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Drug Annotation

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Selexipag: An Oral and Selective IP Prostacyclin Receptor Agonist for the Treatment of Pulmonary Arterial Hypertension

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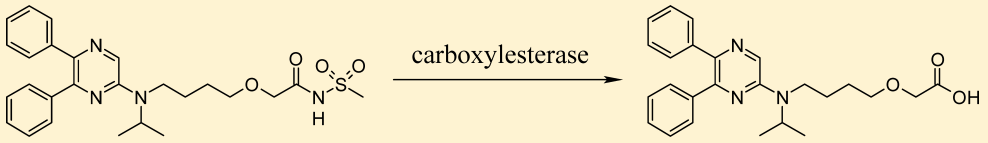
Supporting Information



26a (parent drug) **12b** (active form)

ABSTRACT: Prostacyclin controls cardiovascular function via activation of the prostacyclin receptor. Decreased prostacyclin production occurs in several cardiovascular diseases. However, the clinical use of prostacyclin and its analogues is complicated by their chemical and metabolic instability. A medicinal chemistry program searched for novel nonprostanoid prostacyclin receptor agonists not subject to these limitations. A compound with a diphenylpyrazine structural core was synthesized. Metabolic stability and agonist potency were optimized through modification of the linear side chain. Compound **12b** (MRE-269, ACT-333679) was identified as a potent and highly selective prostacyclin receptor agonist. Replacement of the terminal carboxyl group with an *N*-acylsulfonamide group yielded parent compound **26a** (selexipag, NS-304, ACT-293987), which is orally active and provides sustained plasma exposure of **12b**. Compound **26a** was developed for the treatment of pulmonary arterial hypertension and shown to reduce the risk of the composite morbidity/mortality end point in a phase 3 event-driven clinical trial.

INTRODUCTION

Prostacyclin (PGI_2)[1,2] is an endogenous prostanoid synthesized mainly by vascular endothelial cells. PGI_2 is a potent vasodilator and inhibitor of platelet aggregation. PGI_2 plays an important role in the maintenance of vascular homeostasis.[1,2] It also inhibits vascular smooth muscle cell differentiation, proliferation, and migration.[3] These physiological functions are mediated via activation of the PGI_2 receptor (IP receptor),[4] a G-protein-coupled receptor located on the surface of vascular smooth muscle cells, platelets, and other cells. Down-regulation of the PGI_2 pathway has been implicated in the pathogenesis of several vascular diseases,[5,6] supporting the use of epoprostenol, the synthetic sodium salt of PGI_2, and PGI_2 analogues as therapeutic agents.

However, epoprostenol is chemically and metabolically unstable at physiological temperature and pH.[2] As a consequence, its use requires continuous infusion through a central venous catheter. PGI_2 analogues such as iloprost and treprostinil have been developed in an effort to improve chemical stability, but most of the approved compounds need to be administered by subcutaneous/intravenous infusion or inhalation. The development of orally available PGI_2 analogues with sufficient duration of action has proven challenging.[7,8] Beraprost sodium is an orally available PGI_2 analogue, but its elimination half-life is very short and it has only transient clinical benefits in pulmonary arterial hypertension (PAH).[9] An oral formulation of treprostinil is indicated in the U.S. for the treatment of PAH to improve exercise capacity, but it did not show clinical benefit in combination therapy.[10]

We describe the development of **26a**, a novel orally active IP receptor agonist with a high degree of metabolic stability and an extended duration of action. Compound **26a** was discovered by Nippon Shinyaku Co., Ltd.[11,12] and developed for the treatment of PAH by Nippon Shinyaku and Actelion Pharmaceuticals Ltd.

MEDICINAL CHEMISTRY STRATEGY

The discovery strategy for a long-acting IP receptor agonist was based on the observation that the prototypical prostanoid structure is not necessary for activation of the IP receptor.[11] Octimibate[13] and **1** (BMY-42393),[14,15] both partial agonists at the IP receptor, bear no structural resemblance to PGI_2 and its analogues apart from a carboxylic acid moiety (Figure 1).

Compound **1**, which has a long duration of action in animal models of thrombosis,[16] was chemically modified by replacing the oxazole ring with a pyrazine ring to obtain the lead compound, the 2-amino-5,6-diphenylpyrazine derivative **4c** (Figure 1). The potency of compounds at the human IP receptor was measured by their inhibition of adenosine diphosphate (ADP)-induced platelet aggregation. Compound **4c** inhibited platelet aggregation with a half-maximal inhibitory concentration (IC_{50})

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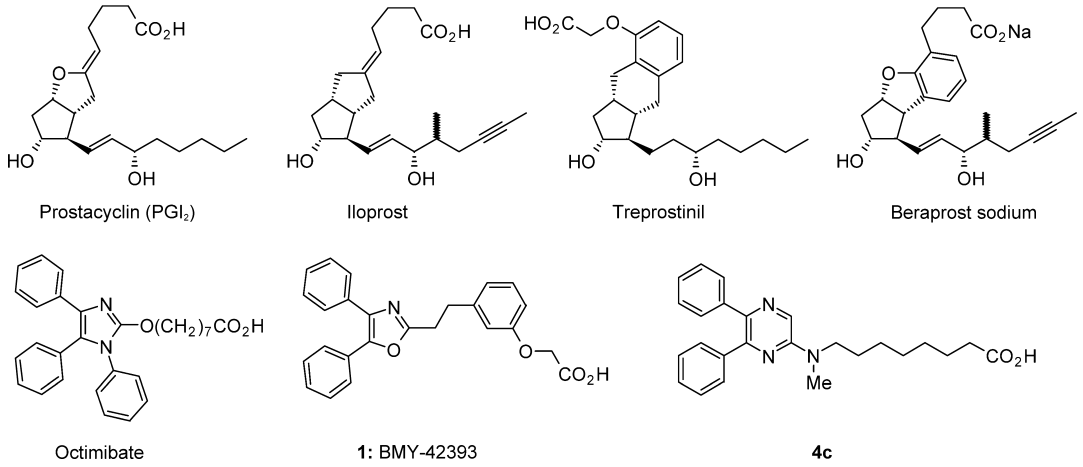
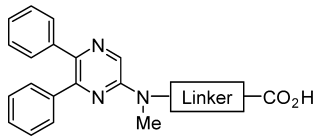
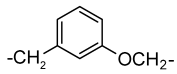
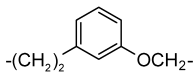


Figure 1. Chemical structures of prostacyclin (PGI_2), PGI_2 analogues, and nonprostanoid prostacyclin (IP) receptor agonists including lead compound **4c**.

value of 1.5 μM. On the basis of the observation that the topological relationship between the diphenyl heterocycle and the carboxylic acid moiety in the oxazole[14,15] and pyrazole[17] series determines agonist activity, the effects of the length and rigidity of the alkylene linker were investigated (Table 1).

Table 1. Effect of Linker Variation on Inhibition of ADP-Induced Human Platelet Aggregation[b]



Compound	Linker	Inhibition of human platelet aggregation IC_{50} (μM)[a]
4a	-$(CH_2)_5$-	8
4b	-$(CH_2)_6$-	0.3
4c	-$(CH_2)_7$-	1.5
4d	-$(CH_2)_8$-	1.5
12a	-$(CH_2)_4OCH_2$-	0.2
19a		5
19b		2

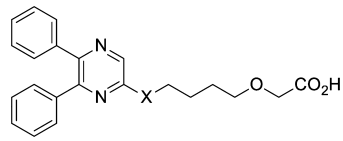
[a]Inhibition of platelet aggregation induced by ADP (10 μM) in human platelet rich plasma. [b]Reproduced with permission from ref 11. Copyright Pergamon 2007.

Structure–activity relationship studies on **4a–d** demonstrated that the presence of a hexamethylene linker in **4b** provided the optimal length, with 5-fold more potent inhibition of platelet aggregation (IC_{50} = 0.3 μM) than was shown by **4c**.

Replacing the methylene unit in the β-position with respect to the carboxyl group with an oxygen atom[18] was envisaged to increase metabolic stability in vivo. This approach generated **12a**, which maintained high in vitro potency (IC_{50} = 0.2 μM).

