Bioorganic & Medicinal Chemistry 25 (2017) 6024–6038

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Synthesis and structure activity relationships of carbamimidoylcarbamate derivatives as novel vascular adhesion protein-1 inhibitors

Susumu Yamaki *, Hiroyoshi Yamada, Akira Nagashima, Mitsuhiro Kondo, Yoshiaki Shimada, Keitaro Kadono, Kosei Yoshihara [a]

Drug Discovery Research, Astellas Pharma Inc., 21 Miyukigaoka, Tsukuba, Ibaraki 305-8585, Japan

ARTICLE INFO

Article history:
Received 24 August 2017
Revised 21 September 2017
Accepted 22 September 2017
Available online 23 September 2017

Keywords:
Vascular adhesion protein-1
Diabetic nephropathy
Carbamimidoylcarbamate
Stability in plasma
Aqueous solubility

ABSTRACT

Vascular adhesion protein-1 (VAP-1) is a promising therapeutic target for the treatment of diabetic nephropathy. Here, we conducted structural optimization of the glycine amide derivative **1**, which we previously reported as a novel VAP-1 inhibitor, to improve stability in dog and monkey plasma, and aqueous solubility. By chemical modification of the right part in the glycine amide derivative, we identified the carbamimidoylcarbamate derivative **20c**, which showed stability in dog and monkey plasma while maintaining VAP-1 inhibitory activity. We also found that conversion of the pyrimidine ring in **20c** into saturated rings was effective for improving aqueous solubility. This led to the identification of **28a** and **35** as moderate VAP-1 inhibitors with excellent aqueous solubility. Further optimization led to the identification of 2-fluoro-3-{3-[(6-methylpyridin-3-yl)oxy]azetidin-1-yl}benzyl carbamimidoylcarbamate (**40b**), which showed similar human VAP-1 inhibitory activity to **1** with improved aqueous solubility. **40b** showed more potent ex vivo efficacy than **1**, with rat plasma VAP-1 inhibitory activity of 92% at 1 h after oral administration at 0.3 mg/kg. In our pharmacokinetic study, **40b** showed good oral bioavailability in rats, dogs, and monkeys, which may be due to its improved stability in dog and monkey plasma.

1. Introduction

Diabetic nephropathy develops in up to 40% of patients with type 1 and type 2 diabetes, and along with diabetic retinopathy and diabetic neuropathy, is a major diabetic microvascular complication.[1] As symptoms progress, dialysis is required, which markedly lowers the quality of life (QOL) of patients. Further, the number of diabetic patients has continued to increase; over 400 million people suffered from diabetes worldwide in 2015, and this number is estimated to increase to more than 600 million by 2040.[2] The increase in diabetes cases is paralleled by an increase in people with diabetic nephropathy. Furthermore, diabetic nephropathy has become the primary reason for starting dialysis in recent years.[1] Therefore, prevention of the development and progression of diabetic nephropathy is an important issue. Although the exact cause of diabetic nephropathy is not clear, its development and progression have been hypothesized to be linked with hyperglycemia and resulting factors, such as acceleration of advanced glycation end-product (AGE) formation, oxidative stress, and (glomerular) hypertension, along with genetic susceptibility.[3] Current therapeutic strategies for diabetic nephropathy are glycemic control, correction of (glomerular) hypertension, and dietary restriction of protein.[1] Recently, glycemic control has been achieved to some extent by the approval of novel class of drugs with various mechanisms of action, such as dipeptidyl peptidase 4 (DPP4) inhibitors and sodium-glucose cotransporter 2 (SGLT2) inhibitors.[4] Inhibition of the renin-angiotensin system (RAS) also has a beneficial effect on the progression of diabetic nephropathy.[1] However, as many patients still progress to end-stage renal disease and start dialysis, these current therapeutic strategies are not sufficient. Therefore, development of novel therapeutic strategies for diabetic nephropathy are needed.

