Design, Synthesis and Biological Evaluation of Imidazo[1,2-a]pyridine Derivatives as Novel DPP-4 Inhibitors

Qing Li¹, Muxing Zhou¹, Li Han¹, Qing Cao¹, Xinning Wang¹, LeiLei Zhao¹, Jinpei Zhou² and Huibin Zhang¹,³,*

¹Center of Drug Discovery, China Pharmaceutical University, Nanjing 210009, China
²Department of Medicinal Chemistry, China Pharmaceutical University, 24 Tongjia Xiang, Nanjing 210009, China
³Jiangsu Key Laboratory of Drug Discovery for Metabolic Disease, China Pharmaceutical University, Nanjing 210009, China
*Corresponding author: Huibin Zhang, zhanghb80@cpu.edu.cn

A new series of DPP-4 inhibitors with imidazo[1,2-a]pyridine scaffold were designed by exploiting scaffold hopping strategy and docking study. Based on docking binding model, structural modifications of 2-benzene ring and pyridine moieties of compound 5a led to the identification of compound 5d with 2,4-dichlorophenyl group at the 2-position as a potent (IC₅₀ = 0.13 μM), selective (DPP-8/DPP-4 = 215 and DPP-9/DPP-4 = 192) and in vivo efficacious DPP-4 inhibitor. Further, molecular docking revealed that compound 5d could retain key binding features of DPP-4 with the pyridine moiety of imidazo[1,2-a]pyridine ring providing an additional π-π interaction with Phe357 of DPP-4. Compound 5d might be a promising lead for further development of novel DPP-4 inhibitor treating T2DM.

Key words: dipeptidyl peptidase-4 inhibitor, docking study, imidazo[1,2-a]pyridine derivatives, type 2 diabetes mellitus

Abbreviations: T2DM, type 2 diabetes mellitus; DPP-4, dipeptidyl peptidase-4; GIP, insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; OGTT, oral glucose tolerance test.

Received 20 January 2015, revised 6 March 2015 and accepted for publication 12 March 2015

Type 2 diabetes mellitus (T2DM) is a chronic, progressive metabolic disease characterized by hyperglycemia resulting from insulin deficiency and insulin resistance (1). T2DM affects more than 370 million people worldwide⁶ (2) and is associated with harmful microvascular and macrovascular complications such as retinopathy, nephropathy and coronary artery disease (3). Incretin hormones, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) play a key role in the maintenance of normal glucose homeostasis (4). Both incretins can glucose dependently stimulate insulin secretion and inhibit glucagon release, and also delay gastric emptying and suppress appetite (5). In plasma, the active forms of these incretins are rapidly metabolized by dipeptidyl peptidase-IV (DPP-4). Thus, inhibition of DPP-4 can increase their plasma endogenous levels of both incretins and consequently improve glycemic control of T2DM (6). DPP-4 inhibitors such as sitagliptin(7), linagliptin(8), alogliptin(9) and teneligliptin(10) (Figure 1) have been approved for the treatment of T2DM (11,12). With comparable efficacy to sulfonylureas, DPP-4 inhibitors can control hyperglycemia alone or in combination with other agents (13). They show a good safety and tolerability profile, have no intrinsic risk of causing hypoglycemia and are weight neutral (14). However, these agents still do not meet clinic and commercial demands, especially for elderly patients with T2DM (15). We report herein the design, synthesis and biological evaluation of imidazo[1,2-a]pyridine derivatives as novel DPP-4 inhibitors.
Based on the results stated above, a novel bicyclic scaffold attached with hydrophobic benzene ring and methylamine was proposed to serve as novel DPP-4 inhibitors (Figure 1). This hypothesis was then evaluated by computational docking study of imidazo[1,2-a]pyrimidin-3-yl)methanamine (compound 4, Figure 2) to the DPP-4 receptor (PDB ID: 4JH0, Figure 2). Docking study was carried out using Glide 5.9b (24). The docking results revealed that benzene ring and methylamine moiety of the designed compound 4 can retain two key features and show similar docking mode as compound 3 (Figure 2). Benzene ring interacts with S1 pocket, and methylamine forms salt bridges with Glu205 and Glu206. However, a difference in the binding modes of the two compounds was observed in hydrogen binding interactions where methylamine does not form hydrogen binding interactions with Tyr 662 (Figure 2).

