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# Dipeptidyl peptidase-4 inhibitor with β-amino amide scaffold: Synthesis, SAR and biological evaluation

Heung Jae Kim [a,b], Woo Young Kwak [a], Jong Pil Min [a], Si Young Sung [a], Ha Dong Kim [a], Mi Kyung Kim [a], Hae Sun Kim [a], Kyung Jin Park [a], Moon Ho Son [a], Soon Hoe Kim [a], Bong Jin Lee [b,*]

[a] Dong-A Pharm. Co., Ltd, 47-5, Sanggal-dong, Giheung-gu, Yongin-si, Gyeonggi-do, 446-905, Republic of Korea
[b] Research Institute of Pharmaceutical Sciences, College of Pharmacy, Seoul National University, 599 Gwanak-Ro, Seoul 151-742, Republic of Korea

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ABSTRACT

Inhibitors of dipeptidyl peptidase-4 (DPP4) have been shown to be effective treatments for type 2 diabetes. Several series of β-amino amide containing piperazine derivatives have been prepared and evaluated as a inhibitor of DPP4. Finally compound **5m** was selected for further evaluation.



Inhibition of the serine protease dipeptidyl peptidase 4 (DPP4) has proven to be an effective treatment for improving glycemic control in type 2 diabetic patients.[1] Recovery of glucose homeostasis by DPP4 inhibition results from the prolonged action of the incretins glucagon-like peptide 1 (GLP-1) that are normally rapidly inactivated by the N-terminal cleavage of the peptide by the aminopeptidase dipeptidyl peptidase-4 (DPP4, CD26, EC 3.4.14.5), a sequence-specific non-classical serine protease.[2] Glucagon-like peptide-1 (GLP-1) stimulates glucose-dependent insulin secretion, inhibits hepatic glucose production, lowers blood glucose levels, and promotes the growth and differentiation of β-cells. GLP-1 may also play a role in suppressing appetite in humans and in modulating peripheral glucose uptake.[3] Inhibition of DPP4 leads to increased levels of endogenous GLP-1 by prolonging its half-life and consequently enhances the beneficial effects of GLP-1 in glucose dependent insulin secretion and β-cell restoration.[4] Since Type 2 diabetes is a disease characterized by elevated blood glucose levels and a relative insufficiency of insulin, a therapeutic agent that extends the duration of GLP-1 action may aid in controlling glucose homeostasis, by enhancing β-cell glucose-stimulated insulin release and promoting insulin gene expression and its biosynthesis.[5] Several DPP4 inhibitors have been developed for the treatment of type 2 diabetes including Sitagliptin[6] (Fig. 1: Vildagliptin,[7] Saxagliptin,[8] Alogliptin[9] and Linagliptin[10])

From previous report,[11] it is well known that β-amino acid substituted piperazines showed desirable inhibition activity for DPP4. We started our initial derivatization from substituted piperazine, compound **4**.

As shown in Scheme 1, the target compound **4** was prepared from commercial Boc protected β-amino acid **1** and N-substituted piperazine **2** using standard peptide coupling conditions followed by deprotection of amine.

Table 1 summarizes the DPP4 inhibitory properties of these β-amino amide piperazines. Several phenyl derivatives of this kind of piperazine showed inhibitory activity in the range of

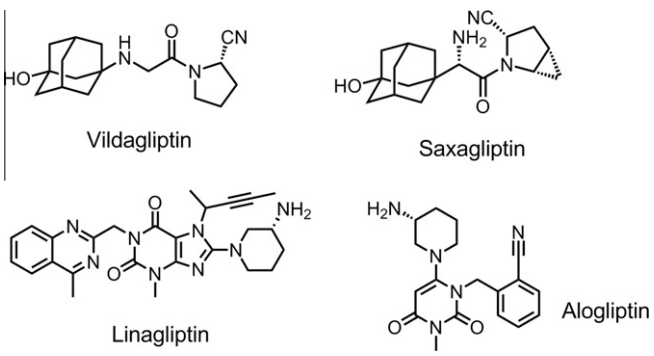


**Figure 1.** DPP4 inhibitors in market.

* Corresponding author. Tel.: +82 2 880 7869; fax: +82 2 872 3632.
*E-mail address:* lbj@nmr.snu.ac.kr (B.J. Lee).

