Bioorganic & Medicinal Chemistry Letters xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Design, synthesis and biological evaluation of novel pyrimidinedione derivatives as DPP-4 inhibitors

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ARTICLE INFO

Keywords:
Pyrimidinedionederivatives
DPP-4 inhibitor
Molecular hybrid
SARs
Type 2 diabetes

ABSTRACT

A series of novel pyrimidinedione derivatives were designed and evaluated for *in vitro* dipeptidyl peptidase-4 (DPP-4) inhibitory activity and *in vivo* anti-hyperglycemic efficacy. Among them, the representative compounds **11**, **15** and **16** showed excellent inhibitory activity of DPP-4 with IC_{50} values of 64.47 nM, 188.7 nM and 65.36 nM, respectively. Further studies revealed that compound **11** was potent *in vivo* hypoglycemic effect. The structure–activity relationships of these pyrimidinedione derivatives had been discussed, which would be useful for developing novel DPP-4 inhibitors as treating type 2 diabetes.

Diabetes is a major global problem nowadays. It currently affects nearly 425 million people worldwide in 2017, and this number will rise to 700 million in 2045.[1] Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are both incretin hormones increasing insulin biosynthesis and therefore contributing to glycemic control.[2,3] However, both hormones are rapidly inactivated by the serine protease DPP-4, leading to limiting their therapeutic practicality. The idea of inhibiting DPP-4 was developed as a promising new therapy for type 2 diabetes mellitus (T2DM) 20 years ago.[4] To date, twelve DPP-4 inhibitors (sitagliptin, vildagliptin, saxagliptin, alogliptin, linagliptin, anagliptin, gemigliptin, teneligliptin, evogliptin, omarigliptin, trelagliptin and gosogliptin) have been approved for the treatment of T2DM (Fig. 1).[5] Yet, there is still strong enthusiasm in developing novel DPP-4 inhibitors since some undesirable side effects exist in current drugs.[6]

Bromophenols are a set of natural products widely distributed in seaweed, most of which exhibit many biological activities, including hypoglycemic effect, anticancer, antioxidant, antimicrobial and other potent bioactivities.[7] In our previous study, a variety of bromophenols (Fig. 2), isolated from the red marine algae of the Rhodomelaceae family or synthesized derivatives, showed potent hypoglycemic effects *in vitro* and *in vivo*.[8–11] Herein, bromophenols are used as potent hypoglycemic agents and are considered as part of a new therapeutic strategy for treatment of type 2 diabetes.

The fully understanding of the interaction between DPP-4 enzyme and the bioactive substances plays a significant role in designing novel DPP-4 inhibitors. From the binding models we found that pyrimidinedione forms π-π interactions with Tyr547, which undergoes a conformational change in the S1 subsite; in addition, the cyanobenzyl group or the butynyl group binds to the S1 subsite.[12,13] Moreover, aminopiperidine is crucial for DPP-4 inhibitory activity, which forms salt bridge with Glu205, Glu206 and Tyr662 in S2 pocket.[14,15]

Synergistic activity is often attributed to molecular hybrids with different pharmacophores. Apart from pyrimidinedione core, we determined bromophenol, butynyl group, fluorocyanobenzyl or (*R*)-3-aminopiperidine as substituents. The widespread application of fluorine in drug design benefits from distinctive properties, including lipophilicity, eletrophilicity, metabolic stability, chemical stability, et al.[16] In view of these observations, a novel series of pyrimidinedione derivatives were designed and evaluated for *in vitro* DPP-4 inhibitory activity. Among these pyrimidinedione derivatives, compound **11** showed the most potent inhibitory activity of DPP-4 and was potent *in vivo* hypoglycemic effect. Structure-activity relationships (SARs) of these pyrimidinedione derivatives are also discussed in this paper.