To explore a suitable central scaffold for investigation, eleven new core scaffolds B–L that can preserve the benzene ring and methylamine of compound 4 in similar positions were evaluated by docking study using Glide SP algorithmb (24). Glide searches for possible locations of the ligand in the active-site region of the receptor using a series of hierarchical filtersb that evaluate the ligand’s interaction with the receptor (25). The pose of the ligand was scored using GlideScore scoring function, which is based on ChemScore, but includes a steric-clash term with other rewards and penalties (24). As shown in Figure 2, the GlideScore of compounds incorporating scaffolds A, B, G and H are higher than that of the rest of scaffolds, in which the scaffold B exhibits the highest GlideScore of −7.886 kcal/mol. Considering both the synthetic feasibility for structural modification and the docking results, we decided to investigate the imidazo[1,2-a]pyridine scaffold B. Thus, imidazo[1,2-a]pyridine analogues 5a–q were synthesized and evaluated for DPP-4 inhibitory activity (Figure 3).

Methods and Materials

General chemistry
All reagents were purchased from commercial sources and used without further purification. Analytical thin-layer chromatography (TLC) was performed on the glass-backed silica gel sheets (silica gel 60 Å GF 254). Melting points were measured on capillary tube and were uncorrected. IR spectra (in KBr pellets) were taken using Shimadzu FT-IR-8400S spectrophotometer. 1H NMR and 13C NMR spectra (DMSO-d6, CDCl3) were recorded with a Bruker AV-300 spectrometer (Bruker Instruments, Inc., Billerica, MA, USA) in the indicated solvents (TMS as internal standard): the values of the chemical shifts are expressed in δ values (p.p.m.) and the coupling constants (J) in Hz. High-resolution mass spectra were recorded using an Agilent QTOF 6520 (Agilent, Santa Clara, CA, USA); element analysis was performed on CHN-O-Rapid instrument (Elementar, Hanau, Germany).

Detailed synthetic procedures and characterization data for all synthesized compounds are provided in the Supporting Information of this article.

In vitro assay for inhibition of DPP-4, DPP-8 and DPP-9
The DPP-4 Drug Discovery Kit (Enzo Life Sciences International, Inc.) was used for the assay of inhibition of DPP-4 activity. The assay is based on the cleavage of 7-amino-4-methylcoumarin (AMC) moiety from the C-terminus of the peptide substrate (H-Gly-Pro-AMC), which increases its
fluorescence intensity at 460 nm. The DPP-4 inhibitor P32/98 was selected as a control. The substrate and DPP-4 enzyme were diluted 1/50 with assay buffer (50 mM Tris, pH = 7.5). 25 μL of assay buffer, 15 μL of enzyme solution and 10 μL of appropriately diluted solutions of the test compounds were added subsequently to 96-well microtitre plates. After incubation at 37 °C for 10 min, 50 μL of diluted substrate solution was added. Fluorescence was measured using an excitation wavelength of 380 nm and an emission wavelength of 460 nm by a Synergy H1 Multi-Mode Reader (BioTek, Beijing, China). The inhibitory rate relative to the control without inhibitor was calculated, and IC50 values were determined by nonlinear regression fitted by GRAPHPAD PRISM 5 (Graph-Pad Software, Inc, La Jolla, CA, USA). The assays for inhibition of DPP-8 and DPP-9 activity were performed in similar procedure using DPP4-Glo™ Assay kit (Cat.No.G8531; Promega, Madison, WI, USA), DPP-8 and DPP-9 enzymes (BPS, Cat. NO. 80080 and 80080).

**In vivo oral glucose tolerance test (OGTT) in ICR mice**

Male ICR mice aged 10 weeks (18–22 g) were purchased from Comparative Medicine Centre of Yangzhou University. The mice were kept under conventional temperature and humidity conditions (12 h/12 h light–dark cycle) and had free access to food and tap water for 1 week before the experimental period. The male ICR mice were fasted overnight (12 h), weighted and randomized into groups (n = 8). Mice were dosed orally with vehicle (0.5% methylcellulose aqueous solution), linagliptin (suspended in vehicle; 3 mg/kg) or compound 5d (suspended in vehicle; 3, 10 and 30 mg/kg) at t = 30 min. Two blood samples were collected at t = 30 and 0 min by tail bleeding for glucose determinations. Glucose (20% aqueous glucose solution, 2.5 g/kg) was subsequently administered orally (at 0 min). Additional blood samples were collected at 30, 60 and 120 min after glucose load for glucose determinations. The blood glucose was measured by blood glucose test strips (SanNuo ChangSha, ChangSha, China). All animal procedures were performed in accordance with the applicable institutional and governmental regulations concerning the ethical use of animals.