Replacing the flexible alkylene linker in **4b** and **4c** with a more rigid meta-disubstituted phenyl linker as shown in **19a** and **19b** led to weaker activity in the platelet aggregation assay.

Table 2. Effect of Concatenating Atoms between the Pyrazine and the Linker on Inhibition of ADP-Induced Human Platelet Aggregation[c]



compd	X	inhibition of human platelet aggregation IC_{50} (μM)[a]
12a	NMe	0.2
22[b]	CH_2	17
25a	O	**34**
25b	S	2
25c[b]	SO	37
25d	SO_2	>100

[a]Inhibition of platelet aggregation induced by ADP (10 μM) in human platelet rich plasma. [b]The biological activity of the sodium salt was evaluated. [c]Reproduced with permission from ref 11. Copyright Pergamon 2007.

Next, the impact of the concatenating nitrogen atom between the pyrazine ring and the linker was investigated (Table 2). Replacing the *N*-methylamino group in **12a** with methylene, oxygen, sulfur, sulfoxide, or sulfone reduced potency in this assay, suggesting that the nitrogen atom adjacent to the pyrazine ring is important for the pharmacological action of **12a**.

Further optimization of **12a** focused on exploring the substituent attached to the exocyclic nitrogen. As illustrated by the examples in Table 3, small groups such as an ethyl, cyclopropyl, or isopropyl groups were generally preferred (**5a**, IC_{50} = 0.5 μM; **5c**, IC_{50} = 0.2 μM; **12b**, IC_{50} = 0.2 μM), while a benzyl group led to a substantial loss of activity (**12d**, IC_{50} = 50 μM).

The prostanoid receptor binding profile of **12b** was characterized because this compound, in addition to being orally bioavailable with a long plasma half-life in animals (Table 4), displayed low potential for causing off-target side effects (low toxicity in animal studies).[19] Compound **12b** bound to the human IP receptor with high affinity (binding affinity (K_i) of 20 nM) and with more than 130-fold selectivity over other prostanoid receptors[19] (prostaglandin E receptors, EP_{1-4}; prostaglandin D receptor, DP_1; prostaglandin F_2 receptor, FP; thromboxane A_2 receptor, TP).

Efforts were then directed toward extending the in vivo exposure of **12b**. Replacement of the carboxyl group with acidic

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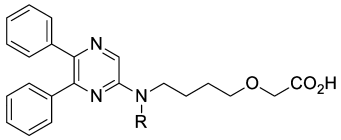
Table 3. Effect of Substituents at the N-Atom Adjacent to the Pyrazine Ring on Inhibition of ADP-Induced Human Platelet Aggregation[c]



compd	R	inhibition of human platelet aggregation IC_{50} (μM)[a]
12a	Me	0.2
5a[b]	Et	0.5
5b[b]	allyl	1
5c	cyclopropyl	0.2
12b	*i*-Pr	0.2
12c	cyclopentyl	0.8
12d[b]	benzyl	50

[a]Inhibition of platelet aggregation induced by ADP (10 μM) in human platelet rich plasma. [b]The biological activity of the sodium salt was evaluated. [c]Reproduced with permission from ref 11. Copyright Pergamon 2007.

Table 4. Pharmacokinetic Parameters of 12b after Intravenous or Oral Administration to Rats, Dogs, and Monkeys[a]

route	parameter	rats[b]	dogs[b]	monkeys[b]
iv	dose (mg/kg)	3	1	*c*
	$t_{1/2}$ (h)	4.9 ± 1.4	5.9 ± 0.2	
	AUC (μg·h/mL)	5.2 ± 0.7	72.5 ± 9.1	
po	dose (mg/kg)	10	3	1
	T_{max} (h)	0.8 ± 0.3	0.8 ± 0.4	2.3 ± 1.5
	C_{max} (μg/mL)	1.9 ± 0.5	14.9 ± 3.6	0.11 ± 0.04
	$t_{1/2}$ (h)	3.6 ± 0.7	6.2 ± 1.0	5.6 ± 1.5
	AUC (μg·h/mL)	17.7 ± 1.9	176 ± 4 1	0.65 ± 0.12
	bioavailability (%)	102	80	

[a]Reproduced with permission from ref 11. Copyright Pergamon 2007. [b]Each value is the mean ± SD (n = 3). [c]Not tested; iv, intravenous; AUC, area under the curve; C_{max}, maximum observed plasma concentration; $t_{1/2}$, apparent terminal half-life; po, per os; T_{max}, time to reach maximum observed plasma concentration.

surrogates such as *N*-acylsulfonamide is a recognized structural modification to improve pharmacokinetic properties of carboxylic acid drugs.[20] Compound **26a**, the *N*-methylsulfonamide derivative of **12b** (Figure 2), bound to the human IP receptor with 13-fold lower affinity than **12b** and was 26-fold less potent than **12b** in platelet aggregation assays.[19] However, in vitro, **26a** was slowly hydrolyzed to the active form **12b** by animal and human hepatic microsomes (Table 5), suggesting that **26a** is converted to the carboxylic acid in vivo. The serine hydrolase inhibitor phenylmethylsulfonyl fluoride completely inhibited

Table 5. Hydrolysis of 26a to 12b in Plasma or by Hepatic Microsomes from Animals and Humans[a,c]

	hydrolysis rate (nmol/min/mL or nmol/min/mg protein)			
	rats	dogs	monkeys	humans
plasma	0.500	*b*		
hepatic microsomes	0.090	0.030	0.057	0.019

[a]**26a** was incubated with plasma or hepatic microsomes at 37 °C for 10 min. [b]Below the limits of detection of **12b**. [c]Reproduced with permission from ref 12. Copyright Pergamon 2007.

hydrolysis of **26a** to **12b** in monkey hepatic microsomes, suggesting that conversion is mediated by a carboxylesterase. In vivo hydrolysis to **12b** following oral administration of **26a** was confirmed in monkeys and humans.[12]

These results prompted investigation of the pharmacokinetic properties of additional sulfonamide and sulfonylurea analogues of **26a**: the *N*-isopropylsulfonamide **26b**, the trifluoromethanesulfonamide **26c**, the *N*-arylsulfonamides **26d** and **26e**, and the sulfonylureas **26f** and **26g** (Figure 2).[12] Compounds **26b**, **26c**, and **26f** displayed low rates of in vitro hydrolysis, similar to that of **26a** in hepatic microsomes from monkeys and humans. In vivo conversion of all three compounds to **12b** was confirmed after oral administration in monkeys (Table 6). Two important differences in the measured pharmacokinetic properties of **12b** were observed following administration of parent compounds compared to administration of **12b**: 10−30% lower maximum plasma concentration (C_{max}) values and a more than 1.5-fold increase in apparent terminal half-life ($t_{1/2}$). These compounds are therefore potential parent drugs with long-lasting agonistic activity due to sustained formation and prolonged exposure of **12b**. It is expected that slower hydrolysis of the parent compound prolongs its pharmacological activity while potentially reducing adverse effects.

This medicinal chemistry program culminated in the discovery of **26a**, a novel IP receptor agonist with properties that differentiate it from PGI_2 and its analogues:

- chemical and metabolic stability are provided by the nonprostanoid structure
- potency is provided by the optimized linker length, heteroatom, and *N*-substituent
- prolonged exposure to the potent active metabolite (carboxylic acid) is provided by the *N*-acylsulfonamide parent drug, which is slowly converted to the carboxylic acid in vivo

TARGET DISEASE: PULMONARY ARTERIAL HYPERTENSION

Pulmonary arterial hypertension is a progressive disease that is characterized by increased resistance in the pulmonary

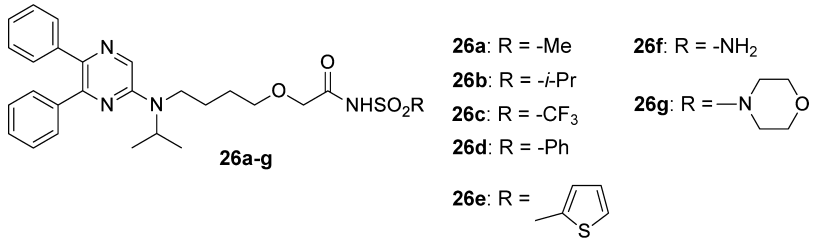


Figure 2. Chemical structures of sulfonamide and sulfonylurea analogues.

