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Piperidine derivatives as nonprostanoid IP receptor agonists 2

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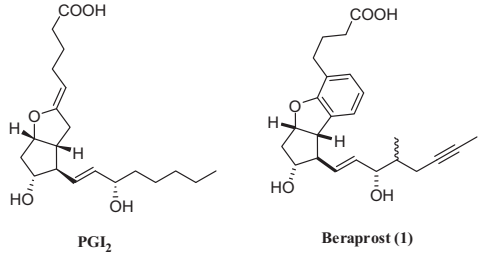
ABSTRACT

We searched for a strong and selective nonprostanoid IP agonist bearing piperidine and benzanilide moieties. Through optimization of substituents on the benzanilide moiety, the crucial part of the agonist, **43** (2-((1-(2-(*N*-(4-tolyl)benzo[*d*][1,3]dioxole-5-carboxamido)ethyl)piperidin-4-yl)oxy)acetic acid monohydrate monohydrochloride) was discovered and exhibited strong platelet aggregation inhibition (IC_{50} = 21 nM) and 100-fold selectivity for IP receptor over other PG receptors. The systemic exposure level and bioavailability after oral administration of **43** were also good in dog.

Prostacyclin inhibits platelet activation and acts as a vasodilator.[1,2] Prostacyclin also plays an important role in biological homeostasis as an endogenous autacoid widely distributed in various tissues. Interest in prostacyclin as an antithrombotic agent has prompted an intensive search for mimics or other prostaglandin I_2 receptor (IP receptor) agonists with high chemical and metabolic stability. Our original approach led us to create the stable prostacyclin mimic beraprost (**1**, Fig. 1), which is used to treat chronic occlusive disease and primary pulmonary hypertension.[3–6]

On the other hand, novel IP agonists that are structural different from prostacyclin, nonprostanoid prostacyclin mimetic, have been sought in order to obtain metabolically stable IP agonists (Fig. 2).[7–13]



PGI_2 Beraprost (1)

Figure 1. Structure of beraprost.

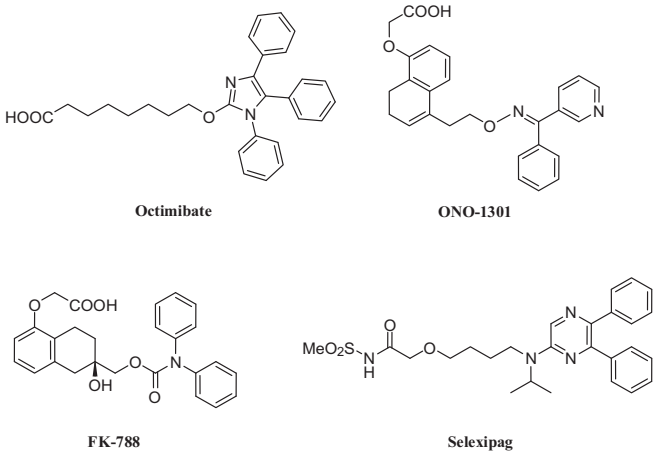


Figure 2. Nonprostanoid IP agonists.

We have been investigating not only prostacyclin mimics but also nonprostanoid IP agonists. We previously reported novel IP agonists bearing a piperidine moiety, such as **2** and **3** (Fig. 3).[14]

The unique piperidine derivative **2** ((2-((1-(2-(*N*-(4-tolyl)benzamido)ethyl)piperidin-4-yl)oxy)acetic acid)) was a good IP receptor agonist and was 50-fold more selective for the human IP receptor than for other human prostanoid receptors. In addition, the pharmacokinetic profile of **2** is particularly favorable, but its platelet aggregation inhibition activity resulting from its IP agonistic action

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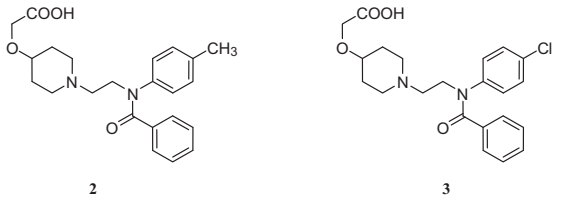
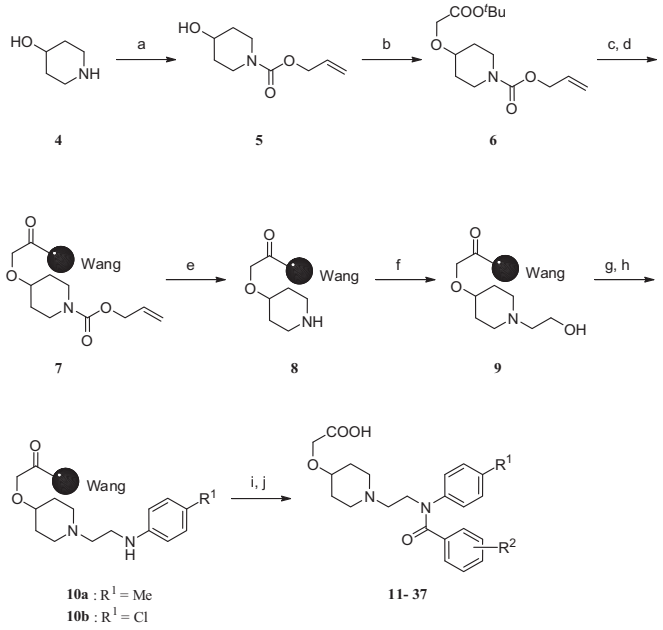