**Molecular docking**

Docking studies were carried out using GLIDE 5.9 in Schrödinger 2013 suite (Schrödinger LLC, NY, USA) (24). The DPP-4 protein was extracted from RCSB...
Protein Data Bank (PDB ID: 4JH0)(20). Protein structures were prepared using Maestro protein preparation wizard (Schroedinger LLC, Maestro 9.4, NY, USA) applying the default parameters. Ligands were built using Maestro build panel (Schroedinger LLC, Maestro9.4, NY, USA) and prepared by LigPrep application. A docking grid was constructed using the centroid of the bound ligand and a maximum size of 10 Å. Molecular docking of all molecules into the generated grid was performed using the standard precision (SP) docking mode. To validate the docking procedure, a self-docking of 11 selected crystallized ligands into their DPP-4 protein was carried out. The root mean square deviations (RMSD) between the predicted conformation and the observed X-ray crystallographic conformation of all compounds were below 2.00 Å (Table S1), which revealed Glide is successful in reproducing the binding position. The Glide scores were correlated well with the reported pIC50 values. (Figure S2); thus, it was found to be promising in predicting the potency of new DPP-4 inhibitors.

Results and Discussion

Chemistry

The synthetic route adopted to obtain imidazo[1,2-a]pyridine 5a-q is diagrammed in Scheme 1. According to reported procedure (26,27), core imidazo[1,2-a]pyridine derivatives 8a-n were conveniently obtained by the reaction of 2-aminopyridine and substituted phenacyl bromides 7a-k that were synthesized by bromination of acetophenones 6a-k with copper (II) bromide (28). Vilsmeier formulation using N, N-dimethylformamide and phosphoryl chloride of these cyclic products 8a-n produced imidazo [1,2-a]pyridine aldehydes 9a-n with good yield. These aldehydes were later converted into oximes 10a-n by con-

Figure 3: Design of novel imidazo[1,2-a]pyridine derivatives and docking binding mode of compound 5a (light pink).
Imidazo[1,2-a]pyridine DPP-4 Inhibitors

DPS-4 inhibition studies and structure–activity relationships

The synthesized compounds 5a–q were evaluated for the inhibition of human recombinant DPP-4 in an in vitro assay at 500 nM using linagliptin and alogliptin as positive controls. Inhibitory potency was measured by following the increase of fluorimetric intensity at 460 nm upon hydrolysis of H-Gly-Pro-aminomethylcoumarin (H-Gly-Pro-AMC). The compounds with good inhibition rates at 500 nM were further selected to determine their IC50 values. The inhibitory activities are depicted in Table 1.

Primary docking studies showed that 2-benzene ring was bound to DPP-4 in the S1 pocket (Figure 3); our SAR analysis started with substitutions on the benzene ring. 2-Phenyl derivative (5a) showed moderate inhibitory activity (IC50 = 13.69 μM). Introducing substituents on the 3’-position of the phenyl ring led to decrease of activity, regardless of electron-donating or withdrawing groups (5a versus 5b, 5g and 5i). Bromine and chlorine atoms substituted on the 4’-position of the phenyl ring group (5j, IC50 = 0.94 μM, 5c, IC50 = 6.46 μM) improved activity compared with unsubstituted 5a. When the chlorine and methyl were introduced into the 2’-position, the inhibitory activities of the resulting compounds 5d 5e and 5h were improved dramatically compared with that of unsubstituted 5a. The activity of 2’-methyl-substituted 5h (IC50 = 0.29 μM) was 47-fold more potent than that of compound 5a. 2’-4’-dichlorine atoms substituted phenyl imidazo[1,2-a]pyridine (5d) displayed the most potent activity with IC50 values of 0.13 μM, which was 100-fold more than that of compound 5a. Meanwhile, the effects of substitutions on the pyridine moiety of 2-phenylimidazo[1,2-a]pyridine were further explored. Introducing of small hydrophobic methyl group on 6-, 7- and 8-position of imidazo[1,2-a]pyridine (5k, 5l and 5m) retained inhibitory activities. However, hydrophilic groups (5o, 5p and 5q) on 7-position of imidazo[1,2-a]pyridine led to losing inhibitory activities.

Docking study

To gain further insight into the observed SAR and the binding mode of the imidazo[1,2-a]pyridine derivatives, a docking study was conducted. We used the molecular docking program GLIDE 5.9b to dock compounds into a DPP-4 crystal structure (PDB ID: 4JH0) (20). The docking
results were shown in Figure 4. Compound 5d fits in the enzyme pocket topologically very well. The overlay of 5d against compound 3 shows two molecules to interact with DPP-4 in a similar way (Figure 4B). The primary amino group at the 3-position of the imidazo[1,2-a]pyridine ring can make salt bridge interaction with the Glu205 and Glu206 and hydrogen bond with Tyr662. The 2, 4-dichlorophenyl group at the 2-position of the imidazo

![Figure 5: Effect of compound 5d during an oral glucose tolerance test in male ICR mice.](image_url)

(A) shows time-dependent changes of blood glucose after oral administration of compounds, followed by 2.5 g/kg oral glucose challenge. Data in (B) represent AUC0–120 min of blood glucose levels. Values are mean ± SEM (n = 8). **p ≤ 0.01 compared to vehicle-treated ICR mice by Student’s t-test; ***p ≤ 0.001 compared to vehicle-treated ICR mice by Student’s - test.

