Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 2661–2664
Bioorganic & Medicinal Chemistry Letters

Lead discovery of quinoxalinediones as an inhibitor of dipeptidyl peptidase-IV (DPP-IV) by high-throughput screening

Hyae-Gyeong Cheon,[a] Chul-Min Lee,[b] Beom-Tae Kim[b] and Ki-Jun Hwang[b,*]

[a]*Biomedicinal Science Division, Korea Research Institute of Chemical Technology, PO Box 107, Yusung, Taejeon 305-706, South Korea*

[b]*Department of Chemistry and Research Center of Bioactive Materials, College of Natural Science, Chonbuk National University, Dukjindong 664-14, Chonju 561-756, South Korea*

Received 9 December 2003; accepted 12 February 2004

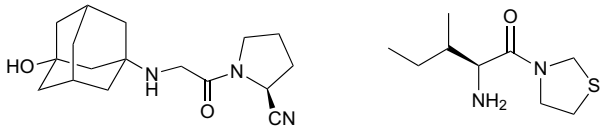
Abstract—*N*-Ureido-quinoxalinedione derivatives have been discovered as leads for a novel series of dipeptidyl peptidase-IV (DPP-IV) inhibitors through high-throughput screening of our chemical library. A brief structure–activity relationship of the compounds was investigated. Among them, entry 5 showed the most potent inhibitory activity. The nitro group in quinoxaline moiety and the aromatic sulfonyl substituted ureido functional group seem to be important to increase the potency dramatically.

1. Introduction

Glucagon-like peptide-1 (GLP-1) is an incretin secreted from the small intestine in response to food ingestion, and exhibits several biological effects including stimulation of insulin secretion, inhibition of glucagon secretion, slowing gastric emptying and induction of satiety.[1] However, GLP-1 action has a very short half life about 1 min due to the degradation by DPP-IV.[2] DPP-IV is a serine protease, which removes the dipeptide from the N-terminus of substrate proteins by cleaving post proline or alanine residues.[3] DPP-IV is expressed in many tissues and body fluids, and exists as either a membrane-bound or a soluble enzyme.[4]

Since the discovery that GLP-1 secretion is impaired in type 2 diabetes, several approaches have been employed to enhance GLP-1 action, thereby ameliorating type 2 diabetes.[5] Among them, DPP-IV inhibition has been proved to be an effective method for type 2 diabetes pharmacotherapy.[6] Several peptide-like DPP-IV inhibitors including LAF-237 and P-32/98 are under clinical trials (Fig. 1).[7]

In order to discover novel nonpeptide DPP-IV inhibitors, a chemical library of approximately 32,000 compounds (Korea Chemical Bank) was screened by high throughput screening (HTS) techniques. DPP-IV HTS was carried out on 96-well plates with HTS automation instruments using rat plasma as the enzyme source and Ala-Pro-7-amino-4-trifluoromethyl-coumarin (AFC) as the substrate. As a result, we found a novel scaffold of quinoxalinedione as a DPP-IV inhibitor. Here, we report structure–activity relationship of quinoxalinediones on in vitro DPP-IV inhibitory activity.



LAF-237 P-32/98

Figure 1. DPP-IV inhibitors.

2. Chemistry

Quinoxalinediones **2** were synthesized by the reaction of aryldiamines **1** with oxalic acid in 3 N HCl under reflux.[8] The yields were generally over 90%. The next amination step was not trivial. Among several other aminating agents, hydroxylamine sulfonic acid[9] was successfully utilized to produce the key intermediate **3** by treating quinoxalinedione **2** with it under basic conditions

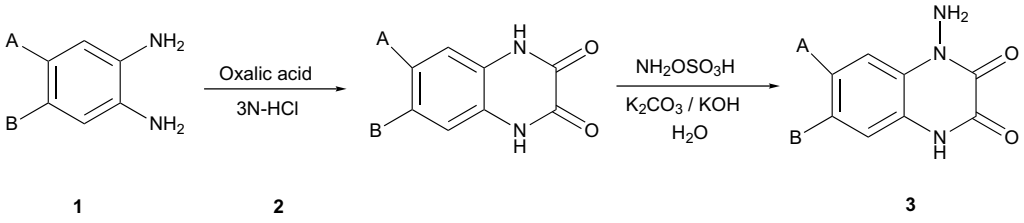
* Corresponding author. Tel.: +82-63-270-3577; fax: +82-63-270-4322; e-mail: kijun@moak.chonbuk.co.kr