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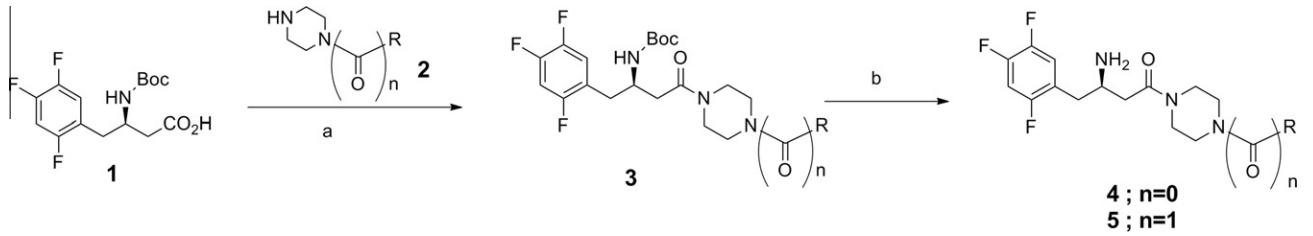
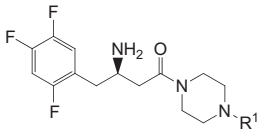
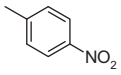
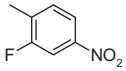
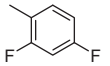
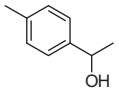
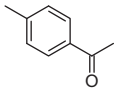
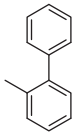
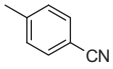
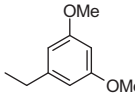
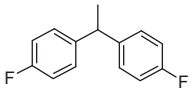



**Scheme 1.** Overall design of β-amino amide containing substitutied piperazine derivatives. Reagents: (a) EDC, HOBt, DIPEA/DMF or $CH_2Cl_2$, (b) HCl in ether/MeOH.

**Table 1**
DPP4 inhibition activities of various substituted compound **4**



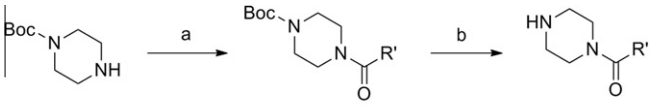
| Compound | $R^1$ | DPP4 $IC_{50}$[12] (nM) |
|---|---|---|
| **4a** |  | 85.7 |
| **4b** |  | 194 |
| **4c** |  | 291 |
| **4d** |  | 264 |
| **4e** |  | 99.7 |
| **4f** |  | 569 |
| **4g** |  | 50.4 |
| **4h** |  | 101 |
| **4i** |  | 103 |
| **4j** |  | 212 |

0.05–0.5 μM. The fluoro substituted compounds **4b** was less potent than compound **4a**. In compound **4c**, displacement of nitro to fluoro resulted in further decrease of inhibitory activity. From the results of compound **4d** and **4e**, carbonyl group was more potent than hydroxyl group. *ortho*-Substitution with phenyl and carbonitrile (**4f**, **4g**) showed 10-fold activity difference. Additional substitution to compound **4i** with bulky phenyl (**4j**) led to decrease of the inhibitory activity.

In case of compound **4b**, from the results of mouse OGTT (oral glucose tolerance test) study,[13] it has a glucose lowering effect of 37.0%, 44.6% at the dose of 3, 10 mg/kg, respectively. It also has good mouse pharmacokinetic profile ($t_{1/2}$ = 7.06 h, BA ~100%), however the evaluation was discontinued due to the limitation of in vivo efficacy. In mouse DPP4 inhibition assay, compound **4c** has an $IC_{50}$ of 94.4 nM as well as human DPP4 inhibition study ($IC_{50}$ of 291 nM). And the compound showed excellent OGTT study result (66% reduction of glucose AUC, at the dose of 10 mg/kg) so dose dependent in vivo DPP4 inhibition study[14] was performed. The dose for 50% plasma DPP4 inhibition ($ED_{50}$) of compound **4c** was calculated to 0.87 mg/kg (For the dose of 0.3, 1, 3, 10 mg/kg, DPP4 inhibition % were 33.8, 52.2, 68.6 and 85.9, respectively). But the compound **4c** doesn't have good selectivity over DPP9 ($IC_{50}$ = 1763 nM, 18-fold selectivity) and has strong CYP3A4 inhibition (81.5, 94.0, 100% inhibition at the concentration of 1, 3, 10 μM), so we stopped the assessment of the compound **4c**.