### Table 1: In vitro activities of compounds 5a-q for DPP-4

<table>
<thead>
<tr>
<th>Compd</th>
<th>R1</th>
<th>R2</th>
<th>%Inhibition at 0.50 μM</th>
<th>IC50 (μM)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>5a</td>
<td>H</td>
<td>H</td>
<td>8.33 ± 1.06</td>
<td>13.69 ± 2.10</td>
</tr>
<tr>
<td>5b</td>
<td>3’-Cl</td>
<td>H</td>
<td>2.17 ± 0.93</td>
<td>&gt;50</td>
</tr>
<tr>
<td>5c</td>
<td>4’-Cl</td>
<td>H</td>
<td>10.46 ± 1.19</td>
<td>6.46 ± 0.20</td>
</tr>
<tr>
<td>5d</td>
<td>2’,4’-diCl</td>
<td>H</td>
<td>75.33 ± 3.43</td>
<td>0.13 ± 0.03</td>
</tr>
<tr>
<td>5e</td>
<td>2’,5’-diCl</td>
<td>H</td>
<td>42.98 ± 2.13</td>
<td>0.65 ± 0.05</td>
</tr>
<tr>
<td>5f</td>
<td>3’,4’-dif</td>
<td>H</td>
<td>-1.98 ± 1.76</td>
<td>NT</td>
</tr>
<tr>
<td>5g</td>
<td>3’-Me</td>
<td>H</td>
<td>-2.70 ± 1.70</td>
<td>NT</td>
</tr>
<tr>
<td>5h</td>
<td>2’-Me</td>
<td>H</td>
<td>59.27 ± 2.22</td>
<td>0.29 ± 0.06</td>
</tr>
<tr>
<td>5i</td>
<td>3’-CF3</td>
<td>H</td>
<td>-1.19 ± 0.96</td>
<td>NT</td>
</tr>
<tr>
<td>5j</td>
<td>4’-Br</td>
<td>H</td>
<td>23.7 ± 1.56</td>
<td>0.94 ± 0.12</td>
</tr>
<tr>
<td>5k</td>
<td>4’-Me</td>
<td>6-Me</td>
<td>10.31 ± 0.87</td>
<td>7.46 ± 0.22</td>
</tr>
<tr>
<td>5l</td>
<td>4’-Me</td>
<td>7-Me</td>
<td>34.08 ± 2.31</td>
<td>0.84 ± 0.12</td>
</tr>
<tr>
<td>5m</td>
<td>4’-Me</td>
<td>8-Me</td>
<td>3.70 ± 1.44</td>
<td>&gt;50</td>
</tr>
<tr>
<td>5n</td>
<td>4’-Me</td>
<td>6-COOH</td>
<td>0.54 ± 0.83</td>
<td>&gt;50</td>
</tr>
<tr>
<td>5o</td>
<td>4’-Me</td>
<td>6-COOH</td>
<td>-3.40 ± 0.90</td>
<td>NT</td>
</tr>
<tr>
<td>5p</td>
<td>–</td>
<td>–</td>
<td>-4.65 ± 1.35</td>
<td>NT</td>
</tr>
<tr>
<td>5q</td>
<td>–</td>
<td>–</td>
<td>-9.09 ± 1.42</td>
<td>NT</td>
</tr>
<tr>
<td>Linagliptin</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.001</td>
</tr>
<tr>
<td>Alogliptin</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.007</td>
</tr>
</tbody>
</table>

NT, not tested.

aMeasured in three independent experiments.