It has been reported about the synthesis of benzyl bromide in our previous reports, as shown in Scheme 1 [17–19] The synthetic route of

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https://doi.org/10.1016/j.bmcl.2018.05.022
Received 30 March 2018; Received in revised form 2 May 2018; Accepted 9 May 2018

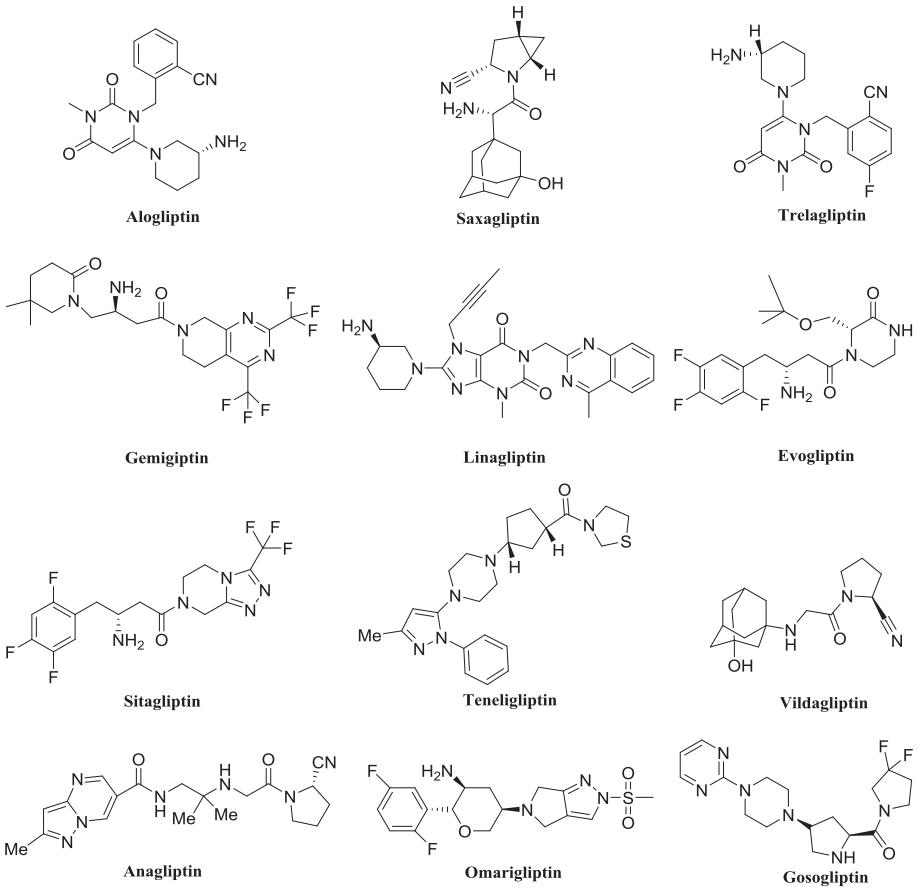


Fig. 1. Marketed DPP-4 inhibitors.

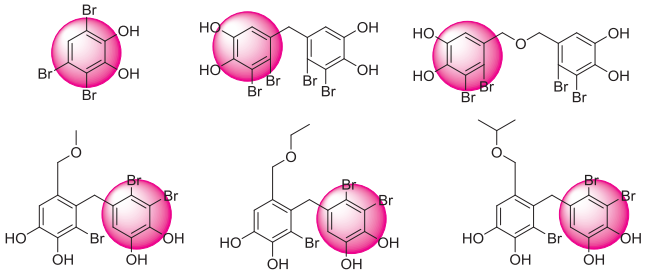
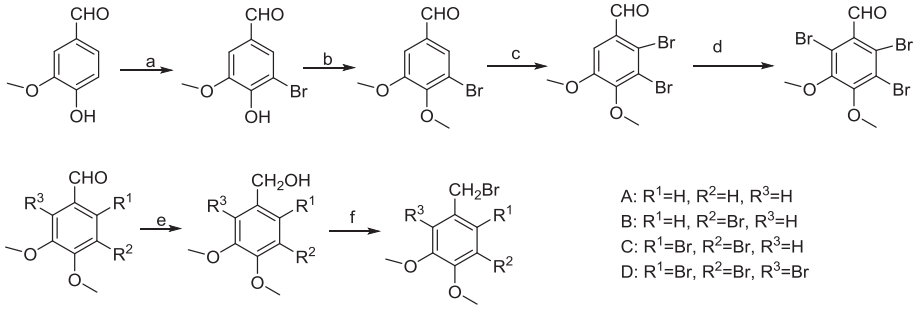


Fig. 2. Structures of bromophenol possessing hypoglycemic effect.