Compound **4** series has some limitation with in vivo efficacy, selectivity and undesirable CYP inhibition, we designed another series of β-amino amide which has moiety of direct carbonyl extension at the position of -N of piperazine. This design is based on the concept that through the carbonyl linker, direction to the S2 pocket of DPP4 active site will be more desirable for the inhibition activity and the peptidomimetic modification will be more susceptible to the enzymatic recognition.

The compound N-substituted piperazine with carbonyl function group was prepared as shown in Scheme 2.




**Scheme 2.** Reagents: (a) R′$CO_2H$, PyBOP, $Et_3N$, $CH_2Cl_2$ or R′$CO_2Cl$, $Et_3N$, $CH_2Cl_2$; (b) HCl in ether/MeOH.

Compound **6**, Commercial Boc-protected piperazine, was coupled with acid or acid chloride to give compound **7**. Deprotection of Boc derivatives **6** by treatment with hydrogen chloride in ether provided compound **8**.

The crystallographic data (PBD id: 1X70) showed that S2 pocket in active site was composed of key amino acids with phe357, Arg358, Ser209. And according to following molecular modeling studies (Fig. 2), simple phenyl ring was introduced after carbonyl unit and modification of *para*-position was followed for efficient targeting on interaction with S2 pocket. Compounds in previous SAR have little chance to make hydrophobic interaction with Phe357[6,15] and more chance to have steric hindrance with

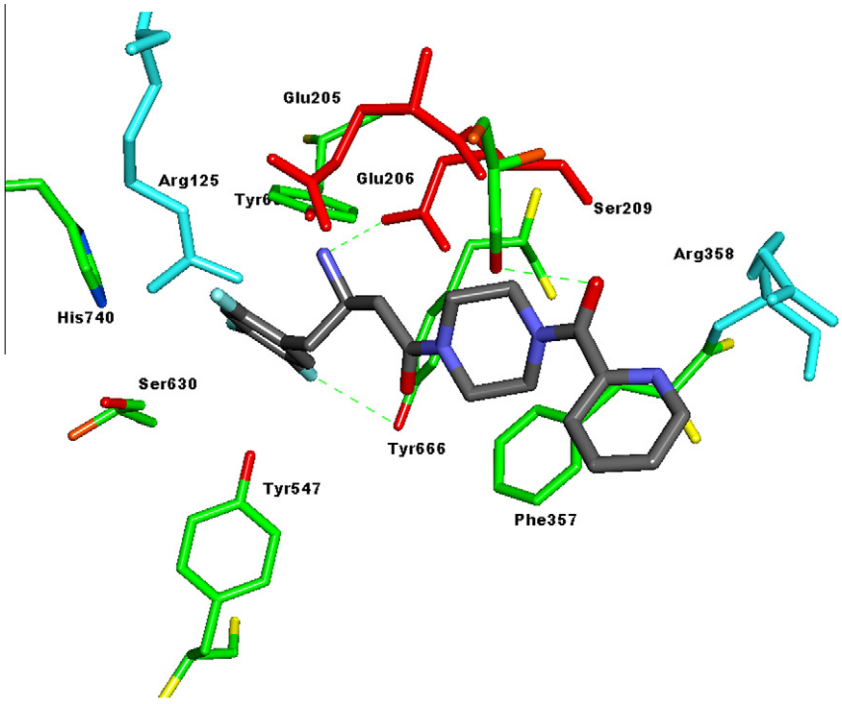
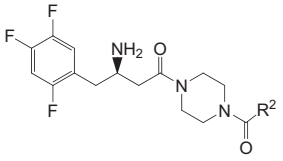
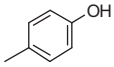
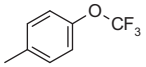
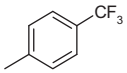
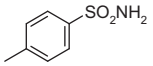
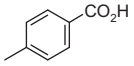
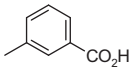
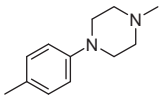



**Figure 2.** Molecular modeling of compound **5m** with DPP4 active site. Substituted pyridine ring of 5 m interacts with the side chain of Phe357 and forms pi–pi interaction. Carbonyl linker enabled to detour steric hindrance with Arg358 and H-bond was formed between carbonyl group and Ser209.