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Table 6. Pharmacokinetic Parameters for 12b after Oral Administration of 12b, the Sulfonamides 26a−c, or the Sulfonylurea 26f (1 mg/kg) to Monkeys[a,e]

compd administered	compd measured	n	T_{max} (h)	C_{max} (ng/mL)	AUC_{0-24h} (ng·h/mL)	$t_{1/2}$ (h)
12b[b]	**12b**	3	2.3	105	652	5.6
26a	**12b**	3	14.0	35	859	10.7
	26a	3	6.7	47	384	4.9
26b	**12b**	2	10	13	170	14.5
	26b	2	10	17	128	2.3
26c[c]	**12b**	3	4	31	308	8.5
26f[c]	**12b**	3	6	20	374	*d*

[a]Each value is the mean of 2 or 3 animals. [b]See ref 11. [c]The plasma concentrations were not determined because the compound was insufficiently ionized in liquid-chromatography−mass spectrometry. [d]Not calculated because the plasma concentrations of **12b** after administration of **26f** continued to increase until the end of the sampling period. [e]Reproduced with permission from ref 12. Copyright Pergamon 2007. AUC_{0-24h}, area under the curve from 0 to 24 h after dosing; C_{max}, maximum observed plasma concentration; $t_{1/2}$, apparent terminal half-life; T_{max}, time to reach maximum observed plasma concentration.

circulation caused by pulmonary artery constriction and gradual pulmonary artery remodeling. Pulmonary arterial hypertension ultimately leads to right heart failure and premature death.[21] Alterations of several signaling pathways contribute to the development and progression of PAH. Current therapeutic management of PAH targets pathways mediated by PGI_2, endothelin, and nitric oxide. The PGI_2 pathway is activated when PGI_2 stimulates the IP receptor, leading to increased cyclic adenosine monophosphate (cAMP) levels and subsequent vasodilatory and antiproliferative effects in pulmonary arterial smooth muscle cells.[3] Dysregulation of the PGI_2 pathway in PAH[5,22,23] results in a decrease in PGI_2 production and an imbalance between relaxant PGI_2 and contractile mediators such as endothelin-1 and thromboxane A_2. These studies support the use of IP receptor agonists in management of PAH.

There are currently three PGI_2 analogues, in addition to epoprostenol, approved for the treatment of PAH: treprostinil (intravenous, subcutaneous, and inhaled; oral in U.S. only), iloprost (inhaled), and beraprost (oral, in Japan and South Korea only).[24−26] Among these, only epoprostenol has demonstrated improved patient survival in a randomized clinical trial in treatment-naïve New York Heart Association functional class III and IV patients.[27] However, the use of epoprostenol is limited by its short half-life, requiring continuous intravenous infusion and by the risk of life-threatening infections.[28] Inhaled and subcutaneously administered PGI_2 analogues have greater chemical stability compared to epoprostenol but require frequent dosing for inhaled iloprost (six to nine times daily) and may induce pain at the injection site for subcutaneous treprostinil, in addition to the usual prostanoid-associated side effects such as headache, flushing, diarrhea, and jaw pain.[7] An IP receptor agonist with oral activity, such as **26a**, could provide an important advance in the pharmacological treatment of PAH.

An oral IP receptor agonist could also have potential applications in other clinical diseases such as chronic thromboembolic pulmonary hypertension, peripheral artery disease, or Raynaud's syndrome. In part because of their pharmacokinetic properties, orally available PGI_2 analogues, such as treprostinil and beraprost, have shown inconsistent clinical benefits in PAH[9,25] and other diseases.[29−31]

PRECLINICAL EVALUATION OF 26A

Compounds 26a and 12b are Agonists at the Human IP Receptor. Compounds **26a** and **12b** are both agonists at the human IP receptor. Subsequent cAMP accumulation assays using recombinant Chinese hamster ovary (CHO) cells demonstrated that **12b** is 16-fold more potent than **26a** at the human IP receptor (EC_{50} = 11 nM for **12b** vs 177 nM for **26a**). These results combined with the observation that **12b** is present at 3−4-fold higher levels than the parent drug at steady-state in humans[32] demonstrate that the active metabolite **12b** is the major contributor to the efficacy of **26a** in man.

Compound 12b Is a Functional Agonist in Human Pulmonary Arterial Smooth Muscle Cells. Pulmonary arterial smooth muscle cells (PASMCs) display increased contractile and proliferative activity and contribute to the pathology of PAH. The functional effects of **12b** mediated through activation of the IP receptor were therefore measured in human PASMCs. Compound **12b** potently induces accumulation of intracellular cAMP, leading to long-lasting (>10 h) membrane hyperpolarization (EC_{50} = 32 nM) which contributes to cell relaxation.[33] Compound **12b** also causes long-lasting and pronounced relaxation of PASMCs as measured by changes in cell shape (EC_{50} = 4.3 nM). In addition, **12b** potently inhibits platelet-derived growth factor-BB-stimulated proliferation of human PASMCs (IC_{50} = 2.9 nM). These in vitro results demonstrate that **12b** possesses characteristics that may be beneficial in the treatment of PAH.

Compound 26a Is Effective in Experimental Pulmonary Hypertension. The effects of repeated oral administration of **26a** (1 mg/kg administered twice daily) on disease progression were measured in a rat model of pulmonary hypertension induced by monocrotaline (MCT). Compound **26a** administered for 19 days reduces the increase in right ventricular systolic pressure evoked by MCT without affecting the heart rate.[34] Compound **26a** also restores vascular endothelial function (as assessed by improvement of endothelium-dependent relaxation responses to acetylcholine), decreases pulmonary artery wall thickness, and reduces right ventricular hypertrophy. The survival of MCT rats at 45 days is significantly increased by **26a** (Figure 3). These results indicate that **26a** suppresses various pathological processes associated with pulmonary hypertension in the rat.

Compound 26a Does Not Cause Tachyphylaxis. Receptor desensitization followed by internalization has been observed following chronic exposure of the IP receptor to PGI_2 analogues in vitro.[35] Furthermore, dose escalation may be required to maintain the efficacy of PGI_2 analogues in patients with PAH although disease progression may also contribute to this apparent loss of efficacy (tachyphylaxis).[36] The potential of **26a** to cause tachyphylaxis was evaluated in normotensive and hypertensive rats following repeated oral administration.[19,37]

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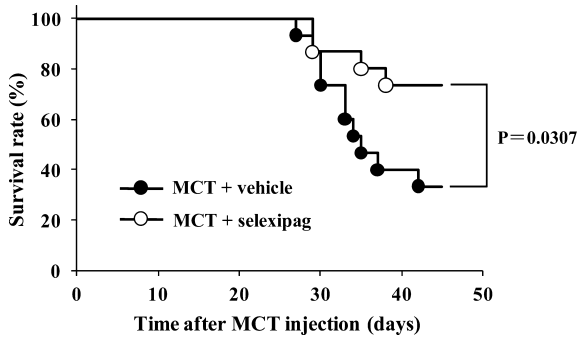

Figure 3. Effect of **26a** (selexipag) on the survival of MCT rats. Compound **26a** at 1 mg/kg or vehicle was administered twice daily for 45 days starting on day 0. Survival curves were calculated by the Kaplan–Meier method and compared by the log-rank test (n = 15). Reproduced with permission from ref 34. Copyright ASPET 2008.

