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Design and synthesis of β-amino-α-hydroxy amide derivatives as inhibitors of MetAP2 and HUVEC growth

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Abstract—The rational design and synthesis of β-amino-α-hydroxy amide derivatives as reversible inhibitors of methionine aminopeptidase-2 (MetAP2) with anti-proliferative activity against human umbilical vein endothelial cells (HUVECs) is described.

1. Introduction

Folkman proposed in 1974 that the inhibition of angiogenesis is potentially a promising approach for the treatment of cancer.[1] The antibiotic fumagillin (**1a**), along with related natural analogues, was shown to have potent anti-angiogenic activity and the synthetic analogue TNP-470 (**1b**) has been in clinical trials against a variety of cancer types.[2] The target for fumagillin has been identified as a type 2 methionine aminopeptidase (MetAP2), a metal-dependent enzyme that is involved in post-translational protein processing.[3]

The natural product fumagillin irreversibly binds to MetAP2 and has been shown to inhibit the growth of endothelial cells.[4] Binding is highly selective towards MetAP2 over MetAP1. However, the irreversible binding of TNP-470 to MetAP2 could be responsible for the toxicity that was observed in patients. Consequently, a variety of different reversible MetAP2 inhibitors has been investigated.[5,6]

β-Amino-α-hydroxy amide based (bestatin-based) inhibitors of methionine aminopeptidases have been reported as reversible inhibitors in the literature.[5] Their activity towards growth inhibition of human umbilical vein endothelial cells (HUVECs) had not been disclosed to our knowledge.[7] Therefore, our studies focused on the design and synthesis of reversible bestatin-based inhibitors of MetAP2, which showed growth inhibition properties against HUVECs.

The structure–activity relationship (SAR) around bestatin-based analogues revealed that the hydrophobic side chain (P1) contributes significantly to the binding. For example, the ethyl sulfide **2** (Fig. 1) with an $IC_{50} = 100\,nM$ against MetAP2 is one of the more potent analogues in its class.[5c,8] SAR around fumagillin

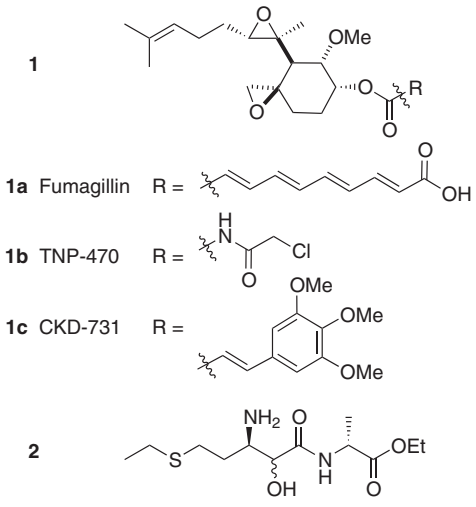

Figure 1. Known MetAP2 inhibitors.

Keywords: Angiogenesis; MetAP2; Reversible inhibitor; HUVEC.
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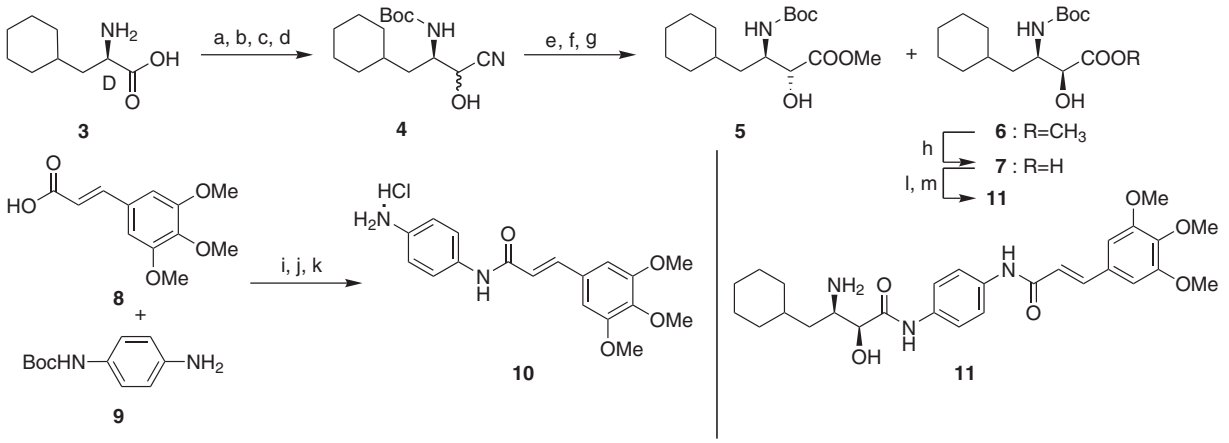


