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Structure–activity studies on diphenylpyrazine derivatives: A novel class of prostacyclin receptor agonists

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Abstract—To develop nonprostanoid prostacyclin receptor agonists with a high degree of metabolic resistance and an extended duration of action, a novel series of diphenylpyrazine derivatives was synthesized and evaluated for their inhibition of ADP-induced human platelet aggregation. Structure–activity relationship studies on the side chain containing the carboxylic acid moiety of the lead compound **5c** showed that the length of the linker and the presence of the concatenating nitrogen atom adjacent to the pyrazine ring are critical for the antiaggregatory activity. This study led to the discovery of 2-amino-5,6-diphenylpyrazine derivatives **8c**, **15a**, and **15b**, which showed potent inhibition of platelet aggregation with IC_{50} values of 0.2 μM. Among these compounds, **15b** is an orally available and long-lasting prostacyclin receptor agonist which is promising for the treatment of various vascular diseases.

1. Introduction

Prostacyclin (PGI_2) (**1**; Fig. 1) is an endogenous mediator produced primarily in the vascular endothelial cells. It plays an important role in vascular homeostasis as an inhibitor of platelet aggregation and a potent vasodilator.[1] These physiological functions are mediated by the activation of a specific G-protein-coupled receptor, the PGI_2 receptor (IP receptor).[2] IP receptor agonists are thought to be clinically useful agents for the precise control of platelet and vascular function, and the sodium salt of PGI_2 itself is available as Flolan® (GlaxoSmithKline) for the treatment of arterial pulmonary hypertension. However, its therapeutic application has been limited because of its chemical lability and complicated administration by continuous infusion through a central venous catheter. To overcome such drawbacks, much effort has been directed toward developing chemically and metabolically stable IP receptor agonists. Beraprost sodium (**2**),[3] a representative chemically stable PGI_2 analogue, is now in clinical use as an orally available drug for the treatment of arteriosclerosis obliterans and idiopathic pulmonary arterial hypertension in the Asian area, including Japan, but its duration of action is insufficient owing to its metabolic instability ($t_{1/2}$ = 1 h in

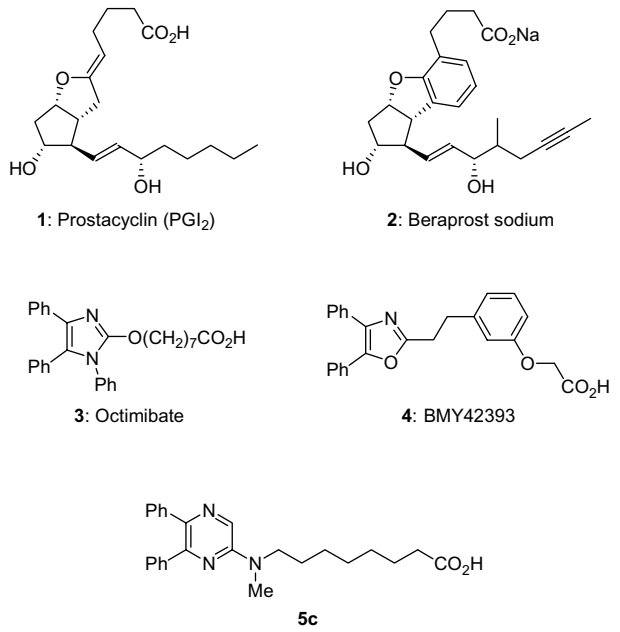

Figure 1. Chemical structures of prostacyclin (PGI_2) and prostacyclin mimetics.