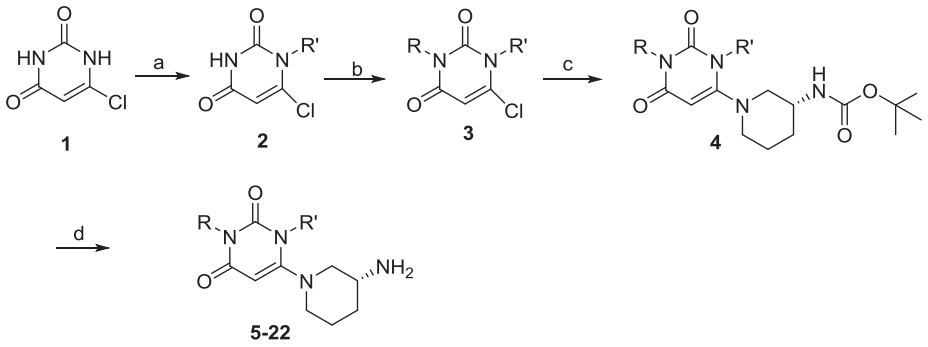
compounds **5–22** is depicted in Scheme 2[20,21] Briefly, the synthesis of pyrimidinedione derivatives was started from commercially available compound **1**. After alkylation of material **1** with 1-bromo-2-butyne or 2-cyanobenzo-5-fluorobenzyl bromide, the resulting precursor **2** was transformed into **3** through the *N*-alkylation. Compound **4** was obtained by a process of replacing the 6-chloro group with (*R*)-3-(Boc-Amino) piperidine. Finally, removal of Boc with TFA produced the final compounds **5–22**.

The inhibitory effects of compounds on DPP-4 activity were evaluated based on fluorescent probe.[22] In this part, we found that three compounds **11**, **15**, **16** showed potent inhibitory activity of DPP-4 at 100 nM with inhibitory rate of (100.12 ± 0.54)%, (95.59 ± 3.7)%, (98.84 ± 0.66)%, respectively (Table 1). As shown in Fig. 3, compounds **11**, **15** and **16** exhibited potent DPP-4 inhibitory activity, with IC_{50} values of 64.47 nM, 188.7 nM and 65.36 nM.

From the data of Table 1, we found that the degree of bromination of these compounds may have a close relationship with their DPP-4 inhibitory activity. For example, the number of bromine of compounds **11–14** gradually increases from 0 to 3. However, the DPP-4 inhibition rates of compounds **11–14** decrease from 100.12% to 0.66%. Similarly, compounds **6–8** were less potent than compound **5**. It could be found



Scheme 1. Reagents and conditions (a):Br_2, CH_3OH, 0 °C; (b): CH_3I, K_2CO_3, DMF, rt; (c): Br_2, CH_3COOH, Fe, 60 °C; (d): NBS, H_2SO_4, 0 °C; (e): $NaBH_4$, CH_3OH, 0 °C; (f):PBr_3, Et_3N, CH_2Cl_2, 0 ~ 15 °C;



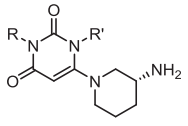
Scheme 2. Reagents and conditions (a):R'Br, DIPEA, DMF, rt; (b) RBr, K_2CO_3, KI, DMF, rt; (c): (*R*)-3-(Boc-Amino)piperidine, K_2CO_3, DMF, 90 °C, N_2 protection; (d): CH_2Cl_2, TFA, rt.