Figure 3. Our nonprostanoid IP agonists.



Scheme 1. Synthesis of compounds **11**–**37**. Reagents and conditions: (a) allyl chloroformate, NEt_3, CH_2Cl_2, rt, 15 h, 74%; (b) $BrCH_2COO^tBu$, Bu_4NBr, toluene, 10 M NaOH aq, 50 °C, 14 h, 62%; (c) TFA, CH_2Cl_2, rt, 3 h; (d) Wang resin, DMP, DIC, DMF, rt, 15 h; (e) 1,3-dimethylbarbituric acid, $Pd(PPh_3)_4$, THF, rt, 15 h; (f) 2-bromoethanol, K_2CO_3, DMF, rt, 12 h; (g) MsCl, NEt_3, CH_2Cl_2, rt, 2 h; (h) substituted aniline, KI, CH_3CN, 70 °C, 12 h; (i) substituted BzCl, NEt_3, THF, rt, 12 h; (j) TFA, CH_2Cl_2, rt, 1 h.

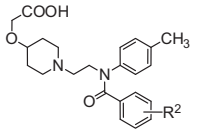
is unsatisfactory (IC_{50} = 130 nM). To obtain promising drug candidates, the inhibitory activity of IP agonists needs to be improved. In this letter, we report the design and synthesis of compounds that maintained the favorable pharmacokinetic profile of **2** while exhibiting higher platelet aggregation inhibitory activity (IC_{50} <100 nM).

We previously showed that replacement of substituents on the aniline moiety of **2** affected the level of platelet aggregation inhibitory activity, but the same approach was not tested for the benzoyl moiety, although a similar effect was expected. Accordingly, in this study, we aimed to discover compounds with substituted benzoyl moieties that increased platelet aggregation inhibitory activity.

Various substituents (e.g., halogen, electron-donating, electron-withdrawing, and aromatic groups) at several positions on the benzoyl moiety were tested. As in the case of **2** and **3**, the 4-position of the aniline moiety was substituted with a methyl or chloro. We used the same solid-phase approach as in our previous study to efficiently obtain a variety of compounds bearing the above-mentioned substituents (Scheme 1).

After protecting the nitrogen atom of commercially purchased 4-hydroxypiperidine by treatment with allyl chloroformate, the bromoacetic acid *t*-butyl ester structure was introduced by O-alkylation to produce **6**. The ester moiety of **6** was hydrolyzed, and **7** was immobilized on Wang resin. Compound **9** was obtained from **7** by nitrogen deprotection and then hydroxyethylation. The hydroxyl group of **9** was mesylated, and an aniline moiety and a benzoyl moiety were sequentially introduced. The final compounds (**11**–**37**) were obtained by the cleaved from the Wang resin. All compounds were purified by HPLC and identified by LC/MS. Platelet aggregation inhibition activities of synthesized compounds are shown in Tables 1 and 2.[15]

Table 1
In vitro platelet inhibitory activity of **11**–**21**[a]



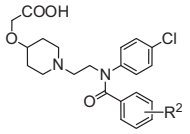
Compound	R^2	Inhibition of human platelet aggregation IC_{50}[b,c] (nM)
11	H	220
12	2-F	970
13	2-Cl	690
14	2-OCH_3	1200
15	2-CF_3	>10,000
16	2-Ph	>10,000
17	3-F	160
18	3-Cl	100
19	3-CH_3	160
20	3-OCH_3	32
21	3-CF_3	20,000

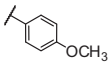
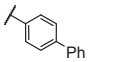
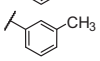
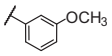
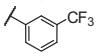
[a] IC_{50} represents the concentration that inhibited induced platelet aggregation by 50%.
[b] Platelet aggregation was induced by ADP (5 μM) in human platelet rich plasma.
[c] Values are means of two experiments.