In rats that have received prior repeated administration of **26a** for up to 4 weeks, vasodilation of the femoral artery in response to **26a** is not significantly diminished. Additional experiments measured the hemodynamic effects of repeated administration of **26a** in spontaneously hypertensive rats. Compound **26a** (10 to 300 mg/kg/day) decreases mean arterial pressure (MAP) in a dose-dependent manner, and the effect at each dose is sustained for 4 days without any loss of response.[37] MAP rapidly returns to predrug levels after cessation of administration of **26a**, and no rebound effect is observed. These findings indicate that repeated oral administration of **26a** does not cause tachyphylaxis.

In vivo tachyphylaxis on continued exposure to G protein-coupled receptor agonists can be caused by recruitment of β-arrestin to the activated and phosphorylated receptor, leading to uncoupling of receptor signaling and subsequent internalization and removal of the receptor from the cell surface. To estimate their receptor uncoupling potential, the potency and efficacy of **26a** and **12b** at the human IP receptor were measured with respect to G-protein signaling (adenylate cyclase activation) and β-arrestin recruitment/receptor internalization (uncoupling) in CHO cells expressing the human IP receptor. Comparison was made with PGI_2 analogues (iloprost, treprostinil, and beraprost).[38] All compounds induce comparable maximal responses for increased cAMP production. However, the compounds differ markedly in their effects on β-arrestin recruitment. Compounds **26a** and **12b** have a low efficacy for recruitment of β-arrestin in contrast to the PGI_2 analogues. This difference between PGI_2 analogues on the one hand and **26a** and **12b** on the other is substantiated by receptor internalization assays. Compounds **26a** and **12b**, even at high concentrations, induce only minimal internalization of human IP receptors from the cell surface in recombinant cells, whereas PGI_2 analogues induce pronounced receptor internalization. These in vitro studies demonstrate that **26a** and **12b** induce limited β-arrestin recruitment and minimal receptor internalization. Together, these findings might explain why **26a** does not cause tachyphylaxis in animal models.

Compounds 26a and 12b are Selective Agonists at the IP Receptor. Compounds **26a** and **12b**, contrary to PGI_2 analogues, are highly selective for the IP receptor.[19,39] The binding affinity of **26a** for the human IP receptor is 260 nM, whereas K_i values >10000 nM are measured at the other human G-protein-coupled prostanoid receptors (EP_{1-4}, DP, FP, and TP) (Table 7). Compound **12b** binds the human IP receptor with 13-fold higher affinity than **26a** (K_i = 20 nM) and binds other prostanoid receptors with at least 130-fold lower affinity. In contrast, the PGI_2 analogues beraprost, treprostinil and iloprost bind much less selectively to prostanoid receptors.[19,39]

The seven non-IP prostanoid receptors fall into two categories: (i) contractile receptors (EP_1, EP_3, TP, and FP), which contract smooth muscle in the gastrointestinal tract and blood vessels, and (ii) vasodilating receptors (EP_2, EP_4, and DP), which are not functional in human pulmonary arteries and have immunosuppressive effects by inhibiting functions of macrophages, natural killer cells, T cells, and other immune cell types.[40]

The high degree of selectivity of **26a** and **12b** for the IP receptor may provide benefits over PGI_2 analogues in terms of both agonist efficacy and patient tolerability. Vasorelaxation in response to **12b** is comparable in pulmonary arteries isolated from MCT pulmonary hypertensive rats and in vessels isolated from normal rats, whereas vasorelaxation induced by the non-selective PGI_2 analogues beraprost[34] and treprostinil[41] is significantly lower in MCT pulmonary hypertensive rats than in normal rats because of coactivation of the contractile EP_3 receptor in pathological conditions.[41] The mRNA expression and pharmacological activity of the EP_3 receptor is up-regulated in experimental pulmonary hypertension models, as it is in human PAH lungs.[42] The effective pulmonary vasodilatation evoked by **12b** in pathological conditions such as PAH supports the therapeutic potential of **26a**.

Oral administration of **26a** does not significantly alter gastric function, and neither **26a** nor **12b** contract rat gastric fundus ex vivo. These findings suggest that the high selectivity of **26a** and **12b** for the IP receptor along with the lower potency of **26a**, the main molecular entity before absorption, may help minimize the severity of gastric side effects such as nausea and vomiting, which are mediated through activation of EP_3 receptors.[43,44] In contrast, the PGI_2 analogues iloprost, beraprost, and treprostinil contract gastric smooth muscle via the stimulation of EP_3 receptors.[44] Iloprost and beraprost also activate contractile EP_1 receptors, and orally administered beraprost slows gastric emptying and gastrointestinal transport.[44]

Table 7. Binding Affinity and Receptor Selectivity of 26a, 12b, and PGI_2 Analogues for Human Prostanoid Receptors

	binding affinity (K_i, nM)							
	IP	EP_1	EP_2	EP_3	EP_4	DP	FP	TP
26a[a]	260	>10000	>10000	>10000	>10000	>10000	>10000	>10000
12b[a]	20	>10000	5800	>10000	4900	2600	>10000	>10000
beraprost[a]	39	>10000	>10000	0.68	7.2	>10000	>10000	>10000
treprostinil[b]	32	212	3.6	2505	826	4.4	4680	>10000
iloprost[b]	3.9	1.1	1172	208	212	1016	131	3778

[a]See ref 19. [b]See ref 39. EP_{1-4}, prostaglandin E receptors; DP, prostaglandin D receptor; FP, prostaglandin F_2 receptor; TP, thromboxane A_2 receptor.

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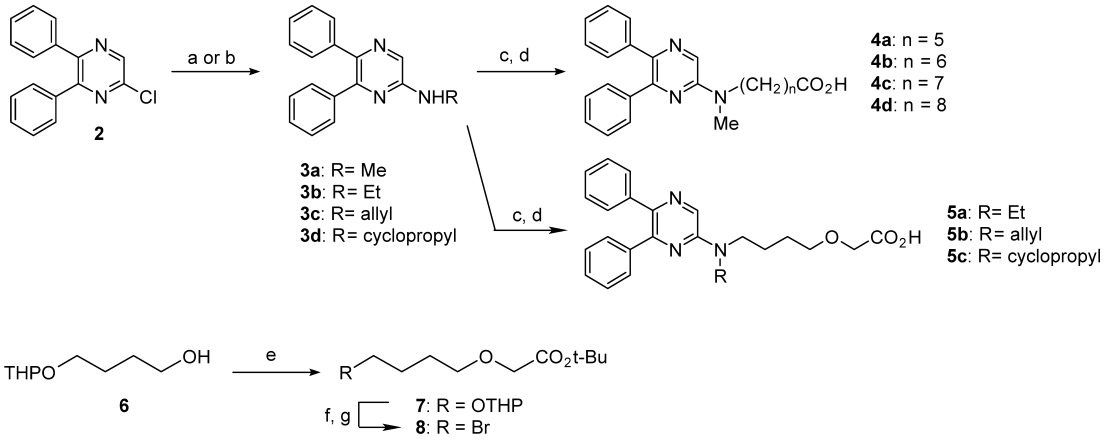
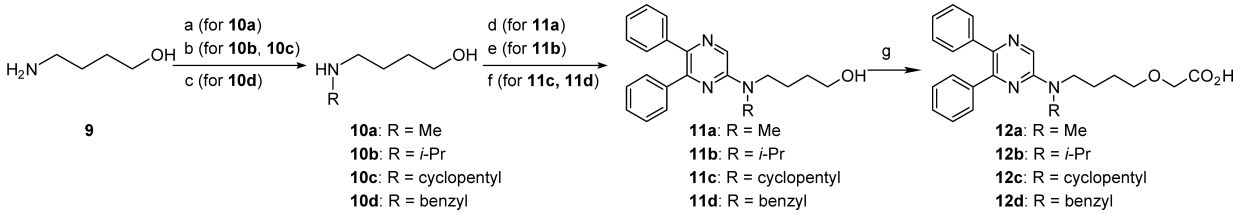
Scheme 1[a]



[a](a) methylamine, MeOH, 80 °C for 12 h, for **3a** (57%); (b) amines, 1-butanol, 150 °C for 24 h, for **3b**–**d** (48–76%); (c) (i) NaH, DMF, 80 °C for 30 min, (ii) $Br(CH_2)_nCO_2R$, room temperature (rt) for 2 h, for **4a**–**d** (8–21% in 2 steps), **8** for **5a**–**c** (24–26% in 2 steps); (d) 1 N NaOH, MeOH, heated at reflux for 1 h; (e) $BrCH_2CO_2t$-Bu, *n*-Bu_4NHSO_4, aq KOH, benzene, <10°C for 45 min, rt for 1 h (61%); (f) *p*-TsOH, MeOH, rt for 30 min (66%); (g) Ph_3P, CBr_4, CH_2Cl_2, rt for 1 h (72%). Reproduced with permission from ref 11. Copyright Pergamon 2007.