that their inhibitory rates decreased with the increase of the degree of bromination on phenol moiety. Furthermore, the degree of bromination of these compounds may have a close relationship with their lipid/water partition coefficient (Log P). For example, the DPP-4 inhibition rates of compounds **5–8** decrease from 86.8% to -0.87% with the increase of Log P. In addition, the compounds (**11**, **16**) with fluorocyanobenzyl substituent were more active than the compounds (**5**, **10**) with butynyl group (Table 1). SAR studies showed that fluorocyanobenzyl was crucial since it formed a hydrogen bond with Arg125. Moreover, π-π interaction also explained its enhanced binding affinity (Fig. 4).

To further study the hypoglycemic effect of compound **11**, blood glucose levels were measured during 4 weeks treatment.[11] In order to improve the solubility, the succinate salt of compound **11** was prepared according to the method of Hu et al.[23] The dynamic effects of compound **11** on water intake and body weight were recorded during four weeks treatment in BKS *db* mice. There was no difference in water intake and body weight at the baseline (Fig. 5A and B). As shown in Fig. 5A, there was a decrease trend in mean water intake from the second week in compound **11**- or metformin-treated group. However, both compound **11** and metformin had no effect on body weight of BKS *db* mice (Fig. 5B). A lot of abdominal fat was developed in BKS *db* mice at the fourth week (Fig. 5C). Compound **11** or metformin was unable to block the increased ratio of abdominal fat (Fig. 5D). In comparison with BKS mice, diabetic BKS *db* mice exhibited hyperglycaemia with blood levels of about 30 mM (Fig. 6). A trend towards decrease in blood glucose levels was revealed in compared with vehicle and compound **11**/metformin-treated groups. The blood glucose levels were reduced from the second week in both compound **11** and metformin treated group (Fig. 6). These results reveal that compound **11** has anti-diabetic potency with an effective blood glucose-lowing effect.

In summary, a series of pyrimidinedione derivatives were designed and evaluated for *in vitro* DPP-4 inhibitory activity and *in vivo* anti-hyperglycemic efficacy. The preliminary SARs analysis exhibits that: (a) hydrophobic property was important for the inhibitory activity of DPP-4. Lipid/water partition coefficient may affect their inhibitory activity

Table 1
Inhibition of DPP-4 by compounds 5–22 at 100 nM.



Compound	R	R′	Log P	Inhibitory rate%	IC_{50}/nM
5	3,4-Dimethoxybenzyl	Butynyl	1.55	86.8 ± 2.1	–[a]
6	3-Bromo-4,5-dimethoxybenzyl	Butynyl	2.38	28.54 ± 2.03	–
7	2,3-Dibromo-4,5-dimethoxybenzyl	Butynyl	3.21	2.7 ± 10.64	–
8	2,3,6-Tribromo-4,5-dimethoxybenzyl	Butynyl	4.04	−0.87 ± 2.14	–
9	3,5-Dimethylbenzyl	Butynyl	2.78	3.61 ± 16.56	–
10	3,5-Difluorobenzyl	Butynyl	2.12	72.98 ± 18.8	–
11	3,4-Dimethoxybenzyl	Fluorocyanobenzyl	2.66	100.12 ± 0.54	64.47
12	3-Bromo-4,5-dimethoxybenzyl	Fluorocyanobenzyl	3.49	73.89 ± 24.03	–
13	2,3-Dibromo-4,5-dimethoxybenzyl	Fluorocyanobenzyl	4.32	8.9 ± 9.42	–
14	2,3,6-Tribromo-4,5-dimethoxybenzyl	Fluorocyanobenzyl	5.15	0.66 ± 14.95	–
15	3,5-Dimethylbenzyl	Fluorocyanobenzyl	3.89	95.59 ± 3.7	188.7
16	3,5-Difluorobenzyl	Fluorocyanobenzyl	3.23	98.84 ± 0.66	65.36
17	Ethyl	Butynyl	0.41	21.49 ± 1.2	–
18	Propyl	Butynyl	0.90	10.16 ± 5.63	–
19	Hexyl	Butynyl	2.15	47.51 ± 3.26	–
20	Decyl	Butynyl	3.82	5.54 ± 2.61	–
21	Hexyl	Fluorocyanobenzyl	3.26	15.78 ± 10.6	–
22	Decyl	Fluorocyanobenzyl	4.93	−25.27 ± 17.51	–
Sitagliptin				100.49 ± 1.17	37.96

[a] The IC_{50} value was not measured.