**Table 2**
DPP4 inhibition activities of various substituted compound **5**



| Compound | $R^2$ | DPP4 $IC_{50}$ (nM) |
|---|---|---|
| **5a** |  | 52.6 |
| **5b** |  | 45.3 |
| **5c** |  | 39.0 |
| **5d** |  | 64.6 |
| **5e** |  | 56.4 |
| **5f** |  | 45.3 |
| **5g** |  | 53.4 |
| **5h** |  | 56.9 |
| **5i** |  | 47.7 |

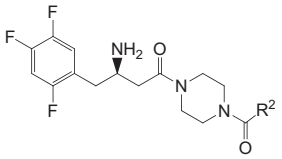
Arg358. Compound **5** series which have carbonyl moiety get the opposite character to the previous compound **4** series. In Table 2, most compounds showed excellent DPP4 inhibitory activities around 50 nM. But there was similar problem in CYP3A4 inhibition ($IC_{50}$; 1–5 μM) as well as in compound **4** series.

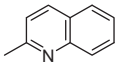
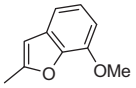
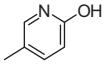
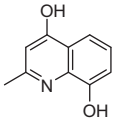
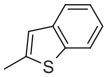
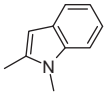
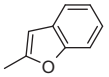
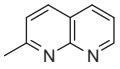
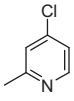
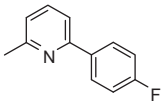
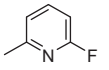
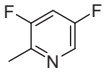
Replacement phenyl ring with heteroaromatic ring resulted in very similar DPP4 inhibitory activity to phenyl ring derivatives. But we could get the compound that has more than 50-fold selectivity over DPP9. In addition to selectivity index, through in vivo OGTT screening and solubility test, compound **5m** was selected as a representative analog in this series for further assessment. Table 3 summarizes the results of DPP4, DPP9 inhibition and OGTT studies.

In vivo DPP4 inhibition study (in Sprague–Dawley rats, Fig. 3) of compound **5m**, maximum inhibition was reached within 30 min. Duration time of inhibition after single oral administration of the dose of 10 mg/kg were measured. Until 6 h DPP4 inhibition was maintained up to 60% and after 24 h, DPP4 inhibition in plasma was around 30%. Compound **5m** showed dose dependency in OGTT study. At the dose of 0.3 mg/kg, glucose AUC reduction was 29.2% compared to control group. The glucose AUC reduction showed 35.8%, 40.9% at the dose of 1, 3 mg/kg (Fig. 4). We calculated the dose which reduce glucose AUC to 30% of control group glucose AUC ($ED_{30}$), 0.38 mg/kg. In separate experiment, plasma DPP4 inhibition were measured in C57BL/6 mice at 1 h after oral administration (five doses were used; 0.1, 0.3, 1.0, 3.0, 10 mg/kg). At the dose of 1, 3, 10 mg/kg, in vivo DPP4 inhibition were 32.5%, 59.1% and 84.6%, respectively (Fig. 4). Compound **5m** was submitted to the advanced test including pharmacokinetic studies in rats. Table 4 summarizes the preliminary ADME data of compound **5m**.

In summary, we have shown that the series of β-amino amide containing the piperazine-phenyl and piperazine carbonyl linker scaffold can have excellent DPP4 inhibitory activity through the efficient targeting to S2 pocket in active site and showed desirable in vivo efficacy with results of oral glucose tolerance test in C57BL/

**Table 3**
DPP4, DPP9 inhibition activities and OGTT study results of various substituted compound **5**



| Compound | $R^2$ | DPP4 $IC_{50}$ (nM) | DPP9 $IC_{50}$ (nM) | OGTT % (3 mg/kg) |
|---|---|---|---|---|
| **5j** |  | 67.7 | 2609 | 40.3 |
| **5k** |  | 24.9 | 1368 | 25.9 |
| **5l** |  | 65.4 | 318 | 21.2 |
| **5m** |  | 57.6 | 4862 | 44.2 |
| **5n** |  | 74.4 | 4746 | 43.6 |
| **5o** |  | 91.5 | 2740 | 15.7 |
| **5p** |  | 31.2 | 471 | 30.4 |
| **5q** |  | 50.6 | 1013 | 17.3 |
| **5r** |  | 33.9 | 427 | 28.6 |
| **5s** |  | 76.2 | 885 | 29.4 |
| **5t** |  | 36.7 | 1449 | 38.5 |
| **5u** |  | 42.2 | 4814 | 29.1 |
| **5v** |  | 30.5 | 658 | 11.6 |
| **5w** |  | 113.9 | 4226 | 20.2 |
| **5x** |  | 57.4 | 2096 | 48.1 |

6 mice. Among the compound **5** series, compound **5m** showed a very promising activity profile including excellent in vivo efficacy and other ADME parameters (liver microsomal stability, plasma stability, permeability and solubility), so further evaluation will be conducted.