0960-894X/$ - see front matter © 2004 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2004.02.065

(10 equiv KOH and K_2CO_3 in water). The yields were at the range of 40–62% depending upon the functional group. With the strong electron withdrawing substituents like nitro group, the yield was generally low (Scheme 1). The amino-quinoxalinediones represented as **3** deserve some mentions due to the unsymmetrical case such as compound **3** (A = H, B = NO_2). It was envisioned that monoamination of compound **2** (A = H, B = NO_2) gave 1-amino-7-nitro-quinoxalinedione (**3**; A = H, B = NO_2) rather than 1-amino-8-nitro-quinoxalinedione (**3**; A = NO_2, B = H) among the possible regioisomers, judging from the character of resonance stabilized anion of **2a** by contrast with anion of **2b** (Fig. 2) and from chemical shifts comparison of the aromatic protons between the observed values[10] and simulated ones by ChemDraw® program.

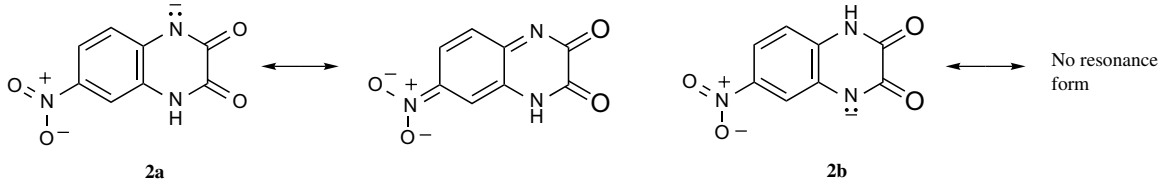
Ureido compounds **4** and **5** were synthesized with no incidence simply by treating aminoquinoxalinediones **3** with appropriate isocyanates and isothiocyanates in DMF, respectively, in 52–75% yields. However, compounds **5** with nitro group could not be synthesized due to the low electrophilicity of isothiocyanate (Scheme 2).

Encouraged by the strong biological activity of some compounds of **4**, the synthesis of compounds **7**, whose nitrogen atom of side chain of **4** is replaced with carbon, were examined next. Quinoxalinediones **2** were treated with methyl bromoacetate and sodium hydride in DMF to give compounds **6** in 40–55% yields with the exception of nitro containing compound. It is assumed that the nucleophilicity of the anion derived from **2** containing nitro group was not strong enough to react with methyl bromoacetate. The conversion of **6** to **7** was accomplished by activating the carboxyl group of **6** with carbonyldiimidazole (CDI)[11] followed by treatment with appropriate sulfonamides (Scheme 3).



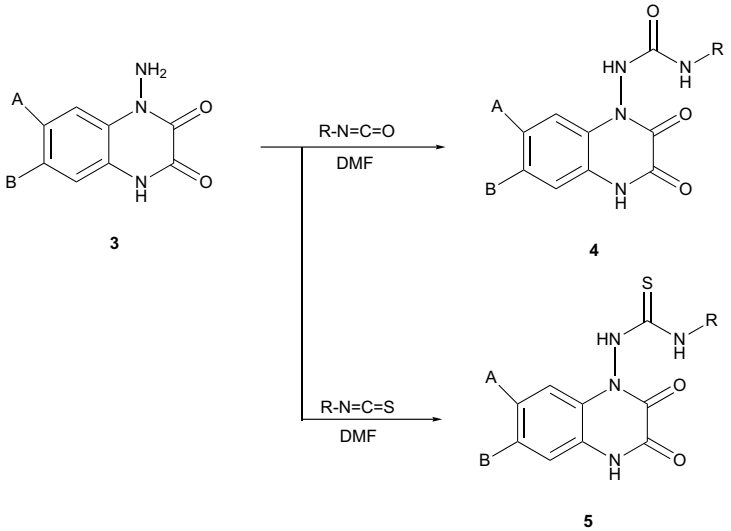
1 **2** **3**

Scheme 1. Synthesis of key intermediates **3**.