Scheme 2[a]



[a](a) (i) HCO_2Et, EtOH, heated at reflux for 18 h, (ii) $LiAlH_4$, THF, slow reflux for 1.5 h (58%); (b) acetone for **10b**, cyclopentanone for **10c**, H_2, rt for 48 h, PtO_2, EtOH (quantitative); (c) (i) PhCHO, MeOH, CH_2Cl_2, H_2, rt overnight, (ii) $NaBH_4$, MeOH, CH_2Cl_2, rt overnight; (d) **2**, K_2CO_3, DMF, 80 °C for 40 h (82%); (e) **2**, neat, 190 °C for 10 h (56%); (f) **2**, triisopropanolamine, sulfolane, 190 °C for 22 h (60% for **11c**, 69% for **11d**); (g) (i) $BrCH_2CO_2t$-Bu, aq KOH, benzene, <10°C for 45 min, rt for 1 h, (ii) 1 N NaOH, MeOH, heated at reflux for 1 h (36–62% in 2 steps). Reproduced with permission from ref 11. Copyright Pergamon 2007.

A role for EP_3 receptors has also been suggested in the development of peripheral pain,[45,46] a side effect reported in patients treated with PGI_2 analogues.[7] Treprostinil exacerbates whereas **12b** inhibits vasoconstriction of rat peripheral arteries in response to sympathetic nerve stimulation.[47] The contractile effect of treprostinil is EP_3-receptor-dependent and may accentuate ischemic muscle pain under conditions of heightened adrenergic activity, as observed in patients with PAH.[48] Compound **26a** is not expected to evoke the side effects associated with concomitant stimulation of other prostanoid receptors and may demonstrate improved tolerability in patients with PAH.

■ CLINICAL EVALUATION OF 26A

A microdose study of oral **26a** (100 μg) conducted in five healthy volunteers[19] showed that it is metabolized to **12b** in the human body with an elimination half-life of 7.9 h, permitting twice-daily dosing.

The safety, tolerability, pharmacokinetics, and pharmacodynamics of **26a** were subsequently evaluated in healthy male subjects in a double-blind, placebo-controlled, randomized, multiple-ascending-dose, up-titration study (400–1800 μg twice daily for 3 days each, in steps of 200 μg; n = 12) or placebo (n = 4).[32] Compound **26a** was well tolerated at doses up to 1600 μg. Steady state was reached after 3 days with no evidence of accumulation of **26a**. At the highest dose (1800 μg), the geometric mean terminal half-lives of **26a** and **12b** were 1.4 and 8.7 h, respectively.

Compound **26a** was investigated in a randomized, double-blind, placebo-controlled, phase 2, proof-of-concept trial in 43 patients with PAH[49] receiving an endothelin receptor antagonist, a phosphodiesterase type-5 inhibitor, or both, as background therapy. Compound **26a** was up-titrated in 200 μg twice daily increments to the maximum tolerated dose or to the maximal dose of 800 μg. More than 60% of patients reached a final dose of **26a** of 600–800 μg twice daily (12%, n = 4:200 μg bid; 18%, n = 6:400 μg bid; 21%, n = 7:600 μg bid; 42%, n = 14:800 μg bid; 2 patients discontinued prematurely). At week 17, **26a** significantly lowered pulmonary vascular resistance (treatment effect: −30.3%; p = 0.0045) and increased cardiac index (treatment effect: +0.5 $L \cdot min^{-1} \cdot m^{-2}$; p = 0.01). In addition, the 6 min walk distance improved by 24.7 m in the **26a** treatment group (95% confidence limits: −23.7 to 72.2). Compound **26a** was well tolerated, and the majority of adverse events were mild or moderate in severity. The high selectivity of **26a** for the IP receptor, the pharmacokinetic properties that reduce peak-trough fluctuations, and the up-titration regimen used are collectively considered to minimize the adverse effects. A decrease over time in the prevalence of adverse events frequently associated with PGI_2 analogue treatment, such as headache, pain in an extremity, pain in the jaw, nausea, and diarrhea, was observed in patients treated with **26a**.

The long-term effect of **26a** on the composite end point of morbidity/mortality was investigated in the double-blind,

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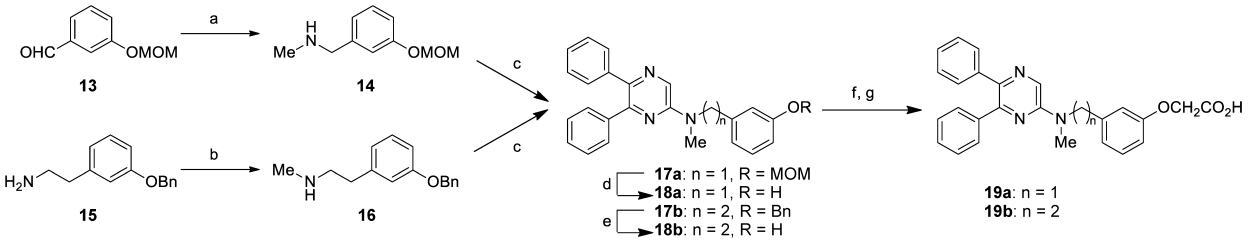
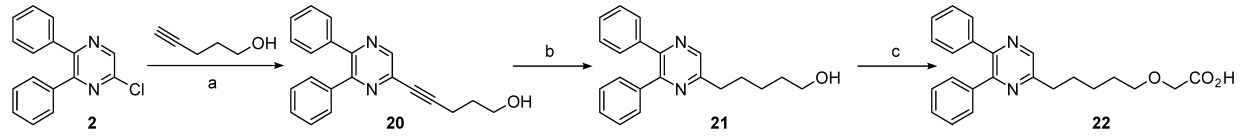
Scheme 3[a]



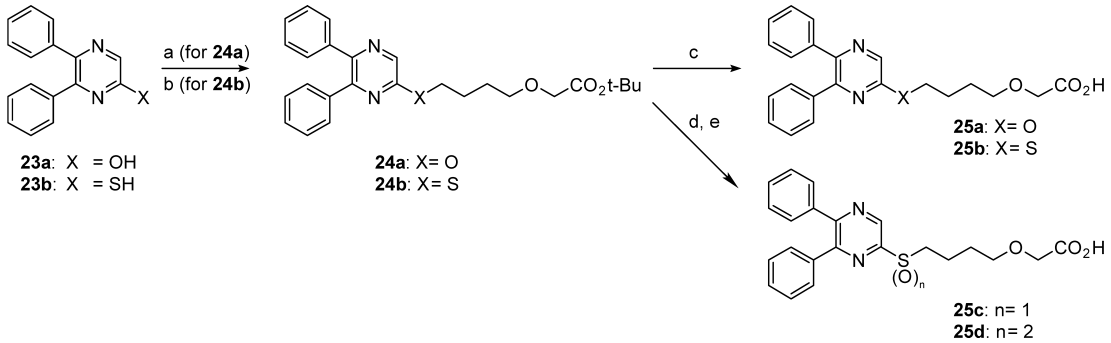
[a](a) $MeNH_2$, H_2, 5% Pd−C, MeOH, rt for 7 h, warmed to 70 °C and stirred for 15 h (77%); (b) (i) HCO_2Et, EtOH, heated at reflux for 22 h (42%), (ii) $LiAlH_4$, THF, rt, reflux for 2 h (80%); (c) **2**, K_2CO_3, DMF, 80 °C for 40 h (60% for **17a**, 71% for **17b**); (d) HCl, MeOH, rt for 3 h (81%); (e) *c*-HCl, EtOH, 80 °C for 17 h (92%); (f) $BrCH_2CO_2Me$, K_2CO_3, cat. KI, MeCN, heated at reflux for 3 h; (g) 1 N NaOH, MeOH, heated at reflux for 1 h (69% for **19a**, 51% for **19b** in 2 steps). Reproduced with permission from ref 11. Copyright Pergamon 2007.