The type of substituent on the aniline moiety (4-methyl or 4-chloro) did not influence the effect of substituents on the benzoyl moiety, and introduction of a 4-methoxy or 4-chloro substituent on the benzoyl moiety reduced the inhibitory activity. Introduction of a CF_3 or phenyl substituent on the benzoyl moiety resulted in an especially strong reduction in the inhibitory activity (Table 1, 15 and 16; Table 2, 26 and 27). On the other hand, substitution at the 3-position of the benzoyl moiety, except introduction of a CF_3 substituent, increased the inhibitory activity. Since introduction of a strongly electron-withdrawing CF_3 group at any position resulted in substantially decreased the inhibitory activity, lower electron density in the benzoyl moiety possibly induced the decreases in platelet aggregation inhibition activity (Table 1, 15 and 21; Table 2, 26 and 32).

Also, an electron-donating methoxy group increased the inhibitory activity of the compound when introduced at the 3-position, but decreased it when introduced at the 4-position (Table 1, 14 and 20; Table 2, 25 and 31). Tables 1 and 2 showed that the 3-substituted benzoyl derivatives were more potent than the

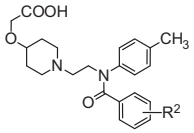
Table 2
In vitro platelet inhibitory activity of **22**–**32**[a]

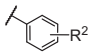
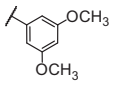
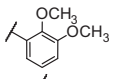
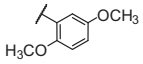
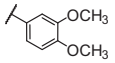


Compound		Inhibition of human platelet aggregation IC_{50}[b,c] (nM)
22		440
23		2000
24		1500
25		3100
26		>10,000
27		>10,000
28		340
29		350
30		310
31		74
32		>10,000

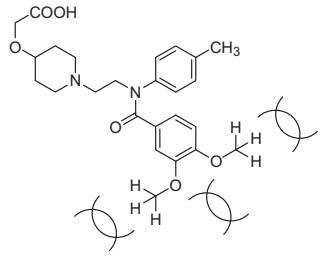
[a] IC_{50} represents the concentration that inhibited induced platelet aggregation by 50%.
[b] Platelet aggregation was induced by ADP (5 μM) in human platelet rich plasma.
[c] Values are means of two experiments.

Table 3
In vitro platelet inhibitory activity of **33**–**37**[a]



Compound		Inhibition of human platelet aggregation IC_{50}[b,c] (nM)
33		530
34		1600
35		>3000
36		>3000
37		21

[a] IC_{50} represents the concentration that inhibited induced platelet aggregation by 50%.
[b] Platelet aggregation was induced by ADP (5 μM) in human platelet rich plasma.
[c] Values are means of two experiments.



36 **37**

Figure 4. Speculation of bulkiness: comparison of **36** with **37**.

4-substituted ones. Both strong electron-withdrawing groups and bulky groups decreased inhibition activities.

Methoxy group at the 3-position was optimal. So some derivatives with methoxy groups and the related substituents were synthesized using the same solid-phase approach described above. Table 3 shows newly synthesized **33**–**37** with their platelet aggregation inhibitory activities.

Substitution at the 2-, 4-, 5- or 6-position of the benzoyl moiety with a methoxy group resulted in decreased inhibitory activity (Table 3 and **33**–**36**) compared with the starting compound (**20**, IC_{50} = 32 nM). In contrast, **37** bearing a methylenedioxy group between the 3- and 4-positions showed increased activity. Interestingly, the activities of **36** and **37** were very different even though the 3- and 4-positions of both the compounds had alkoxy substituents.

The methylenedioxy group forms a ring structure, that is, rigid and relatively compact, while the dimethoxy groups are free to rotate and bulkier. Such differences may partially explain the difference in inhibitory activities between these two compounds (Fig. 4).