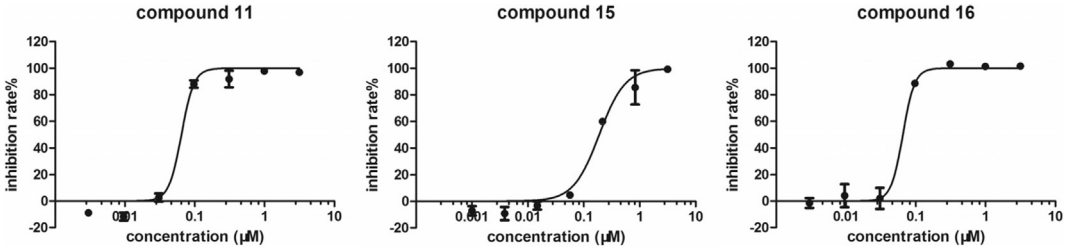


Fig. 3. Dose-dependent inhibition of DPP-4 by compounds **11**, **15**, **16.**

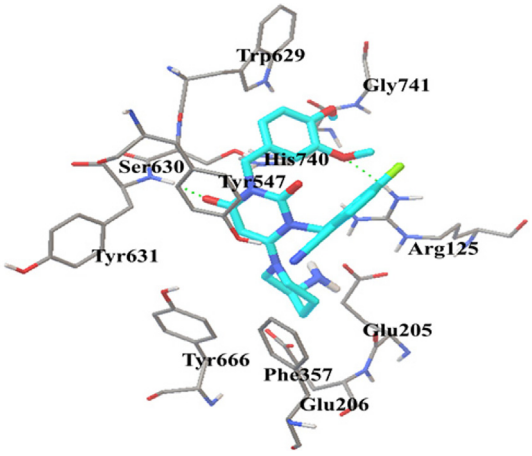


Fig. 4. Compound **11** in the active site of DPP-4 with key interactions.

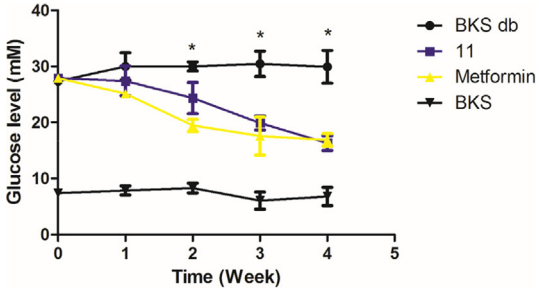


Fig. 6. Hyperglycaemic effect of compound **11**. Data are expressed as mean ± SD (n = 8). *$p < 0.05$ versus BKS *db* group.