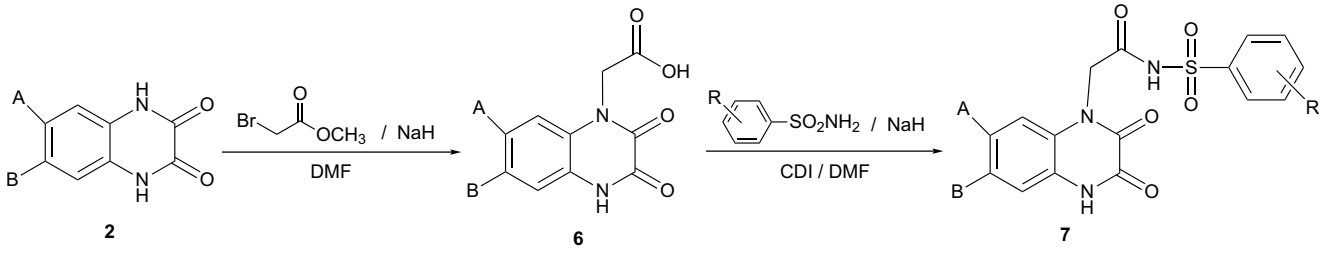
2a **2b**

Figure 2. Resonance stabilized form of **2a**.



3 **4** **5**

Scheme 2. Synthesis of ureido compounds **4** and thioureido compounds **5**.



Scheme 3. Synthesis of compounds **7**, carbon analogues of compounds **4**.

3. Results and discussion

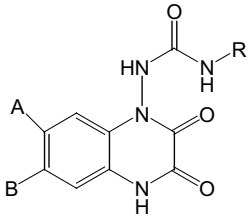
DPP-IV enzyme assay was carried out using rat plasma by measuring 7-amino-4-trifluoromethyl-coumarin (AFC) liberated from Ala-Pro-AFC in the presence or absence of a test compound.[7,12] Rat plasma preparation (20 μL) was incubated with Ala-Pro-AFC (40 μM) at room temperature, pH 7.8 for 1 h in the presence or absence of test compounds (20 μM). Test compounds were dissolved in DMSO. DMSO concentration in the assay mixture was 5%, which did not affect enzyme activity. After 1 h incubation, the fluorescence of AFC released by the reaction was measured at 360 nm (excitation wavelength) and at 485 nm (emission wavelength). P-32/98 was used as a reference compound.

Analysis of structure–activity relationship of ureido compounds **4** revealed that nitro group on quinoxaline ring moiety (entries 1–5) influenced the DPP-IV activity greatly. Simple substitution of chlorine or hydrogen for the nitro group (entries 6–16) diminished the potency. In addition, the sulfonyl group in ureido part appears to be essential since entries 17–20 exhibited very weak inhibition when compared with the activities of entries 1–5 (Table 1). For the most active compounds (entries 1–5) their IC_{50} values were determined,[13] although they are not included in Table 1 for the purpose of table clarity.

We moved on the structural modification by substituting sulfur atom for the oxygen of the ureido side chain of compounds **4** to produce the thioureido compounds **5** (entries 21–26), which deserves the following mention. Although the compounds **5** containing nitro and sulfonyl group (like entries 1–4 type compounds) are highly desirable in terms of biological activity, the synthetic attempts to couple nitro group containing quinoxaline **3** with several isothiocyanates were failed possibly due to the weak electrophilicity of the isothiocyanates. However, most of the thioureido compounds **5** showed moderate to good activities in spite of the absence of the nitro and sulfonyl groups (Table 2). Next, the strong biological activity exhibited by the compounds of entries 1–5 of Table 1 prompted us to synthesize the carbon analogues represented by compounds **7**. However, the biological activity shown by this class of compounds is too weak to make comments.

In conclusion, we discovered a novel scaffold for DPP-IV inhibitor containing quinoxalinedione moiety through high throughput screening of chemical library of approximately 32,000 compounds (Korean Chemical

Table 1. Activity data of ureido compounds **4**



4

Entry	A	B	R	% Inhibition
1	H	NO_2	SO_2–Ph	57.2
2			SO_2–Ph(2-CH_3)	79.1
3			SO_2–Ph(4-CH_3)	48.0
4			SO_2–Ph(4-Cl)	66.8
5			CO–Ph	72.2
6	H	Cl	SO_2–Ph	17.3
7			SO_2–Ph(2-CH_3)	26.4
8			SO_2–Ph(4-CH_3)	20.5
9			CO–Ph	14.8
10	Cl	Cl	SO_2–Ph	23.7
11			SO_2–Ph(2-CH_3)	10.4
12			SO_2–Ph(4-CH_3)	21.0
13	H	H	SO_2–Ph	14.1
14			SO_2–Ph(2-CH_3)	12.3
15			SO_2–Ph(4-CH_3)	12.3

(*continued on next page*)

Table 1 (*continued*)