### Table 2: The inhibitory activity of compounds 5d and 5h for DPP-8 and DPP-9

<table>
<thead>
<tr>
<th>Compound</th>
<th>DPP-4 IC50 (μM)</th>
<th>DPP-8 IC50 (μM)</th>
<th>DPP-9 IC50 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5d</td>
<td>0.13</td>
<td>28</td>
<td>25</td>
</tr>
<tr>
<td>5h</td>
<td>0.29</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Alogliptin</td>
<td>0.007</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>
[1,2-a]pyridine ring fits tightly into the hydrophobic S1 pocket well (Figure 4B). These two key binding interactions may explain the observed potent increase in compound 5d compared to compound 5a and the decreasing activity of compound 5q. Compound 5a and 5q may not form two key binding interactions efficiently, in which compound 5a forms salt bridges to Glu205 and Glu206 and does not exhibit hydrogen bond with Tyr662 (Figure 4A), while phenyl ring of compound 5q cannot fully occupy S1 pocket due to the strong hydrogen binding interaction of trifluoromethyl of triazolo[4,3-a]pyrazine ring with Arg358 (Figure 4C); thus, compound 5q lost inhibitory activity. In addition, the pyridine moiety of imidazo[1,2-a]pyridine ring of compound 5d provides an additional π–π interactions with the Phe357 (Figure 4B). This result may explain the significantly enhanced potency of compound 5d.

Selectivity for DPP-4 over DPP-8 and DPP-9

Compounds 5d and 5h were chosen for further evaluation of inhibition selectivity for DPP-4 over DPP-8 and DPP-9. The inhibitory activities for DPP-8/9 are listed in Table 2. As shown in Table 2, compounds 5d and 5h showed good selectivity against other serine proteases. The selectivity ratio of compound 5d for DPP-4 over DPP-8 and DPP-9 were 215 and 192, respectively. The I$_{50}$ value of compound 5h for DPP-8 and DPP-9 were more than 100 μM, with ~340-fold selectivity ratio.

Effects of 5d on glucose excursion after an OGTT in ICR mice

Compound 5d was chosen for in vivo evaluation as it showed good in vitro potency and selectivity. Compound 5d (3, 10, and 30 mg/kg), linagliptin (3 mg/kg) or vehicle (0.5% methylcellulose aqueous solution) was orally administered to 12 h-fasted ICR mice (n = 8 in each group) 30 min prior to an oral glucose challenge (2.5 g/kg). Blood glucose values in the mice were measured at ~30, 0, 30, 60 and 120 min. The results are shown in Figure 5. The results indicated that compound 5d produces a dose-dependent improvement of glucose tolerance in ICR after oral administration. Compound 5d (30 mg/kg) reduced area under the curve from 0 to 120 min (AUC)$_{0-120 min}$ to 33.53% (5d, 748.66 ± 88.56; vehicle control, 1,125.49 ± 92.25), which was similar to the hypoglycemic effect of linagliptin (3 mg/kg, 35.0%, 731.57 ± 84.56) (Figure 5).

Conclusion

In this article, we reported the design, synthesis and biological evaluation of new DPP-4 inhibitor with imidazo[1,2-a]pyridine scaffold. First, novel imidazo[1,2-a]pyridine DPP-4 inhibitors were designed using scaffold hopping strategy combining with docking study. Then, based on docking binding model, structure modifications and SAR studies of 2-benzene ring and pyridine moieties of 2-phenylimidazo [1,2-a]pyridine were performed, and compound 5d with 2,4-dichlorophenyl group at the 2-position was identified as a potent, selective and in vivo efficacious DPP-4 inhibitor. Finally, further molecular modelling revealed compound 5d can fit in the enzyme pocket topologically very well with the pyridine moiety of imidazo[1,2-a]pyridine ring providing an additional π–π interactions with the Phe357 of DPP-4. Based on these results, compound 5d might be a promising lead compound for further optimization. Currently, further optimization is underway and will be published in due course.

Acknowledgments

This study was supported by the China National Key Hi-Tech Innovation Project for the R&D of Novel Drugs (No. 2013ZX09301303-002, and supported by Natural Science Foundation of Jiangsu Province (No. BK20141349).

References

Li et al.


Notes


Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Detailed synthetic procedures and characterization data for synthesized compounds.

Table S1. The structures for docking study, pIC50 values, pIC50, GlideScore RMSD and GlideScore.

Figure S1. Superposition of crystal structure of compounds 1 (magenta), 2 (green) and 3 (cyan).

Figure S2. The linear regression between Calculated GlideScore versus experimental pIC50 values for the 10 studied DPP-4 inhibitors.
学霸图书馆
www.xuebalib.com

本文献由“学霸图书馆-文献云下载”收集自网络，仅供学习交流使用。

学霸图书馆（www.xuebalib.com）是一个“整合众多图书馆数据库资源，
提供一站式文献检索和下载服务” 的24小时在线不限IP图书馆。
图书馆致力于便利、促进学习与科研，提供最强文献下载服务。

图书馆导航：
图书馆首页 文献下载 图书馆入口 外文数据库大全 疑难文献辅助工具