Keywords: Prostacyclin; IP receptor agonist; Diphenylpyrazine; Antiaggregatory activity.
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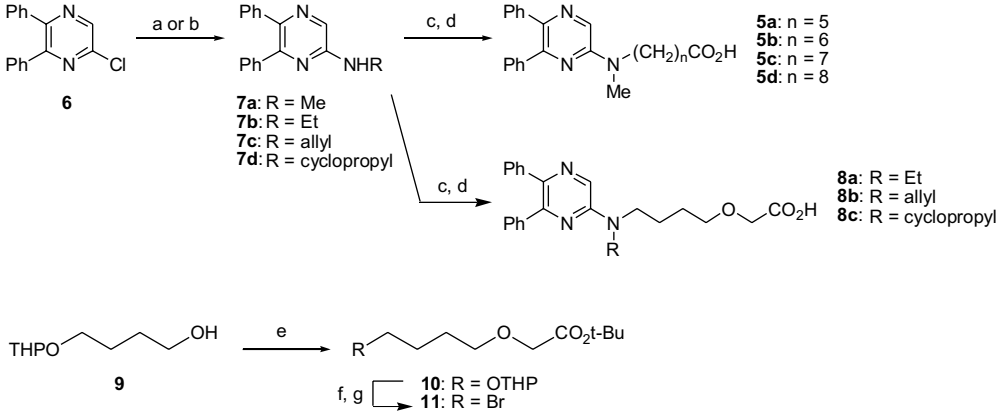
humans).[4] On the other hand, many nonprostanoid IP receptor agonists,[5–11] which do not have the prostanoid skeleton, have been reported. Octimibate **3**[5] and BMY42393 **4**[6] (Fig. 1), partial agonists for the IP receptor, are structurally quite different from PGI_2 and its analogues, except for the carboxylic acid moiety. These observations demonstrate that the prostanoid skeleton is not essential for the activation of the IP receptor. We similarly focused our attention on nonprostanoid IP receptor agonists with a high degree of metabolic resistance and an extended duration of action.

On the basis of the chemical structures of the known nonprostanoid-type agonists, we performed extensive chemical modification of these compounds. As part of our drug-discovery effort to identify new IP receptor agonists, we designed and synthesized the 2-amino-5,6-diphenylpyrazine derivative **5c** (Fig. 1), which has a pyrazine ring instead of the oxazole ring of BMY42393. Our original lead compound **5c** moderately inhibited ADP-induced human platelet aggregation (IC_{50} = 1.5 μM). We next sought to increase the potency of **5c** by optimizing the side chain containing the carboxylic acid moiety. In this report, we describe the synthesis and structure–activity relationships (SAR) of diphenylpyrazine derivatives as a novel class of IP receptor agonists. Our goals were to investigate the SAR based on the structure of **5c**, to maximize potency, and to identify an orally available agent with a good pharmacokinetic profile.

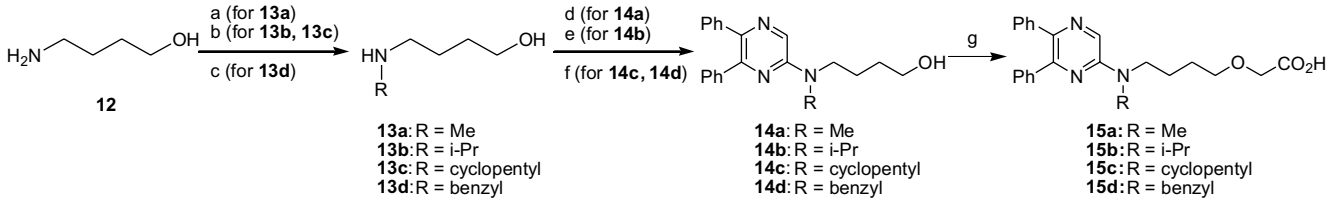
2. Chemistry

The strategy for the synthesis of the pyrazine derivatives related to **5a–d** and **8a–c** involved *N*-alkylation of the 2-amino-5,6-diphenylpyrazines **7a–d** with the appropriate bromides as the key step (Scheme 1). Coupling of 2-chloro-5,6-diphenylpyrazine **6**[12] with various amines provided the aminopyrazines **7a–d**. *N*-Alkylation of **7a–d** by deprotonation with sodium hydride followed by treatment with the esters of ω-bromoalkanoic acids or the bromide **11** (prepared as described below) afforded the corresponding esters, which were converted to the desired compounds **5a–d** and **8a–c** by alkaline hydrolysis. The alcohol **9**[13] was *O*-alkylated with *tert*-butyl bromoacetate under phase-transfer conditions[14] to give the ester **10**. Removal of the tetrahydropyranyl (THP) protecting group on **10** by a standard protocol followed by bromination afforded the bromide **11**.

