Bioorganic & Medicinal Chemistry xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

# Synthesis and structure activity relationships of glycine amide derivatives as novel Vascular Adhesion Protein-1 inhibitors

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ARTICLE INFO

*Article history:*
Received 20 September 2016
Revised 18 October 2016
Accepted 19 October 2016
Available online xxxx

*Keywords:*
Vascular Adhesion Protein-1
Diabetic microvascular complication
Glycine amide
PAMPA

ABSTRACT

Vascular Adhesion Protein-1 (VAP-1) is a promising therapeutic target for the treatment of several inflammatory-related diseases including diabetic microvascular complication. We identified glycine amide derivative **3** as a novel structure with moderate VAP-1 inhibitory activity. Structure-activity relationship studies of glycine amide derivatives revealed that the tertiary amide moiety is important for stability in rat blood and that the position of substituents on the left phenyl ring plays an important role in VAP-1 inhibitory activity. We also found that low TPSA values and weak basicity are both important for high PAMPA values for glycine amide derivatives. These findings led to the identification of a series of orally active compounds with enhanced VAP-1 inhibitory activity. Of these compounds, **4g** exhibited the most potent ex vivo efficacy, with plasma VAP-1 inhibitory activity of 60% after oral administration at 1 mg/kg.



## 1. Introduction

Vascular Adhesion Protein-1 (VAP-1) is a member of the family of copper-containing amine oxidases/semicarbazide-sensitive amine oxidase (AOC/SSAO), found in humans as a membrane-bound form and a soluble form. The membrane-bound form of VAP-1 is mainly expressed in endothelial cells, smooth muscle cells, and adipocytes, whereas the soluble VAP-1 is released into plasma mainly from vascular endothelial cells.[1]

VAP-1 is reported to have two functions. As an adhesion molecule, VAP-1 is involved in leukocyte rolling, adhesion and transmigration, which are central steps in leukocyte extravasation to sites of inflammation.[2] Another function of VAP-1 is to act as an amine oxidase. It possesses topaquinone (TPQ) in the active site as a cofactor, and catalyzes the conversion of primary amines (e.g., methylamine and aminoacetone) into the corresponding aldehydes (e.g., formaldehyde and methylglyoxal), while releasing ammonia and hydrogen peroxide (Eq. (1)).[3]

$$RCH_2NH_2 + O_2 + H_2O \rightarrow RCHO + H_2O_2 + NH_3 \quad (1)$$

Increased plasma and/or membrane associated VAP-1 activities have been found in patients with diabetic mellitus, and even more so in patients with diabetic microvascular complication such as diabetic retinopathy and diabetic nephropathy.[4] Elevated expression of VAP-1 results in increased production of enzymatic products of VAP-1 like aldehyde and hydrogen peroxide. These products have been reported to participate in the development of diabetic microvascular complication, for example, by inducing oxidative stress.[5] Further, plasma and/or membrane bound VAP-1 is also increased, and is suggested to be involved in diseases such as rheumatoid arthritis,[6] atherosclerosis,[7] chronic heart failure[8] and Alzheimer's disease.[9] All of which are associated with inflammation. These facts suggest that VAP-1 is a promising therapeutic target for the treatment of several inflammatory-related diseases, including diabetic microvascular complication.

Several approaches to inhibit VAP-1 have been reported, including small interfering RNAs, function blocking antibodies, and small molecule inhibitors.[1,10] Among these, Bioite Therapeutics is conducting clinical trials with their anti-VAP-1 antibody (BTT-1023) for the treatment of autoimmune inflammatory and fibrotic diseases.[11] As for small molecule inhibitors, PXS-4728A (**1**) ($IC_{50}$ = 5 nM[12]) has recently advanced to clinical trials for treatment of non-alcoholic steatohepatitis (NASH) (Fig. 1).[12]

We have previously reported a novel VAP-1 inhibitor, compound **2**, which showed potent VAP-1 inhibitory activity with an $IC_{50}$ value of 0.019 μM (Fig. 1).[13c] Oral administration of this

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http://dx.doi.org/10.1016/j.bmc.2016.10.025
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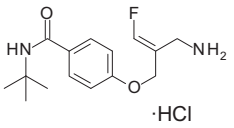
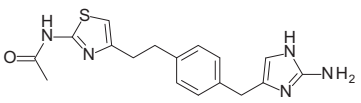
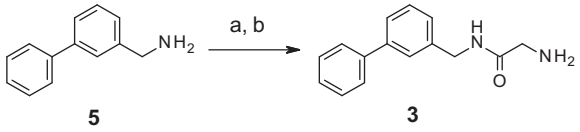


PXS-4728A (**1**)



**2**

**Figure 1.** Structures of PXS-4728A (**1**) and **2**.



**Scheme 1.** Reagents and conditions: (a) *N*-(*tert*-butoxycarbonyl)glycine, WSCD·HCl, HOBt, DMF; (b) 4 M HCl (EtOAc solution).

compound at 10 mg/kg showed an inhibitory effect on ocular permeability in streptozotocin (STZ)-induced diabetic rats. These results suggested that VAP-1 would be a promising therapeutic target for the treatment of diabetic macular edema. However, this compound was not suitable for further clinical development due to its insufficient pharmacokinetic properties in rats ($F = 0.7\%$). These facts prompted us to identify novel VAP-1 inhibitors.