Scheme 4[a]



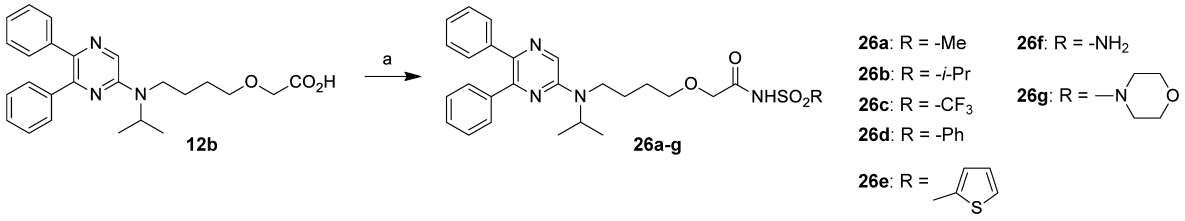
[a](a) $PdCl_2(PPh_3)_2$, CuI, Et_3N, 80 °C for 7 h (60%); (b) H_2, 5% Pd−C, EtOH, rt for 4 h (99%); (c) (i) $BrCH_2CO_2$ *t*-Bu, *n*-Bu_4NHSO_4, aq KOH, benzene, <10 °C for 45 min, rt for 1 h, (ii) 1 N NaOH, MeOH, heated at reflux for 1 h (25% in 2 steps). Reproduced with permission from ref 11. Copyright Pergamon 2007.

Scheme 5[a]



[a](a) (i) NaH, DMF, rt for 0.5 h, (ii) **8**, rt for 18 h (54%); (b) **8**, Na_2CO_3, acetone, rt for 24 h (82%); (c) 1 N NaOH, MeOH, heated at reflux for 1 h (62% for **25a**, 61% for **25b**); (d) *m*CPBA (1 equiv for **25c**, 2 equiv for **25d**), $CHCl_3$, 0 °C for 2 h for **25c**, 19 h for **25d**; (e) 4 N HCl, 1,4-dioxane, 80 °C for 0.5 h (39% for **25c**, 66% for **25d**, in 2 steps). Reproduced with permission from ref 11. Copyright Pergamon 2007.

Scheme 6[a]



[a](a) (i) CDI, THF, rt for 0.5 h, then reflux 0.5 h, (ii) RSO_2NH_2, rt for 10 min, DBU, rt for 12 h for **26a** (77%) and 36 h for **26b**−**g** (43−65%). Reproduced with permission from ref 12. Copyright Pergamon 2007.

randomized, placebo-controlled, phase 3 GRIPHON (Prostaglandin (PGI_2) Receptor Agonist in Pulmonary Arterial Hypertension) study, which enrolled 1156 patients with PAH.[50] The dose was initiated at 200 μg twice daily and increased up to a maximum of 1600 μg twice daily. A large majority of patients (80.5%) were on stable background therapy composed of an endothelin receptor antagonist and/or a phosphodiesterase type-5 inhibitor. In the GRIPHON study, **26a** (selexipag) demonstrated a statistically significant effect on morbidity/mortality, the primary end point.

On the basis of these long-term efficacy and safety studies, the oral selective IP receptor agonist **26a** has the potential to overcome the limitations associated with PGI_2 analogues and, if approved, may represent an important new drug for the treatment of PAH as it would offer long-term outcome benefits with an oral therapy targeting the prostacyclin pathway.

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CHEMISTRY

Syntheses of Diphenylpyrazine Derivatives Leading to Identification of 12b as a Long-Lasting IP Receptor Agonist. The detailed chemistry has been described previously.[11]

N-Alkylation of 2-amino-5,6-diphenylpyrazines **3a−d** with the appropriate bromides was the key step in the synthesis of the pyrazine derivatives related to **4a−d** and **5a−c** as described in Scheme 1.

Alternative synthetic routes for the synthesis of the pyrazine derivatives related to **12a−d** and **19a** and **19b** are summarized in Schemes 2 and 3.

The synthetic route to the alkylated pyrazine **22** is shown in Scheme 4.

Pyrazine derivatives **25a−d** were prepared according to Scheme 5.

Synthesis of Sulfonamide and Sulfonylurea Derivatives of 12b Leading to Identification of 26a As a Parent Drug of 12b. The detailed chemistry has been described previously.[12]

The synthetic route to **26a−g** is shown in Scheme 6. The carboxylic acid **12b** was used to prepare *N*-acylsulfonamides **26a−e** and *N*-acylsulfonylureas **26f** and **26g**.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b00698.

SMILES for all compounds and compound numbers used in the original publications (CSV).

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript

Notes

The authors declare the following competing financial interest(s): The only funding sources for the authors was Actelion Pharmaceuticals Ltd and Nippon Shinyaku Co., Ltd.

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ABBREVIATIONS USED

ADP, adenosine diphosphate; AUC_{0-24h}, area under the curve from 0 to 24 h after dosing; cAMP, cyclic adenosine monophosphate; CHO, Chinese hamster ovary; C_{max}, maximum observed plasma concentration; DP, prostaglandin D; EC_{50}, half-maximal effective concentration; EP_{1-4}, prostaglandin E; FP, prostaglandin F_2; IC_{50}, half-maximal inhibitory concentration; IP/PGI_2, prostacyclin; iv, intravenous; K_i, binding affinity; MAP, mean arterial pressure; MCT, monocrotaline; PAH, pulmonary arterial hypertension; PASMCs, pulmonary arterial smooth muscle cells; po, per os; rt, room temperature; $t_{1/2}$, apparent terminal half-life; T_{max}, time to reach maximum observed plasma concentration; TP, thromboxane A_2

REFERENCES

(1) Moncada, S.; Gryglewski, R.; Bunting, S.; Vane, J. R. An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. *Nature* **1976**, *263*, 663−665.

(2) Moncada, S.; Vane, J. R. Pharmacology and endogenous roles of prostaglandin endoperoxides, thromboxane A2, and prostacyclin. *Pharmacol. Rev.* **1978**, *30*, 293−331.

(3) Fetalvero, K. M.; Martin, K. A.; Hwa, J. Cardioprotective prostacyclin signaling in vascular smooth muscle. *Prostaglandins Other Lipid Mediators* **2007**, *82*, 109−118.

(4) Coleman, R. A.; Smith, W. L.; Narumiya, S. International Union of Pharmacology classification of prostanoid receptors: properties, distribution, and structure of the receptors and their subtypes. *Pharmacol. Rev.* **1994**, *46*, 205−229.

(5) Tuder, R. M.; Cool, C. D.; Geraci, M. W.; Wang, J.; Abman, S. H.; Wright, L.; Badesch, D.; Voelkel, N. F. Prostacyclin synthase expression is decreased in lungs from patients with severe pulmonary hypertension. *Am. J. Respir. Crit. Care Med.* **1999**, *159*, 1925−1932.

(6) Hirsh, P. D.; Campbell, W. B.; Willerson, J. T.; Hillis, L. D. Prostaglandins and ischemic heart disease. *Am. J. Med.* **1981**, *71*, 1009−1026.

(7) Badesch, D. B.; McLaughlin, V. V.; Delcroix, M.; Vizza, C. D.; Olschewski, H.; Sitbon, O.; Barst, R. J. Prostanoid therapy for pulmonary arterial hypertension. *J. Am. Coll. Cardiol.* **2004**, *43*, S56−S61.

(8) Robertson, L.; Andras, A. Prostanoids for intermittent claudication. *Cochrane Database Syst. Rev.* **2013**, *4*, DOI: 10.1002/14651858.CD000986.pub3.