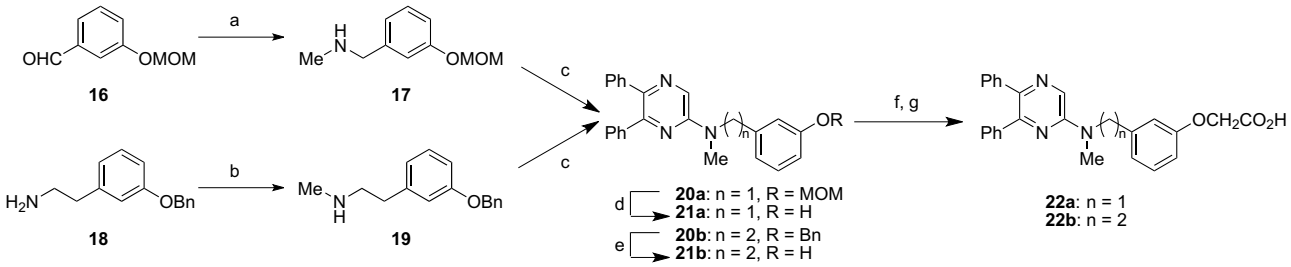
Alternative synthetic routes for the synthesis of the pyrazine derivatives related to **15a–d** and **22a** and **22b** are summarized in Schemes 2 and 3. *N*-Alkylation of 4-amino-1-butanol **12** by formylation followed by $LiAlH_4$ reduction (for **13a**) or reductive alkylation (for **13b** and **13c**) provided the amino alcohols **13a–c**. The benzylaminoalcohol **13d** was prepared according to a literature procedure.[15] The amino alcohols **13a–d** were then coupled with the chloropyrazine **6** to afford the corresponding alcohols **14a–d**, which were transformed into the target compounds **15a–d** according to the *O*-alkylation procedure described for **10** followed by alkaline



Scheme 1. Reagents: (a) methylamine, MeOH for **7a**; (b) amines, 1-butanol for **7b–d**; (c) i—NaH, DMF; ii—$Br(CH_2)_nCO_2R$ for **5a–d**, **11** for **8a–c**; (d) 1 N NaOH, MeOH; (e) $BrCH_2CO_2t$-Bu, n-Bu_4NHSO_4, aq KOH, benzene; (f) *p*-TsOH, MeOH; (g) Ph_3P, CBr_4, CH_2Cl_2.



Scheme 2. Reagents: (a) i—HCO_2Et, EtOH, ii—$LiAlH_4$, THF, for **13a**; (b) acetone for **13b**, cyclopentanone for **13c**, H_2, PtO_2, EtOH; (c) i—PhCHO, MeOH, CH_2Cl_2; ii—$NaBH_4$, MeOH, CH_2Cl_2, for **13d** (see Ref. 15); (d) **6**, K_2CO_3, DMF; (e) **6**, neat; (f) **6**, triisopropanolamine, sulfolane; (g) i—$BrCH_2CO_2t$-Bu, aq KOH, benzene; ii—1 N NaOH, MeOH.



Scheme 3. Reagents: (a) $MeNH_2$, H_2, 5% Pd–C, MeOH; (b) i—HCO_2Et, EtOH; ii—$LiAlH_4$, THF; (c) **6**, K_2CO_3, DMF; (d) HCl, MeOH; (e) *c*-HCl, EtOH; (f) $BrCH_2CO_2Me$, K_2CO_3, cat KI, MeCN; (g) 1 N NaOH, MeOH.

hydrolysis. The transformation from **13c–d** to **14c–d** was carried out by our own original method. To overcome the steric hindrance of the amines, the reaction was carried out at high temperature in the presence of high-boiling triisopropanolamine base with high-boiling sulfolane as the solvent. Under these conditions, the required number of equivalents of the expensive amino alcohols could be reduced. The phenoxyacetic acids **22a** and **22b** were prepared from the methylamines **17** and **19**, which were easily derived from the benzaldehyde **16**[16] and the phenethylamine **18**,[17] respectively. Coupling of **17** with the chloropyrazine **6** followed by *O*-deprotection yielded the phenol **21a**. Subsequent *O*-alkylation of **21a** with methyl bromoacetate followed by alkaline hydrolysis gave the desired compound **22a**. A similar sequence of reactions starting with **19** led to the acid **22b**.