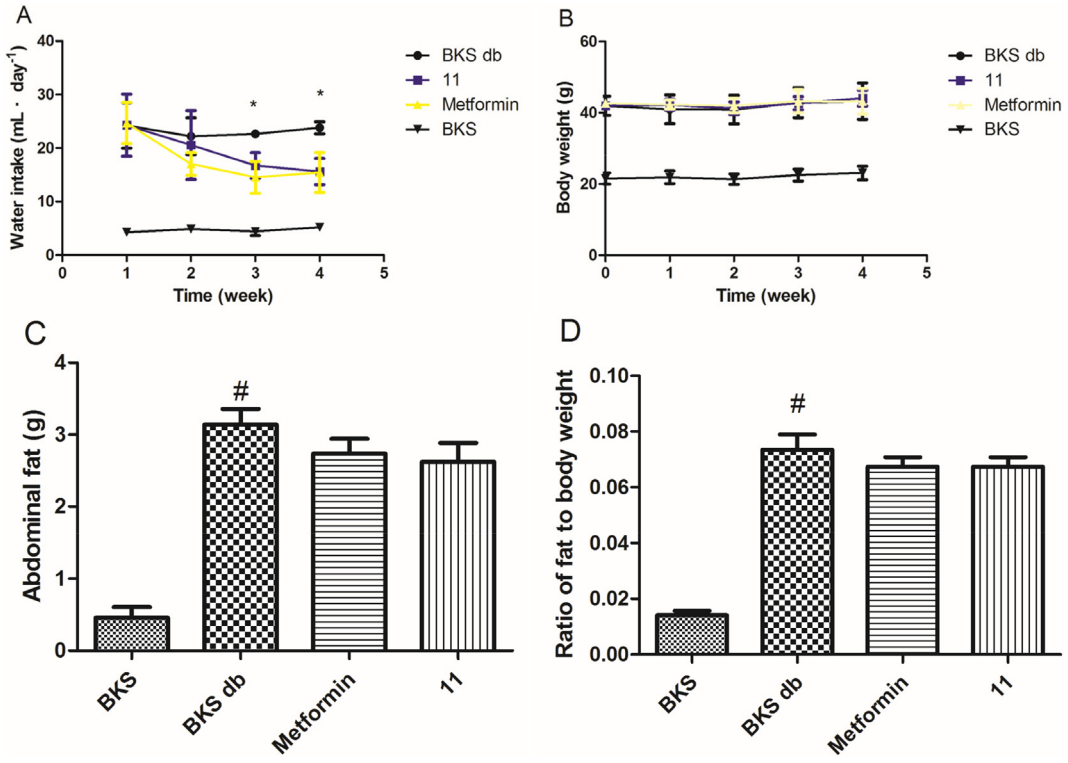


Fig. 5. Effects of 4 weeks treatment with compound **11** on mean water intake and body weight. (A): the water intake in BKS *db* mice during 4 weeks treatment with vehicle, compound **11** and metformin, and in BKS mice treated with vehicle. Data are expressed as mean ± SD (n = 4). *$p < 0.05$ (compound **11** group versus BKS *db* group). (B), (C) and (D): the body weight, abdominal fat weight and the fat ratio at the fourth week respectively. Data are expressed as mean ± SD (n = 8). #$p < 0.05$ versus BKS group.

of DPP-4; (b) the inhibitory rates increase with the decrease of the number of bromine atoms on phenol moiety; (c) the alkyl side chain could affect the DPP-4 inhibitory activity. (d) fluorocyanobenzyl was crucial for the inhibitory activity of DPP-4. Among them, the representative compounds **11**, **15** and **16** showed strong inhibitory activity of DPP-4 with IC_{50} values of 64.47 nM, 188.7 nM and 65.36 nM, respectively. Further studies revealed that compound **11** are potent *in vivo* hypoglycemic effect. Potentially, compound **11** is a promising DPP-4 inhibitor and worth further development.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 81773586, 81703354), and Shandong Provincial Natural Science Foundation for Distinguished Young Scholars (JQ201722), and Key research and development project of Shandong province (2016GSF201193, 2016ZDJS07A13, 2016GSF115002, 2016GSF115009), and Key Research Program of Frontier Sciences, CAS (QYZDB-SSW-DQC014), and the Project of Discovery, Evaluation and Transformation of Active Natural Compounds, Strategic Biological Resources Service Network Program of Chinese Academy of Sciences (ZSTH-026), and National Science Foundation of China (NSFC)-Shandong Joint Fund (U1706213), and National Program for Support of Top-notch Young Professionals, and Taishan scholar Youth Project of Shandong province and Qingdao Marine Biomedical Science and Technology Innovation Center project (2017-CXZX01-1-1, 2017-CXZX01-3-9).

A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2018.05.022.

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