Vascular adhesion protein-1 (VAP-1) was recently linked to diabetic nephropathy; for example, it was reported to induce oxidative stress and accelerate AGE formation.[5] VAP-1 is a member of the family of copper-containing amine oxidases/semicarbazide-sensitive amine oxidase (AOC/SSAO), which is encoded by the AOC3 gene. VAP-1 has been reported to function as both an adhesion molecule and amine oxidase.[6] As an adhesion molecule, VAP-1

* Corresponding author.
E-mail address: susumu.yamaki@astellas.com (S. Yamaki).
[a] Present address: Haupt Pharma Toride Co., Ltd, 5662 Omonmai, Toride, Ibaraki 302-0001, Japan.

https://doi.org/10.1016/j.bmc.2017.09.036
0968-0896/

is involved in leukocyte rolling, adhesion, and transmigration, which are key steps in leukocyte extravasation to sites of inflammation.[7] As an amine oxidase, VAP-1 catalyzes the conversion of biogenic primary amines into their corresponding aldehydes, while releasing ammonia and hydrogen peroxide by the reaction with topaquinone (TPQ) in its active site.[8] Increased VAP-1 activity is found in patients with diabetes mellitus, and even higher activities have been observed in patients with diabetic microvascular complications, such as diabetic retinopathy and diabetic nephropathy.[5] Elevated VAP-1 activity causes the production of large amounts of degradated products, such as aldehydes and hydrogen peroxide, which are (cyto)toxic products known to damage the renovasculature directly or indirectly for example by inducing oxidative stress and accelerating AGE formation.[5] Therefore, VAP-1 is a possible contributing factor for further deterioration of pathologies, suggesting that it is a promising novel therapeutic target for the treatment of diabetic nephropathy.

We previously reported compound **1** (Fig. 1), a glycine amide derivative, as a novel VAP-1 inhibitor that shows potent human VAP-1 inhibitory activity with an IC_{50} value of 0.025 μM, and inhibition of urinary protein excretion in streptozotocin (STZ)-induced diabetic rats after oral administration at 0.3 and 1 mg/kg.[9] While **1** was stable in rat and human plasma, it was unstable in dog and monkey plasma, with less than 1% detected after incubation with dog and monkey plasma for 6 h (Table 1). Since **1** is unstable in dog and monkey plasma, its pharmacokinetic properties in these species is likely to be poor, suggesting that safety assessment of **1** in these species would be difficult. Since the International Conference on Harmonization Guideline ICH M3 states that some nonclinical safety studies should be carried out in both rodent and non-rodent species to support the safe conduct of clinical trials,[10] we proposed that structural optimization of **1** was required to improve its stability in plasma and therefore pharmacokinetic properties in non-rodent species, such as dogs and monkeys.

Since structurally related compounds that have the same right part with **1** (Fig. 1) were also unstable in dog and monkey plasma (data not shown), we suspected that chemical groups in this part of the compound may be responsible for its instability in dog and monkey plasma. As this right part contains a CH_2-NH_2 group, it may be a suitable substrate for SSAO. It was previously reported that mammals other than humans have much higher plasma SSAO activity than humans and rats.[5c,11] For example, the Vmax (mU/L) of plasma SSAO in dogs, humans, and rats are 9000 ± 1000, 352 ± 102, and 27, respectively.[5c] Therefore, we hypothesized that **1**, a potential substrate for SSAO, might be oxidized rapidly by high SSAO activity, making it unstable in dog and monkey plasma. We therefore focused our efforts on identifying novel VAP-1 inhibitors lacking CH_2-NH_2 group in the right part. Inoue et al. reported the guanidine analog **2** as a VAP-1 inhibitor without the CH_2-NH_2 group with moderate human VAP-1 inhibitory activity (IC_{50} = 0.23 μM), and rat plasma VAP-1 inhibitory activity after subcutaneous administration.[12] Since guanidine derivatives cannot be substrates for SSAO due to the absence of CH_2-NH_2 group, we expected that they are stable in dog and monkey plasma. Therefore, we designed acylguanidine, carbamimidoylcarbamate, and carbamimidoylurea analogs as bioisosteres of the right part of **1** without CH_2-NH_2 group.

1 also exhibited poor aqueous solubility (2.3 μM) in the Japanese Pharmacopoeia 2nd fluid for disintegration test (JP2; pH = 6.8) (Table 1). Therefore, improvement of the aqueous solubility of **1** is also required since compounds with low aqueous solubility tend to show poor bioavailability and dose proportionality.[13]

Here, we describe the synthesis of a novel series of carbamimidoylcarbamate derivatives, and the successful development of an orally active VAP-1 inhibitor with improved aqueous solubility and good pharmacokinetic properties in rats, dogs, and monkeys.