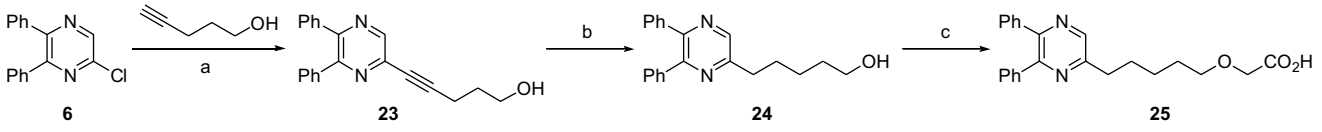
The synthetic route to the alkylated pyrazine **25** is shown in Scheme 4. Sonogashira reaction of the chloropyrazine **6** with 4-pentyn-1-ol gave the coupling product **23**, which was converted to the alcohol **24** by catalytic hydrogenation. The alcohol **24** underwent a two-step reaction sequence as described above that involved introduction of the ester moiety followed by alkaline hydrolysis to give the desired compound **25**.

The pyrazine derivatives **28a–d** were prepared according to Scheme 5. Coupling of the hydroxypyrazine **26a**[12] and the mercaptopyrazine **26b**[18] with the bromide **11** afforded the esters **27a** and **27b**, respectively. The desired acids **28a** and **28b** were synthesized by alkaline hydrolysis of **27a** and **27b**. The sulfoxide **28c** and the sulfone **28d** were prepared by oxidation of the sulfide **27b** by *m*-chloroperbenzoic acid (*m*CPBA) followed by acid hydrolysis.

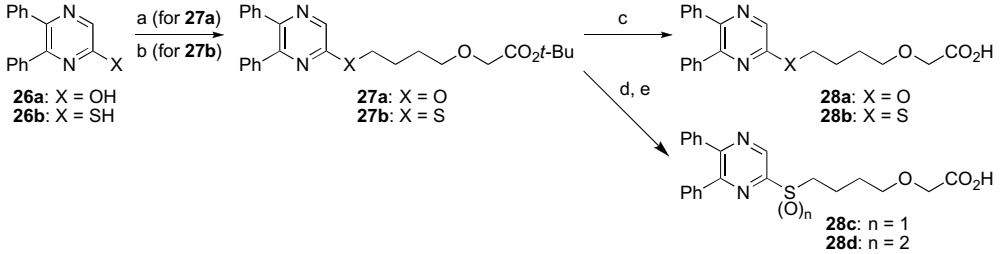
The synthetic routes to the tetrazole **30** and the *N*-methylsulfonamide **31** are shown in Schemes 6 and 7, respectively. *O*-Alkylation of the alcohol **14b** with bromoacetonitrile gave the nitrile **29**, which was converted to the tetrazole **30** by heating with sodium azide. The carboxylic acid **15b** was easily converted to the sulfonamide **31** through condensation with methanesulfonamide in the presence of 1,1′-carbonyldiimidazole (CDI).[11]

3. Results and discussion

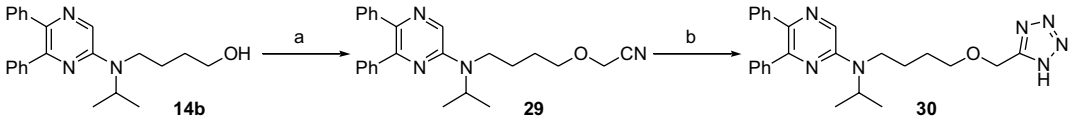
The test compounds prepared were evaluated for their potency to inhibit ADP-induced platelet aggregation in human platelet-rich plasma. The concentration of test



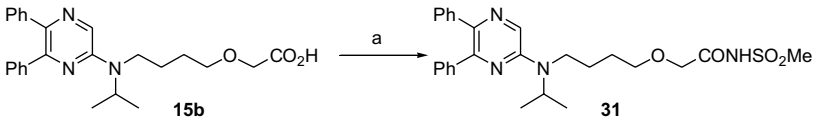
Scheme 4. Reagents: (a) $PdCl_2(PPh_3)_2$, CuI, Et_3N; (b) H_2, 5% Pd–C, EtOH; (c) i—$BrCH_2CO_2$ *t*-Bu, *n*-Bu_4NHSO_4, aq KOH, benzene; ii—1 N NaOH, MeOH.