(9) Barst, R. J.; McGoon, M.; McLaughlin, V.; Tapson, V.; Oudiz, R.; Shapiro, S.; Robbins, I. M.; Channick, R.; Badesch, D.; Rayburn, B. K.; Flinchbaugh, R.; Sigman, J.; Arneson, C.; Jeffs, R. Beraprost therapy for pulmonary arterial hypertension. *J. Am. Coll. Cardiol.* **2003**, *41*, 2119−2125.

(10) Tapson, V. F.; Jing, Z. C.; Xu, K. F.; Pan, L.; Feldman, J.; Kiely, D. G.; Kotlyar, E.; McSwain, C. S.; Laliberte, K.; Arneson, C.; Rubin, L. J. Oral treprostinil for the treatment of pulmonary arterial hypertension in patients receiving background endothelin receptor antagonist and phosphodiesterase type 5 inhibitor therapy (the FREEDOM-C2 study): a randomized controlled trial. *Chest* **2013**, *144*, 952−958.

(11) Asaki, T.; Hamamoto, T.; Sugiyama, Y.; Kuwano, K.; Kuwabara, K. Structure-activity studies on diphenylpyrazine derivatives: a novel class of prostacyclin receptor agonists. *Bioorg. Med. Chem.* **2007**, *15*, 6692−6704.

(12) Nakamura, A.; Yamada, T.; Asaki, T. Synthesis and evaluation of N-acylsulfonamide and N-acylsulfonylurea prodrugs of a prostacyclin receptor agonist. *Bioorg. Med. Chem.* **2007**, *15*, 7720−7725.

(13) Seiler, S.; Brassard, C. L.; Arnold, A. J.; Meanwell, N. A.; Fleming, J. S.; Keely, S. L., Jr. Octimibate inhibition of platelet aggregation: stimulation of adenylate cyclase through prostacyclin receptor activation. *J. Pharmacol. Exp. Ther.* **1990**, *255*, 1021−1026.

(14) Meanwell, N. A.; Rosenfeld, M. J.; Trehan, A. K.; Wright, J. J.; Brassard, C. L.; Buchanan, J. O.; Federici, M. E.; Fleming, J. S.; Gamberdella, M.; Zavoico, G. B.; Seiler, S. M. Nonprostanoid prostacyclin mimetics. 2. 4,5-Diphenyloxazole derivatives. *J. Med. Chem.* **1992**, *35*, 3483−3497.

(15) Meanwell, N. A.; Rosenfeld, M. J.; Trehan, A. K.; Romine, J. L.; Wright, J. J.; Brassard, C. L.; Buchanan, J. O.; Federici, M. E.; Fleming, J. S.; Gamberdella, M.; Zavoico, G. B.; Seiler, S. M. Nonprostanoid prostacyclin mimetics. 3. Structural variations of the diphenyl heterocycle moiety. *J. Med. Chem.* **1992**, *35*, 3498−3512.

(16) Seiler, S. M.; Meanwell, N. A. BMY 42393, An orally active prostacyclin partial agonist of novel Structure. *Cardiovasc. Drug Rev.* **1995**, *13*, 289−304.

(17) Meanwell, N. A.; Rosenfeld, M. J.; Wright, J. J.; Brassard, C. L.; Buchanan, J. O.; Federici, M. E.; Fleming, J. S.; Seiler, S. M. Structure-activity relationships associated with 3,4,5-triphenyl-1H-pyrazole-1-nonanoic acid, a nonprostanoid prostacyclin mimetic. *J. Med. Chem.* **1992**, *35*, 389−397.