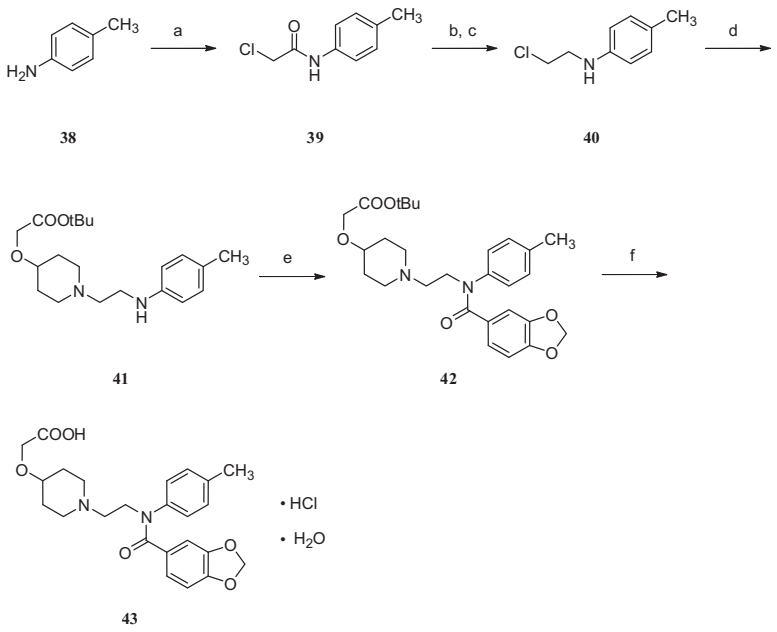
For example, a bulky substituent on the benzoyl moiety would interfere with the direct interaction between the IP receptor and the benzoyl moiety, or might cause an unfavorable conformation of the aniline and benzoyl moieties.

By replacing substituents, we identified target compounds with IC_{50} <100 nM, when tested for platelet aggregation inhibition activity (**20**, **21**, and **37**). Then, we established a large-scale production method for the most active compound (**37**) and prepared its hydrochloride salt (**43**), which can be purified by recrystallization (Scheme 2).

Commercially purchased *p*-toluidine was converted to its chloroacetamide derivative by treatment with the corresponding acid chloride, and then the amide moiety was reduced to obtain *N*-(2-chloroethyl)-4-methylaniline (**40**). Next, *t*-butyl 2-(piperidine-4-yloxy)acetate, prepared as previously reported[16], was *N*-alkylated with **40**, and the resulting compound (**41**) was amidated with piperonyloyl chloride. Acid hydrolysis then produced **43** (the monohydrochloride salt of the monohydrate of **37**).[17] Compound **43**, which can be purified by recrystallization, was examined by prostanoid receptor binding assay (Table 4) and pharmacokinetics testing in fasted dog.

Among several prostanoid receptors tested, only the IP receptor showed affinity for the **43**. Although more receptors (e.g., EP_1 and DP) need to be tested, this compound appears to be a highly selective ligand of the IP receptor. Pharmacokinetics testing of **43** in fasted dog (0.5 mg/kg, p.o.) showed good exposure (C_{max} = 130 ng/mL) and bioavailability (76%).

In conclusion, we used piperidine-type nonprostanoid IP agonists **2** and **3**, developed by our group, as starting materials and successfully produced **43**, a compound that exhibited a favorable pharmacokinetic profile and strong inhibition of platelet aggregation,



Scheme 2. Synthesis of compound **43**. Reagents and conditions: (a) 2-chloroacetyl chloride, pyridine, THF, 0–20 °C, 1 h, 86%; (b) $NaBH_4$, BF_3·THF, THF, 0 °C then reflux, 1 h; (c) HCl–MeOH, rt, 1 h, 98% (2 steps); (d) *tert*-butyl 2-(piperidin-4-yloxy)acetate, DBU, KI, DMF, 95 °C, 6 h, 70%; (e) piperonyloyl chloride, pyridine, CH_3CN, 25 °C, 2 h, 84%; (f) 1 M HCl aq, 60 °C, 1 h, 85%.

Table 4
In vitro binding affinity for human prostanoid receptors of **43**

Compound	K_i value of human prostanoid receptors[a] (nM)					
	IP	EP_2	EP_3	EP_4	FP	TP
43	400	>50,000	>50,000	>50,000	>50,000	>50,000

[a] Values are means of two experiments.

by replacing substituents in the benzoyl moiety of the starting materials.

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17. Compound **43** was fully characterized by spectral methods. Representative data on compound **43**: ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.64–1.76 (1H, m), 1.90–2.03 (2H, m), 2.08–2.18 (1H, m), 2.25 (3H, s), 2.93–3.11 (2H, m), 3.19–3.44 (3H, m), 3.56–3.79 (2H, m), 4.07 (2H, s), 4.16–4.18 (2H, m), 5.98 (2H, s), 6.74 (1H, d, J = 8.0 Hz), 6.79 (1H, dd, J = 8.0, 1.2 Hz), 6.83–6.85 (1H, br s), 7.10–7.16 (4H, m), 10.20–10.32 (1H, br s), 12.60–12.72 (1H, br s).; EI-MS m/z: 441 $[MH]^+$; Elemental analysis: Anal. Calcd for $C_{24}H_{28}N_2O_7$·HCl·H_2O: C, 58.24; H, 6.31; Cl, 7.16; N, 5.66. Found: C, 57.88; H, 6.37; Cl, 7.09; N, 5.58.