Scheme 5. Reagents: (a) i—NaH, DMF; ii—**11**; (b) **11**, Na_2CO_3, acetone; (c) 1 N NaOH, MeOH; (d) *m*CPBA (1 eq for **28c**, 2 eq for **28d**), $CHCl_3$; (e) 4 N HCl, 1,4-dioxane.



Scheme 6. Reagents: (a) $BrCH_2CN$, *n*-Bu_4NHSO_4, aq KOH, benzene; (b) NaN_3, NH_4Cl, DMF.

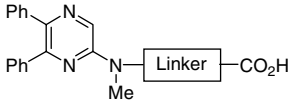


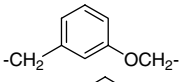
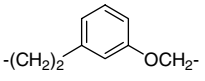
Scheme 7. Reagents: (a) i—CDI, THF; ii—$MeSO_2NH_2$, DBU.

compound giving 50% inhibition of aggregation (IC_{50}) was determined from dose–response curves. In this assay, iloprost, beraprost sodium, and BMY42393 exhibited IC_{50} values of 5 nM, 17 nM, and 1.5 μM, respectively. The biological activity of the compounds synthesized is summarized in Tables 1–4.

Previous studies on nonprostanoid PGI_2 mimetics such as the oxazole[6] and pyrazole series[11] provide key information on the structural requirements for the design of potent IP receptor agonists. These studies demonstrate that the agonist activity strongly depends on the topographical relationship between the diphenylated heterocycle and the carboxylic acid functionality, which comprise the nonprostanoid PGI_2 mimetic pharmacophore. With this information in hand, our first efforts toward optimization of the lead compound **5c** (IC_{50} = 1.5 μM) were focused on altering the conformationally flexible alkylene chain while keeping the other features intact. We investigated the effects of the length and rigidity of the methylene linker (Table 1). Structure–activity studies on **5a**–**d** clearly demonstrated that a hexamethylene linker was of the optimal length. Compound **5b** (hexamethylene linker), which is one carbon shorter

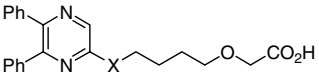
Table 1. Effect of linker variation on inhibition of ADP-induced human platelet aggregation



Compound	Linker	Inhibition of human platelet aggregation IC_{50}[a] (μM)
5a	$-(CH_2)_5-$	8
5b	$-(CH_2)_6-$	0.3
5c	$-(CH_2)_7-$	1.5
5d	$-(CH_2)_8-$	1.5
15a	$-(CH_2)_4-OCH_2-$	0.2
22a		5
22b		2

[a] Inhibition of platelet aggregation induced by ADP (10 μM) in human platelet-rich plasma.

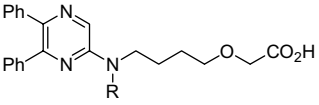
Table 2. Effect of concatenating atoms between the pyrazine and the linker on inhibition of ADP-induced human platelet aggregation



Compound	X	Inhibition of human platelet aggregation IC_{50}[a] (μM)
15a[b]	NMe	0.2
28b[b]	S	2
25	CH_2	17
28a	O	34
28c	SO	37
28d	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.

Table 3. Effect of substituents at the *N*-atom adjacent to the pyrazine ring on inhibition of ADP-induced human platelet aggregation

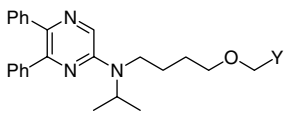


Compound	R	Inhibition of human platelet aggregation IC_{50}[a] (μM)
8a[b]	Et	0.5
8b	Allyl	1
8c	Cyclopropyl	0.2
15a[b]	Me	0.2
15b	*i*-Pr	0.2
15c[b]	Cyclopentyl	0.8
15d	Benzyl	50

[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.

than the lead **5c** (heptamethylene), showed fivefold-more-potent inhibition than **5c** (IC_{50} = 0.3 μM). Reducing the length of **5c** by two carbons, however, resulted in a significant loss of potency (**5a** (pentamethylene), IC_{50} = 8 μM), while extension of the linker by one carbon to give **5d** (octamethylene) had no effect on potency. Replacement of the methylene unit in the β-position