Scheme 1. Exemplary synthesis of β-amino-α-hydroxy amide derivatives. Reagents and conditions: (a) Boc_2O, NEt_3, THF; (b) $HOBt{\cdot}H_2O$, EDC, (MeO)NHMe·HCl, NEt_3; (c) 1 M $LiAlH_4$, Et_2O; (d) acetone cyanohydrin, Et_3N, CH_2Cl_2; (e) conc. HCl, dioxane, reflux; (f) 4 M HCl/dioxane, MeOH; (g) i. Boc_2O, Et_3N, THF; ii. separation of isomers; (h) 1 M aq NaOH, THF, MeOH; (i) **8**, $(COCl)_2$, cat. DMF, EtOAc; (j) **9**, Et_3N, THF; (k) 4 M HCl/dioxane, MeOH; (l) **10**, $HOBt{\cdot}H_2O$, EDC, Et_3N; (m) 4 M HCl/dioxane, MeOH.

uncovered the acyl group as essential for biological activity.[9] The trimethoxy cinnamic ester CKD-731 (**1c**) shows remarkably high anti-proliferative activity against calf endothelial cells ($IC_{50} = 0.03$ pg/mL).[10]

We hypothesized that a molecule bearing both structural fragments, the β-amino-α-hydroxy acyl entity of **2** and cinnamoyl moiety of **1c**, joined by an appropriate linker should have good inhibitory activity against both MetAP2 and endothelial cell growth. Amides with a variety of diamines linkers including *para*-phenylenediamine as a linker were investigated.[11] Several compounds of this scaffold have been prepared and evaluated against MetAP2 and growth of HUVECs.

2. Chemistry

The synthesis of compound **11**, as a representative example of β-amino-α-hydroxy amide derivatives, is outlined in Scheme 1. The required *N*-Boc protected β-amino-α-hydroxy acid (**7**) was obtained from the commercially available α-amino acid in an 8-step sequence.[12,13] Starting from D-3-cyclohexyl alanine (**10**), *N*-Boc protection, Weinreb amide formation, and reduction with $LiAlH_4$ gave the aminoaldehyde. Addition of acetone cyanohydrin to the aldehyde provided a mixture of two diastereomeric cyanohydrines (**4**), which could not be separated at this stage of the synthesis. Hydrolysis of **4** to the acid was accompanied by the deprotection of the amine. Esterification and *N*-Boc protection proceeded in good yields after which diastereomeric **5** and **6** could be readily separated by flash chromatography. The desired acid **7** was obtained by saponification of the corresponding ester **6**.

Linker **9** was coupled first to the cinnamic acid **8** and following *N*-Boc deprotection then coupled to **7**. Final deprotection of the amine gave **11** as its hydrochloride salt, which was purified by HPLC or alternatively by recrystallization.