DOI: 10.1021/acs.jmedchem.5b00698
J. Med. Chem. 2015, 58, 7128−7137

(18) Skuballa, W.; Schillinger, E.; Sturzebecher, C. S.; Vorbruggen, H. Synthesis of a new chemically and metabolically stable prostacyclin analogue with high and long-lasting oral activity. *J. Med. Chem.* **1986**, *29*, 313−315.
(19) Kuwano, K.; Hashino, A.; Asaki, T.; Hamamoto, T.; Yamada, T.; Okubo, K.; Kuwabara, K. 2-[4-[(5,6-diphenylpyrazin-2-yl) (isopropyl)-amino]butoxy]-N-(methylsulfonyl)acetam ide (NS-304), an orally available and long-acting prostacyclin receptor agonist prodrug. *J. Pharmacol. Exp. Ther.* **2007**, *322*, 1181−1188.
(20) Gallant, M.; Carriere, M. C.; Chateauneuf, A.; Denis, D.; Gareau, Y.; Godbout, C.; Greig, G.; Juteau, H.; Lachance, N.; Lacombe, P.; Lamontagne, S.; Metters, K. M.; Rochette, C.; Ruel, R.; Slipetz, D.; Sawyer, N.; Tremblay, N.; Labelle, M. Structure-activity relationship of biaryl acylsulfonamide analogues on the human EP(3) prostanoid receptor. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2583−2586.
(21) Farber, H. W.; Loscalzo, J. Pulmonary arterial hypertension. *N. Engl. J. Med.* **2004**, *351*, 1655−1665.
(22) Christman, B. W.; McPherson, C. D.; Newman, J. H.; King, G. A.; Bernard, G. R.; Groves, B. M.; Loyd, J. E. An imbalance between the excretion of thromboxane and prostacyclin metabolites in pulmonary hypertension. *N. Engl. J. Med.* **1992**, *327*, 70−75.
(23) Stearman, R. S.; Cornelius, A. R.; Lu, X.; Conklin, D. S.; Del Rosario, M. J.; Lowe, A. M.; Elos, M. T.; Fettig, L. M.; Wong, R. E.; Hara, N.; Cogan, J. D.; Phillips, J. A., 3rd; Taylor, M. R.; Graham, B. B.; Tuder, R. M.; Loyd, J. E.; Geraci, M. W. Functional prostacyclin synthase promoter polymorphisms. Impact in pulmonary arterial hypertension. *Am. J. Respir. Crit. Care Med.* **2014**, *189*, 1110−1120.
(24) Frumkin, L. R. The pharmacological treatment of pulmonary arterial hypertension. *Pharmacol. Rev.* **2012**, *64*, 583−620.
(25) Jing, Z. C.; Parikh, K.; Pulido, T.; Jerjes-Sanchez, C.; White, R. J.; Allen, R.; Torbicki, A.; Xu, K. F.; Yehle, D.; Laliberte, K.; Arneson, C.; Rubin, L. J. Efficacy and safety of oral treprostinil monotherapy for the treatment of pulmonary arterial hypertension: a randomized, controlled trial. *Circulation* **2013**, *127*, 624−633.
(26) Galie, N.; Humbert, M.; Vachiery, J. L.; Vizza, C. D.; Kneussl, M.; Manes, A.; Sitbon, O.; Torbicki, A.; Delcroix, M.; Naeije, R.; Hoeper, M.; Chaouat, A.; Morand, S.; Besse, B.; Simonneau, G. Effects of beraprost sodium, an oral prostacyclin analogue, in patients with pulmonary arterial hypertension: a randomized, double-blind, placebo-controlled trial. *J. Am. Coll. Cardiol.* **2002**, *39*, 1496−1502.
(27) Barst, R. J.; Rubin, L. J.; Long, W. A.; McGoon, M. D.; Rich, S.; Badesch, D. B.; Groves, B. M.; Tapson, V. F.; Bourge, R. C.; Brundage, B. H.; Koerner, S. K.; Langleben, D.; Keller, C. A.; Murali, S.; Uretsky, B. F.; Clayton, L. M.; Jöbsis, M. M.; Blackburn, S. D.; Shortino, D.; Crow, J. W. A comparison of continuous intravenous epoprostenol (prostacyclin) with conventional therapy for primary pulmonary hypertension. The Primary Pulmonary Hypertension Study Group. *N. Engl. J. Med.* **1996**, *334*, 296−302.
(28) Sitbon, O.; Humbert, M.; Nunes, H.; Parent, F.; Garcia, G.; Herve, P.; Rainisio, M.; Simonneau, G. Long-term intravenous epoprostenol infusion in primary pulmonary hypertension: prognostic factors and survival. *J. Am. Coll. Cardiol.* **2002**, *40*, 780−788.
(29) Creager, M. A.; Pande, R. L.; Hiatt, W. R. A randomized trial of iloprost in patients with intermittent claudication. *Vasc. Med.* **2008**, *13*, 5−13.
(30) Mohler, E. R., 3rd; Hiatt, W. R.; Olin, J. W.; Wade, M.; Jeffs, R.; Hirsch, A. T. Treatment of intermittent claudication with beraprost sodium, an orally active prostaglandin I2 analogue: a double-blinded, randomized, controlled trial. *J. Am. Coll. Cardiol.* **2003**, *41*, 1679−1686.
(31) Wigley, F. M.; Korn, J. H.; Csuka, M. E.; Medsger, T. A., Jr.; Rothfield, N. F.; Ellman, M.; Martin, R.; Collier, D. H.; Weinstein, A.; Furst, D. E.; Jimenez, S. A.; Mayes, M. D.; Merkel, P. A.; Gruber, B.; Kaufman, L.; Varga, J.; Bell, P.; Kern, J.; Marrott, P.; White, B.; Simms, R. W.; Phillips, A. C.; Seibold, J. R. Oral iloprost treatment in patients with Raynaud's phenomenon secondary to systemic sclerosis: a multicenter, placebo-controlled, double-blind study. *Arthritis Rheum.* **1998**, *41*, 670−677.
(32) Bruderer, S.; Hurst, N.; Kaufmann, P.; Dingemanse, J. Multiple-dose up-titration study to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of selexipag, an orally available selective prostacyclin receptor agonist, in healthy subjects. *Pharmacology* **2014**, *94*, 148−156.
(33) Gatfield, J.; Menyhart, K.; Nayler, O. Anti-contractile and anti-proliferative responses in human pulmonary arterial smooth muscle cells induced by ACT-333679, the active metabolite of selexipag, a non-prostanoid selective prostacyclin receptor agonist. *Eur. Respir. J.* **2014**, *44*, 2353.
(34) Kuwano, K.; Hashino, A.; Noda, K.; Kosugi, K.; Kuwabara, K. A long-acting and highly selective prostacyclin receptor agonist prodrug, 2-{4-[(5,6-diphenylpyrazin-2-yl) (isopropyl)amino]butoxy}-N-(methylsulfonyl)acetam ide (NS-304), ameliorates rat pulmonary hypertension with unique relaxant responses of its active form, {4-[(5,6-diphenylpyrazin-2-yl) (isopropyl)amino]butoxy}acetic acid (MRE-269), on rat pulmonary artery. *J. Pharmacol. Exp. Ther.* **2008**, *326*, 691−699.
(35) Smyth, E. M.; Austin, S. C.; Reilly, M. P.; FitzGerald, G. A. Internalization and sequestration of the human prostacyclin receptor. *J. Biol. Chem.* **2000**, *275*, 32037−32045.
(36) Barst, R. How has epoprostenol changed the outcome for patients with pulmonary arterial hypertension? *Int. J. Clin. Pract. Suppl.* **2010**, *64*, 23−32.
(37) Morrison, K.; Wanner, D.; Gatfield, J.; Gnerre, C.; Hess, P.; Clozel, M. Repeated oral administration of the selective prostacyclin receptor agonist selexipag does not cause tachyphylaxis in spontaneously hypertensive rats. *J. Am. Coll. Cardiol.* **2015**, *65*, A1558.
(38) Gatfield, J.; Menyhart, K.; Morrison, K.; Nayler, O. The non-prostanoid prostacyclin receptor agonist ACT-333679, the active metabolite of selexipag, is characterized by low beta-arrestin recruitment and receptor internalization activity. *J. Am. Coll. Cardiol.* **2015**, *65*, A1542.
(39) Whittle, B. J.; Silverstein, A. M.; Mottola, D. M.; Clapp, L. H. Binding and activity of the prostacyclin receptor (IP) agonists, treprostinil and iloprost, at human prostanoid receptors: treprostinil is a potent DP1 and EP2 agonist. *Biochem. Pharmacol.* **2012**, *84*, 68−75.
(40) Hirata, T.; Narumiya, S. Prostanoid receptors. *Chem. Rev.* **2011**, *111*, 6209−6230.
(41) Morrison, K.; Studer, R.; Ernst, R.; Haag, F.; Kauser, K.; Clozel, M. Differential effects of selexipag and prostacyclin analogs in rat pulmonary artery. *J. Pharmacol. Exp. Ther.* **2012**, *343*, 547−555.
(42) Rajkumar, R.; Konishi, K.; Richards, T. J.; Ishizawar, D. C.; Wiechert, A. C.; Kaminski, N.; Ahmad, F. Genomewide RNA expression profiling in lung identifies distinct signatures in idiopathic pulmonary arterial hypertension and secondary pulmonary hypertension. *Am. J. Physiol. Heart Circ. Physiol.* **2010**, *298*, H1235−1248.
(43) Kan, K. K.; Jones, R. L.; Ngan, M. P.; Rudd, J. A. Actions of prostanoids to induce emesis and defecation in the ferret. *Eur. J. Pharmacol.* **2002**, *453*, 299−308.
(44) Morrison, K.; Ernst, R.; Hess, P.; Studer, R.; Clozel, M. Selexipag: a selective prostacyclin receptor agonist that does not affect rat gastric function. *J. Pharmacol. Exp. Ther.* **2010**, *335*, 249−255.
(45) Minami, T.; Nakano, H.; Kobayashi, T.; Sugimoto, Y.; Ushikubi, F.; Ichikawa, A.; Narumiya, S.; Ito, S. Characterization of EP receptor subtypes responsible for prostaglandin E2-induced pain responses by use of EP1 and EP3 receptor knockout mice. *Br. J. Pharmacol.* **2001**, *133*, 438−444.
(46) Southall, M. D.; Vasko, M. R. Prostaglandin receptor subtypes, EP3C and EP4, mediate the prostaglandin E2-induced cAMP production and sensitization of sensory neurons. *J. Biol. Chem.* **2001**, *276*, 16083−16091.
(47) Morrison, K. J.; Haag, F.; Ernst, R.; Studer, R.; Clozel, M. Selexipag, unlike prostacyclin analogs, does not evoke paradoxical vasoconstriction of rat femoral artery. *Am. J. Respir. Crit. Care Med.* **2014**, *189*, A3319.
(48) Nagaya, N.; Nishikimi, T.; Uematsu, M.; Satoh, T.; Kyotani, S.; Sakamaki, F.; Kakishita, M.; Fukushima, K.; Okano, Y.; Nakanishi, N.; Miyatake, K.; Kangawa, K. Plasma brain natriuretic peptide as a prognostic indicator in patients with primary pulmonary hypertension. *Circulation* **2000**, *102*, 865−870.

DOI: 10.1021/acs.jmedchem.5b00698
J. Med. Chem. 2015, 58, 7128−7137

(49) Simonneau, G.; Torbicki, A.; Hoeper, M. M.; Delcroix, M.; Karlocai, K.; Galie, N.; Degano, B.; Bonderman, D.; Kurzyna, M.; Efficace, M.; Giorgino, R.; Lang, I. M. Selexipag: an oral, selective prostacyclin receptor agonist for the treatment of pulmonary arterial hypertension. *Eur. Respir. J.* **2012**, *40*, 874−880.
(50) McLaughlin, V. V.; Channick, R.; Chin, K.; Frey, A.; Gaine, S.; Ghofrani, A.; Hoeper, M.; Lang, I.; Preiss, R.; Rubin, L. J.; Simonneau, G.; Sitbon, O.; Stefani, M.; Tapson, V.; Galiè, N. Effect of selexipag on morbidity/mortality in pulmonary arterial hypertension: results of the GRIPHON study. *J. Am. Coll. Cardiol.* **2015**, *65*, A1538.

DOI: 10.1021/acs.jmedchem.5b00698
J. Med. Chem. 2015, 58, 7128−7137