Table 4. Effect of alteration of terminal carboxyl group on inhibition of ADP-induced human platelet aggregation



Compound	Y	Inhibition of human platelet aggregation IC_{50}[a] (μM)
15b	CO_2H	0.2
30		0.4
31	$CONHSO_2Me$	6.4

[a] Inhibition of platelet aggregation induced by ADP (10 μM) in human platelet-rich plasma.

with respect to the carboxyl group by an oxygen atom was explored in expectation of increasing metabolic resistance to β-oxidative degradation in vivo.[19] Introduction of an oxygen atom at this position gave **15a** (IC_{50} = 0.2 μM), which was compatible with maintenance of in vitro activity. Next, to reduce the flexibility of the linear alkyl chain, we incorporated a phenyl ring within the methylene linker to obtain **22a** and **22b** and compared their activities with those of **5b** and **5c**, which have a simple methylene linker. We used a *meta*-substitution pattern because the length of the resulting linker is approximately equal to that of the methylene linker in **5b** and **5c** in the extended form. This was to try to ensure that the terminal carboxyl group would be correctly positioned. Compounds **22a** and **22b** showed weaker activity than **5c** (**22a**, IC_{50} = 5 μM; **22b**, IC_{50} = 2 μM).

The marked enhancement in potency observed for **15a** prompted us to explore the SAR of compounds incorporating an oxygen atom in the methylene linker. Keeping this oxygen atom in the linker, we then investigated the necessity of the nitrogen atom which joins the pyrazine ring and the alkyl side chain (Table 2). Replacement of the *N*-methylamino group by methylene or oxygen led to a two-order-of-magnitude reduction in potency (**25**, IC_{50} = 17 μM; **28a**, IC_{50} = 34 μM). A sulfur atom at this site produced a 10-fold reduction in activity (**28b**, IC_{50} = 2 μM), and the sulfoxide **28c** and the sulfone **28d** also showed substantially lower potency. These results show that the *N*-methylamino group played an important role in the biological activity of **15a**.

To explore the steric requirements for the substituent at the nitrogen atom adjacent to the pyrazine ring, the effects of replacing the *N*-methyl group were also evaluated (Table 3). Some sensitivity to variation in this region was observed. Compound **8a** (R = Et) had a slightly lower antiaggregatory activity against human platelets (IC_{50} = 0.5 μM), and compounds **8c** (R = cyclopropyl) and **15b** (R = *i*-Pr) showed activity equal to that of **15a**. Thus, small-sized alkyl groups were well tolerated. In contrast, replacement of the *N*-methyl group by larger substituents gave compounds **8b** (R = allyl) and **15c** (R = cyclopentyl), with 4- to 5-fold lower potency, and the relatively inactive compound **15d** (R = benzyl). These results suggest that the *N*-substituent may interact with a sterically restricted pocket of the receptor ligand-binding domain which can accept only a small hydrophobic group.

Finally, modification of the terminal carboxyl group of **15b** was investigated (Table 4). Tetrazole **30** showed only a marginally reduced potency (IC_{50} = 0.4 μM) compared to **15b**, as observed in early studies of nonprostanoid IP receptor agonists.[6,11] The tetrazole ring therefore functioned as an effective isostere of the carboxyl group in **15b**. Although *N*-acylsulfonamides are well known as carboxylic acid mimics,[20,21] the *N*-methylsulfonamide **31** showed significantly reduced inhibitory activity (IC_{50} = 6.4 μM), suggesting that the terminal carboxyl group is important for biological activity.

When a therapeutic agent targeting the IP receptor is being developed for long-term administration, a critical issue to be considered is the risk of unpredictable side effects mediated by other prostanoid receptors. To avoid such side effects, it is desirable to develop an agent that is selective for the IP receptor. On the basis of its performance in in vitro functional assays and other characteristics, including favorable pharmacokinetic and low toxicity in animal studies, we selected **15b** as a promising candidate and investigated its binding affinity for the IP and other prostanoid receptors. In another paper,[22] we demonstrate that **15b** shows a high binding affinity for the human IP receptor with a K_i value of 20 nM in our receptor binding assay, displaying more than 130-fold selectivity over the other human prostanoid receptors (EP_{1-4}, DP, FP, and TP). In addition, no significant off-target activity in binding or enzyme assays was observed for **15b** (data not shown).