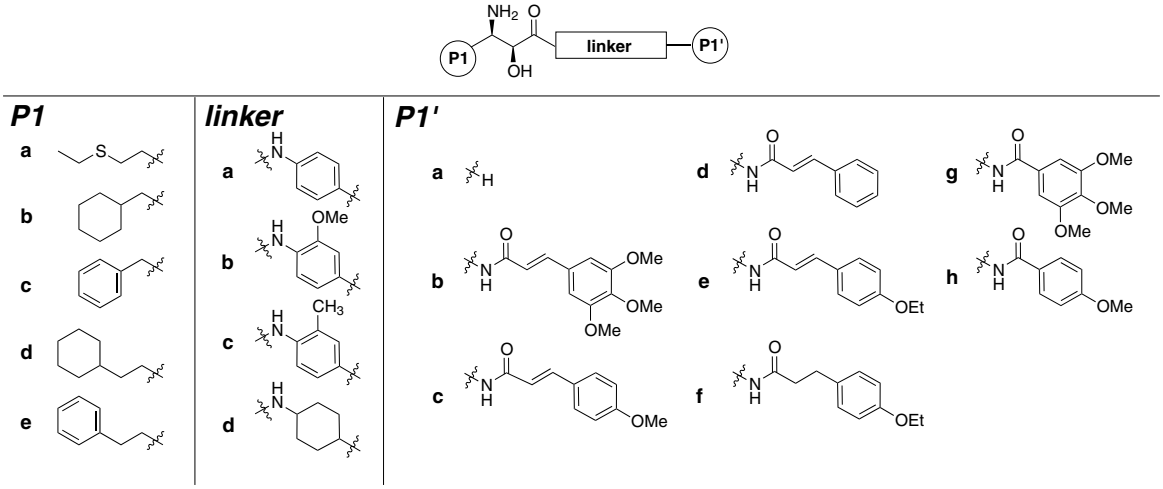
3. Structure–activity relationships

The amino alcohol analogues **12**–**31** synthesized in this study were tested for their ability to inhibit recombinant human MetAP2 (rhMetAP2). The activity assays were performed using a modification of a literature assay.[14] Furthermore, anti-proliferative activities of the analogues were evaluated against HUVECs.[15] The in vitro inhibitory enzyme and cellular activities are summarized in Table 1.

Compound **12** shows, as we hypothesized, inhibitory activity against MetAP2 and endothelial cells. In comparison to **12** structural modifications at the P1 side (e.g., **11**–**15**) revealed significant changes in the enzyme activity but not towards activity of endothelial cell growth. The ethyl sulfide derivative **12** and phenylethylene derivative **15** showed highest binding affinities. Loss of potency was also observed for the *R*-amino-*R*-alcohol **16** compared to its *R*,*S*-epimer **11**.

Changes with additional substituents like methoxy and methyl in the linker are tolerated with respect to activity (e.g., **17**, **18** and **20**, **21**). However, replacement of the phenylenediamine in **15** with a saturated cyclohexylenediamine linker (**22**) is accompanied by a lack of activity towards endothelial cell growth. Modifications at the P1′ side did not alter the enzyme activity (e.g., **23**–**28**), but decreased significantly the activity towards endothelial cells. The 3,4,5-trimethoxy cinnamic amides **12**–**21**, with the exception of **19**, showed the most potent inhibitory activity towards endothelial cells. Complete removal of the cinnamic amide in **12** led to a compound (**28**) without any detectable cellular activity despite demonstrating significant potency against MetAP2. A direct correlation between cellular and enzyme assay results could not be established. Therefore, we hypothesize that the activity against HUVECs is the consequence of a more complex mechanism of action. Similar SAR towards calf pulmonary artery endothelial cells have been described for fumagillin/CKD-731 analogues.[9]

Table 1. Enzyme and cellular activities of derivatives



Compd#	P1–linker–P1′	IC_{50} MetAP2 (nM)	GI_{50} HUVEC (μM)	GI @ 5 μM HUVEC (%)
11	b–a–b	177	0.67	>98
12	a–a–b	56	nd[a]	89
13	c–a–b	755	nd	93
14	d–a–b	356	0.80	>98
15	e–a–b	67	0.85	>98
16	*epi*–b–a–b	2240	2.0	96
17	b–b–b	308	1.2	>98
18	b–c–b	230	1.8	>98
19	b–d–b	2200	nd	52
20	e–b–b	158	0.70	>98
21	e–c–b	82	2.3	92
22	e–d–b	98	nd	46
23	a–a–c	33	nd	43
24	a–a–e	46	nd	91
25	a–a–d	35	nd	48
26	a–a–g	43	nd	20
27	a–a–h	45	nd	15
28	a–a–a	58	nd	0
29	b–a–e	217	1.0	>98
30	b–a–f	209	nd	42
31	e–a–e	169	1.8	>98

[a] nd: not determined.

4. Conclusion

In conclusion, we have successfully designed and synthesized reversible inhibitors of MetAP2 with anti-proliferation activity against HUVECs by introducing the 3,4,5-trimethoxycinnamoyl moiety into β-amino-α-hydroxyamide derivatives. The cellular assay results do not correlate with the inhibition of MetAP2. Further investigation is required to gain more insight into the mechanism of action of these compounds.

Acknowledgements

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15. HUVEC proliferation assay: Cells were seeded at a density of 1500 cells/well in 96-well Wallac flat-bottom isoplates, cultured for 24 h @ 37 °C and then treated with compounds (dose-response ranging from 0.04 to 10 μM) for 24 h. Cell proliferation was measured by ^{3}H-thymidine incorporation using the Scintillation proximity assay (^{3}H-thymidine uptake assay system™; Amersham Life Science). Plates were counted in the Wallac 1450 MicroBeta scintillation counter. GI_{50} values for compounds were determined by plotting the percent control ^{3}H-thymidine uptake against the compound concentration.