because of its important role in tumor-associated processes the uPA-system is an interesting field of intervention.

2.4 Peptidomimetics and Farnesyltransferase Inhibitors

Inhibitors for farnesyltransferase (Ftase) were originally developed with respect to the fact that the Ras protein is

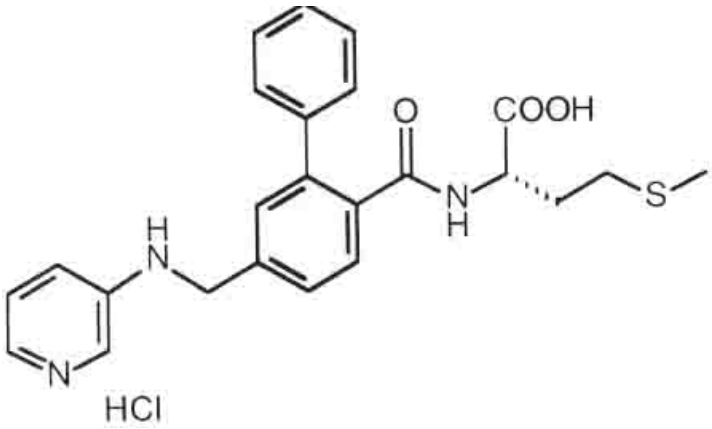


Fig (6). Structure of farnesyltransferase inhibitor A-170634.

mutated in about 20-30% of human tumors. The mechanisms of action of mutated Ras are beyond the scope of this review. For detailed information see [98,99].

In order to perform its biological activity Ras must be localized to the inner surface of the plasma membrane. This attachment is achieved by lipidation of different amino acid residues. Nonlipidated Ras is cytosolic and biologically inactive. In the first step, the cysteine residue of the carboxyterminal "CAAX" motif is enzymatically farnesylated by farnesyltransferase.

Many peptidomimetics were designed on the basis of the CAAX recognition motif of farnesyl transferase. Unfortunately, only a few of these inhibitors were evaluated with regard to their antiangiogenic potential, since *in vivo* antitumor activity against Ras-mutated hyperplasies could partly be caused by inhibition of tumor angiogenesis.

Ng *et al.*. investigated the effect of A-170634 on tumor angiogensis [100,101]. A-170634 turned out to suppress angiogenesis both, directly by inhibiting growth factor induced vascularization and indirectly by reducing the VEGF secretion by the tumor cells.

3. CONCLUSION

Since the publication of the Folkman´s idea that the inhibition of pathological angiogenesis can contribute beneficially to the treatment of malign disorders, over 300 inhibitors of angiogenesis have been discovered to date and some of them have already entered the preclinical and clinical trials with promising results.

In the post genome era particularly, the inhibition of protein-protein interactions emerge as a novel promising strategy to modulate individual biological processes without affecting the intrinsic activity of the single proteins. Most proteins fulfill their function in different biological contexts, but only the temporal and spatial colocalization with other counterparts results in the specific effect. Therefore, small molecules blocking these essential interactions are in great demand. Peptidomimetics are predestinated for this purpose.

These considerations are reflected in the case of angiogenesis. Out of the three main classes discussed in this review the integrins and the localization of the proteases at the cell surface emerge as the most promising target. Because MMPs have also been implicated in the generation of endogenous endostatin, the MMP-inhibition can result in the stimulation rather than the inhibition of angiogenesis [84]. Similar considerations are valid for the uPAs. On the other side, preventing the localization of MMPs and uPA at the plasma membrane disables the endothelial cells from migration and therefore blocks neovascularization without effecting the protease activity, which is necessary for the maintenance of other important physiological processes.

In this context it is notable that some anti-cancer agents, which were initially designed to block tumor growth by direct targeting of tumor cells, "accidentally" turned out to be the potent inhibitors of angiogenesis [102]. This is valid for inhibitors of farnesyltransferase (Ftase)(vide supra), or for many other chemotherapeutica. All these findings implicate that the development of anti-angiogenic therapies are of paramount interest for fighting cancer, either alone or in combination with the established methods.

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