After intravenous and oral administration of **15b** to rats, dogs, and monkeys (Table 5), **15b** displayed good oral bioavailability (BA rat, 102%; dog, 80%) and appropriately extended duration ($t_{1/2}$ rat, 3.6 h; dog, 6.2 h; monkey, 5.6 h).

4. Conclusions

To develop nonprostanoid IP receptor agonists with a high degree of metabolic resistance and a long duration

Table 5. Pharmacokinetic parameters of **15b** after intravenous or oral administration to rats, dogs, and monkeys

Route	Parameter	Rats[a]	Dogs[a]	Monkeys[a]
iv	Dose (mg/kg)	3	1	—[b]
	$t_{1/2}$ (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 ± 41	0.65 ± 0.12
	BA (%)	102	80	—

[a] Each value is the mean ± SD (n = 3).
[b] Not tested.

of action, we performed a SAR study on the side chain containing the carboxylic acid moiety of diphenylpyrazine derivative **5c**. This study revealed that the inhibition of ADP-induced human platelet aggregation was strongly influenced by the length of the linker and the presence of the concatenating nitrogen atom. In addition, we investigated the effects of altering the *N*-substituent and the terminal carboxyl group and identified the structural requirements for effective PGI_2 mimicry in this series. The optimized compounds **8c**, **15a**, and **15b** inhibited platelet aggregation equally potently, with IC_{50} values of 0.2 μM. Compound **15b** displayed good oral bioavailability and fairly long plasma duration in rats, dogs, and monkeys. Further studies on this series of derivatives are in progress to develop clinical candidates for orally available and long-lasting IP receptor agonists.

5. Experimental

Reagents and solvents were used as obtained from the supplier without further purification. Melting points were determined on a Shibata melting-point apparatus and are uncorrected. Column chromatography was carried out on a silica gel column (Wako Wakogel® C-200 or Fuji Silysia PSQ-100B). TLC was carried out on Merck TLC aluminum sheets silica gel 60 F_{254}, and detection was by UV quenching at 254 nm or spraying with phosphomolybdic acid. Yields were not optimized. ^{1}H NMR spectra were recorded on a Varian Gemini 2000 (200 MHz) spectrometer. Chemical shifts (δ) are given in ppm relative to the internal standard, tetramethylsilane, and coupling constants are given in Hertz (Hz). Mass spectra were recorded on a JEOL JMS-700 mass spectrometer and IR spectra on a Shimadzu FT IR-8100 spectrometer.

5.1. Preparation of 5a–d and 8a–c

5.1.1. *N*-(5,6-Diphenylpyrazin-2-yl)methylamine (7a). A mixture of the chloropyrazine **6**[12] (14.00 g, 52 mmol) and a 40% solution of methylamine in methanol (57 mL) was heated at 80 °C for 12 h in a sealed tube. The mixture was cooled to room temperature and concentrated. The residue was then diluted with water and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated in vacuo, after which the residual solid was recrystallized from EtOAc–hexane to give **7a** (7.82 g, 57%) as pale yellow crystals. Mp 137–138 °C [lit. 138–139 °C[23]]. FAB-MS *m*/*z* 262 $[MH]^+$. ^{1}H NMR ($CDCl_3$) δ: 3.04 (3H, d, J = 5.0 Hz), 4.78 (1H, br), 7.10–7.50 (10H, m), 7.94 (1H, s). Anal. Calcd for $C_{17}H_{15}N_3$: C, 78.13; H, 5.79; N, 16.08. Found: C, 78.31; H, 5.97; N, 16.05.