To find a novel class of orally active VAP-1 inhibitors, we screened our in-house compound library for VAP-1 enzymatic inhibitory activity and identified compound **3** as a moderate VAP-1 inhibitor with an $IC_{50}$ value of 0.18 μM (Table 1). Although this compound possesses a novel scaffold with moderate VAP-1 inhibitory activity, **3** showed poor ex vivo efficacy (17% inhibition in rat plasma VAP-1 activity after oral administration at 100 mg/kg). We therefore conducted structural optimization of **3** in order to improve its ex vivo efficacy. In this manuscript, we describe the synthesis and structure-activity relationships of glycine amide derivatives as novel VAP-1 inhibitors.

## 2. Chemistry

The synthesis of glycine amide derivatives is outlined in Schemes 1–6. Condensation of benzylamine **5** with *N*-(*tert*-butoxycarbonyl)glycine followed by deprotection of *tert*-butoxycarbonyl (Boc) group gave **3** (Scheme 1). Scheme 2 shows the synthesis of **4a**–**4h**. Condensation of benzylamine **6** with *N*-Boc-glycine gave **7**. Suzuki coupling of **7** with various boronic acids or esters followed by removal of Boc group afforded **4a**–**4h**.

Compounds **10**, **11a**–**11d**, **12a**–**12b**, and **13** were prepared by the synthetic scheme depicted in Scheme 3. Suzuki coupling between **7** and corresponding boronic acids or esters gave biphenyl analogs **8a**–**8c**. Hydrolysis and removal of Boc group of **8a** by treatment with 6 M HCl at 100 °C yielded **10**. Hydrolysis of **8a** by NaOH gave carboxylic acid **9**, which was then converted into amide derivatives **11a**–**11d** by condensation with a variety of amines and subsequent deprotection of Boc group. Treatment of aniline **8b** with carbamoyl chlorides followed by removal of Boc group yielded **12a**–**12b** respectively. Reductive amination of aldehyde **8c** with diethylamine followed by removal of Boc group gave benzylamine analog **13**.

Scheme 4 shows the synthesis of compounds **17**–**19**. Suzuki coupling between aryl bromide **14** with **15** followed by deprotection of Boc group gave benzylamine **16**. Benzylamine **16** was then converted into **17**–**19** by condensation with the corresponding amino acids and subsequent removal of Boc group.

The synthesis of **27**–**30** is shown in Scheme 5. Condensation of benzylamine **6**, **20** or isoindoline **22** with appropriate amino acids afforded **23**, **24** and **26**. Substitution of benzylbromide **21** with *tert*-butyl [2-(methylamino)ethyl]carbamate gave **25**. Suzuki coupling of the resulting **23**–**26** with **15** followed by deprotection of Boc group gave **27**–**30**.

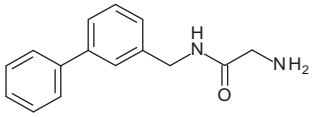
Benzamide derivative **33** was synthesized as shown in Scheme 6. Suzuki coupling between aryl bromide **31** and **15** followed by hydrolysis using NaOH gave carboxylic acid **32**. Condensation of **32** with *tert*-butyl [2-(methylamino)ethyl]carbamate followed by removal of Boc group gave compound **33**.

## 3. Results and discussion

The inhibitory activities of the synthesized compounds against human and rat VAP-1 were measured by a radiochemical-enzyme assay using $^{14}C$-benzylamine as an artificial substrate. Plasma VAP-1 activities in normal rats after oral administration of test compounds were evaluated by using the same radiochemical-enzyme assay.

We identified glycine amide derivative **3** as a novel structure from our in-house compound library. This compound showed moderate VAP-1 inhibitory activity with an $IC_{50}$ value of 0.18 μM. However, it showed poor ex vivo efficacy (17% inhibition in rat plasma VAP-1 activity after oral administration at 100 mg/kg) in spite of the fact that there were no particular problems with solubility, membrane permeability, or metabolic stability in liver microsomes (data not shown). Since an amide bond would be sometimes vulnerable to hydrolysis, we suspected the stability of this compound in vivo and examined its stability in rat blood. As shown in Figure 2, it was found that concentration of **3** was decreased to 33% after incubation with rat blood for 1 h. Therefore, we considered that instability of **3** in rat blood is one of the reasons for low ex vivo activity. Because we needed to confirm the activity of compounds in rodent, such as rat, before advancing to clinical trial, we considered it important to improve stability in rat blood to obtain a compound showing potent ex vivo efficacy. Testa

**Table 1**
In vitro and pharmacodynamic profile of **3**

| Compound | Structure | Human VAP-1 $IC_{50}$ (μM)[a] | Rat ex vivo[b] | |
|---|---|---|---|---|
| | | | Dose (mg/kg, po) | Inhibition ratio (at 1 h) (%) |
| **3**[c] |  | 0.18 | 100 | 17 |

[a] $IC_{50}$ value is shown as the mean of independent experiments ($n = 2$).
[b] Inhibitory effect on plasma VAP-1 activity in rats ($n = 5$) at 1 h after oral administration of compound **3**.
[c] Hydrochloride salt.