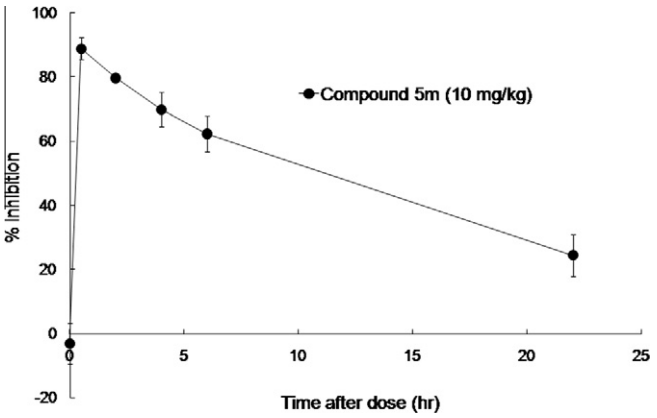



**Figure 3.** Inhibition of DPP4 activity in plasma obtained from Sprague–Dawley rats after single oral administration of compound **5m** at the dose of 10 mg/kg.

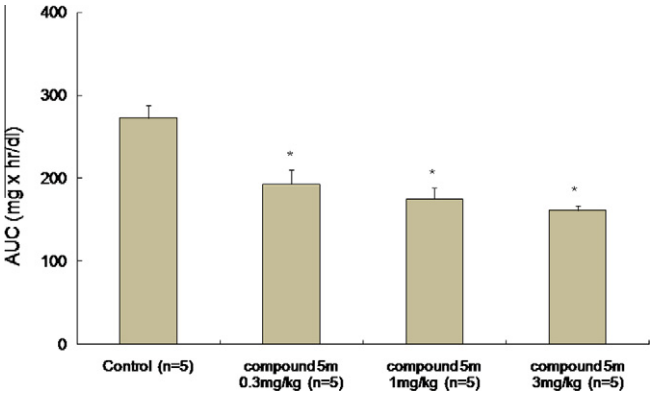



**Figure 4.** Effects of compound **5m** on the change of blood glucose levels in C57BL/6 mice after oral glucose loading.

**Table 4**
Preliminary ADME data of compound **5m**

| | |
|---|---|
| Liver microsomal stability (remaining % at 1 h) | Rat(85), mice(79), dog(88), human(89) |
| CYP3A4 induction (at 50 μM) | 10% < |
| Rat PK (po, 10 mg/kg): $t_{1/2}$, $C_{max}$, BA | 4.74 h, 2031 ng/mL, 65.3% |
| Permeability [Papp($\times 10^{-6}$ cm/s)] | 2.11 |
| Plasma stability (remaining % at 1 h) | Mouse (>97), Rat (>97), Human (97) |
| Protein binding (%) | Mouse (44.3), Rat (27.1), Human (50.3) |
| pH solubility (pH 1–9) | >10 mg/mL |

## References and notes

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12. *DPP-4 inhibition assay*: Inhibitors at various concentrations were incubated with 20 ng/ml rhDPP-4 enzyme and 50 mM H-Gly-Pro-AMC for 1 h at 25 °C. The fluorescence (360 nm excitation/465 nm emission) was measured after 1 h.
13. *OGTT study (in the C57BL/6J mice)*: Compound or vehicle (0.5% methylcellulose) was treated orally and then challenged with glucose (2 g/kg) 30 min post-dose. The blood glucose excursion profile from 0 to 120 min was used to integrate an area under for curve (AUC) and percent inhibition values for each treatment were generated from the AUC data normalized to the non-glucose controls.
14. Compound or vehicle (0.5% methylcellulose) was treated orally. After 1 h, plasma sample were collected and DPP-4 inhibition assay (Ref. 13) followed.
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