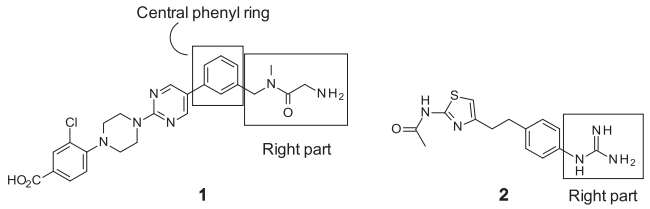

Fig. 1. Structures of compound **1** and **2**.

2. Chemistry

The synthesis of target compounds is outlined in Schemes 1–7. Lithium-halogen exchange of **3** and subsequent formylation gave aldehyde **4**. The Wittig reaction between aldehyde **4** and (3-nitrobenzyl)(triphenyl)phosphonium bromide or (4-nitrobenzyl)(triphenyl)phosphonium bromide followed by hydrogenation in the presence of Pd/C gave **5a**–**b**. The reaction with *N*,*N*′-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboximidamide followed by deprotection of *tert*-butoxycarbonyl (Boc) group yielded **6a**–**b**. Boc protection of **7** followed by benzylic bromination and phosphonium salt formation gave **8**. The Wittig reaction between aldehyde **4** and **8** and subsequent hydrogenation yielded **9**. Removal of Boc group of **9** gave phenethylamine intermediate, which was converted to **10** in a manner similar to that for **6a**–**b**.

Scheme 2 shows the synthesis of acylguanidine analog **14**. The Wittig reaction between aldehyde **4** and phosphonium salt **11** followed by acid-catalyzed esterification gave **12**, which was hydrogenated to yield **13**. Hydrolysis of **13** using NaOH gave the carboxylic acid intermediate, which was activated by carbonyldiimidazole (CDI) and then reacted with guanidinium carbonate to give **14**.

20a–**h** and **24** were prepared according to Scheme 3. Miyaura borylation of compound **15a**–**b** gave boronic ester **16a**–**b**. Suzuki-Miyaura coupling between **17** and various substituted boronic acids or esters, including **16a**–**b**, or between **18** and (3-bromophenyl)methanol gave **19a**–**h**. **20a** was synthesized from ester **19a** in a manner similar to that for **14**. Reductive amination of **19b** with methylamine followed by the reaction with CDI and guanidinium carbonate yielded **20b**. Benzyl alcohol derivatives **19c**–**e** were activated by CDI and then reacted with guanidinium salt to give **20c**–**e**. Reduction of **19f**–**h** using $NaBH_4$ afforded benzyl alcohol intermediates, which were converted to **20f**–**h** in a manner similar to that for **20c**–**e**. Substitution of **21** with morpholine followed by bromination using *N*-bromosuccinimide (NBS) gave **22**, which was reacted with (2-fluoro-3-formylphenyl)boronic acid under Suzuki-Miyaura reaction condition to afford **23**. **23** was converted into **24** in a manner similar to that for **20f**–**h**.

The synthesis of **28a**–**b** and **32a**–**e** is shown in Schemes 4 and 5, respectively. Compound **26** was prepared from **25** by alkylation with 1-(chloromethyl)-4-methoxybenzene. Buchwald-Hartwig cross coupling reaction between **26** and corresponding amines followed by removal of *para*-methoxybenzyl (PMB) group afforded benzyl alcohol intermediates **27a**–**b**, which were converted to **28a**–**b** in a manner similar to that for **20c**–**e**. Compound **30a** was commercially available and **30b** was prepared from **29** by silylation with *tert*-butyl(chloro)dimethylsilane. **30a**–**b** and various amines underwent Buchwald-Hartwig cross coupling reaction followed by removal of *tert*-butyldimethylsilyl (TBS) group gave **31a**–**e**. **31a**–**e** were converted into **32a**–**e** in a manner similar to that for **20c**–**e**.

Scheme 6 shows the synthesis of **35**. Coupling reaction between **30a** and 1-Boc-piperazine gave **33**. Removal of both Boc group and TBS group in **33** using HCl followed by reductive amination with