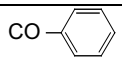
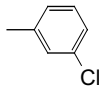
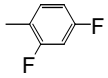
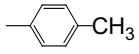
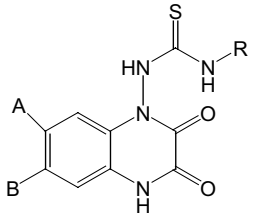
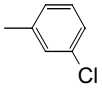
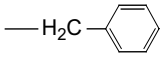
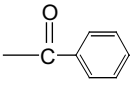
Entry	A	B	R	% Inhibition
16				13.0
17	H	NO_2		6.6
18				3.6
19				5.1
20				1.0

Table 2. Activity data of thioureido compounds **5**



5

Entry	A	B	R	% Inhibition
21	H	H		26.5
22				32.1
23				41.5
24			$-CH_2CH_3$	40.1
25	Cl	Cl		50.0
26				20.5

Bank), and that nitro group in the quinoxaline ring as well as sulfonyl group in ureido part seems to be critical to maintain the inhibitory potency of this series of compounds. The through investigations regarding these quinoxalinedione compounds toward the DPP-IV inhibition are now underway.

References and notes

1. (a) Holst, J. J.; Deacon, C. F. *Diabetes* **1998**, *47*, 1663; (b) Ahren, B. *BioEssays* **1998**, *20*, 642; (c) Willms, B.; Werner, J.; Holst, J. J.; Orskov, C.; Creutzfeldt, W.; Nauck, M. A. *J. Clin. Endocrinol. Metab.* **1996**, *81*, 327.
2. (a) Deacon, C. F.; Prodal, L.; Klarskov, L.; Olesen, M.; Holst, J. J. *Am. J. Physiol.* **1996**, *271*, E458; (b) Mentlein, R.; Gallwitz, B.; Schmidt, W. E. *Eur. J. Biochem.* **1993**, *214*, 829.
3. (a) Schon, E.; Mansfold, H. W.; Demuth, H. U.; Barth, A.; Ansorge, S. *Biomed. Biochim. Acta* **1985**, *44*, K9; (b) Gruher, M.; Scholz, W.; Flad, H. D. *Cell Immunol.* **1988**, *113*, 423.
4. Yaron, A.; Naider, F. *Crit. Rev. Biochem. Mol. Biol.* **1993**, *28*, 31.
5. Vilsboll, T.; Krarup, T.; Deacon, C. F. *Diabetes* **2001**, *50*, 609.
6. Ahren, B.; Simmonsson, E.; Larsson, H.; Landin-Olsson, M.; Torheirsson, H.; Jansson, P. A.; Sandqvist, M.; Bavenholm, P.; Efendic, S.; Eviksson, J. W.; Dickinson, S.; Holmes, D. *Diabetes Care* **2002**, *25*, 869.
7. (a) Villhauer, E. B.; Brinkman, J. A.; Naderi, G. B.; Dunning, B. E.; Mangold, B. L.; Mone, M. D.; Russell, M. E.; Weldon, S. C.; Hughes, T. E. *J. Med. Chem.* **2002**, *45*, 2362; (b) Villhauer, E. B.; Brinkman, J. A.; Naderi, G. B.; Burkey, B. F.; Dunning, B. E.; Prasad, K.; Mangold, B. L.; Russell, M. E.; Hughes, T. E. *J. Med. Chem.* **2003**, *46*, 2774.
8. Cai, S. X.; Kher, S. M.; Zhou, Z.; Espitia, S. A.; Tran, M.; Hawkinson, J. E.; Woodward, R. M.; Weber, E.; Keana, F. W. *J. Med. Chem.* **1997**, *40*, 730.
9. Wallace, R. G. *Org. Prep. Proc. Int.* **1982**, *14*, 269.
10. ^{1}H NMR (DMSO-d_6) at 400 MHz of compound **3** (A = H, B = NO_2) δ 5.69 (s, 2H, $-NH_2$), 6.90 (d, 1H, *-9H*), 7.76 (d, 1H, *-8H*), 8.13 (m, 1H, *-6H*), 12.06 (br, 1H, –NH–).
11. Paquette, L. A. In *Reagents for Organic Synthesis*; John Wiley and Sons, 1995; pp 1007–1009.
12. Balkan, B.; Kwasnik, L.; Milserendino, R.; Holst, J. J. *Diabetologia* **1999**, *42*, 1324.
13. IC_{50} values (μm): entry 1, 22.1; entry 2, 18.7; entry 3, 25.8; entry 4, 18.4; entry 5, 12.7.