5.1.2. *N*-(5,6-Diphenylpyrazin-2-yl)ethylamine (7b). A mixture of **6** (2.00 g, 7.5 mmol) and a 70% solution of ethylamine in water (9.66 g, 150 mmol) in 1-butanol (10 mL) was heated at 150 °C for 24 h in a sealed tube. The mixture was cooled to room temperature and concentrated. The residue was diluted with water and extracted with $CHCl_3$, after which the extract was dried over $MgSO_4$ and evaporated in vacuo. The residual solid was washed with isopropyl ether and filtered to give **7b** (1.56 g, 76%) as colorless crystals. Mp 124–126 °C. FAB-MS *m*/*z* 276 $[MH]^+$. ^{1}H NMR ($CDCl_3$) δ: 1.31 (3H, t, J = 7.2 Hz), 3.47 (2H, qd, J = 7.2, 5.4 Hz), 4.67 (1H, br), 7.10–7.50 (10H, m), 7.93 (1H, s). Anal. Calcd for $C_{18}H_{17}N_3$: C, 78.52; H, 6.22; N, 15.26. Found: C, 78.46; H, 6.23; N, 15.22.

5.1.3. *N*-(5,6-Diphenylpyrazin-2-yl)allylamine (7c). Compound **7c** was prepared from **6** and allylamine in a manner similar to that described for **7b** except that the crude **7c** was purified by silica gel column chromatography with hexane–EtOAc (4:1) as the eluent Yield 48%, pale yellow crystals. Mp 102–103 °C. FAB-MS *m*/*z* 288 $[MH]^+$. ^{1}H NMR ($CDCl_3$) δ: 4.03–4.14 (2H, m), 4.88 (1H, t, J = 5.6 Hz), 5.21 (1H, dt, J = 10.2, 2.2 Hz), 5.32 (1H, dt, J = 17.0, 1.6 Hz), 5.98 (1H, ddt, J = 17.0, 10.2, 5.6 Hz), 7.19–7.50 (10H, m), 7.94 (1H, s). Anal. Calcd for $C_{19}H_{17}N_3$: C, 79.41; H, 5.96; N, 14.62. Found: C, 79.42; H, 5.96; N, 14.57.

5.1.4. *N*-(5,6-Diphenylpyrazin-2-yl)cyclopropylamine (7d). Compound **7d** was prepared from **6** and cyclopropylamine in a manner similar to that described for **7b**. Yield 61%, pale yellow crystals. Mp 136–138 °C. FAB-MS *m*/*z* 288 $[MH]^+$. ^{1}H NMR ($CDCl_3$) δ: 0.60–0.70 (2H, m), 0.82–0.95 (2H, m), 2.60–2.75 (1H, m), 5.20 (1H, s), 7.15–7.45 (10H, m), 8.30 (1H, s). Anal. Calcd for $C_{19}H_{17}N_3$: C, 79.41; H, 5.96; N,14.62. Found: C, 79.49; H, 6.08; N, 14.49.

5.1.5. *tert*-Butyl[4-(2-tetrahydropyranyloxy)butoxy]acetate (10). Compound **10** was prepared from the alcohol **9**[13] essentially according to a previously described method.[14] A mixture of **9** (35.98 g, 0.207 mol), tetra-*n*-butylammonium hydrogensulfate (33.95 g, 0.100 mol), benzene (300 mL), and 40% aqueous KOH (300 mL) was stirred vigorously and then cooled to 5 °C. *tert*-Butyl bromoacetate (48.45 g, 0.248 mol) was added dropwise as rapidly as possible while keeping the temperature below 10 °C. After stirring had been continued for 45 min, the mixture was stirred at room temperature for a further 1 h. The reaction mixture was diluted with ice water and extracted with Et_2O. The extract was washed with water and brine and dried over $MgSO_4$. After the solvent was concentrated in vacuo, the residual oil was subjected to chromatography on silica gel with hexane–EtOAc (4:1) as the eluent to give **10** (36.21 g, 61%) as a colorless oil. FAB-MS *m*/*z* 289 $[MH]^+$.^{1}H NMR ($CDCl_3$) δ: 1.40–1.95 (10H, m), 1.48 (9H, s), 3.30–3.65 (4H, m), 3.69–3.93 (2H, m), 3.96 (2H, s), 4.59 (1H, t, J = 3.3 Hz). Anal. Calcd for $C_{15}H_{28}O_5 \cdot 0.2H_2O \cdot 0.3C_4H_8O_2$: C, 60.76; H, 9.72. Found: C, 60.52; H, 9.30.

5.1.6. *tert*-Butyl(4-bromobutoxy)acetate (11). To a solution of the ester **10** (36.21 g, 0.126 mol) in MeOH (360 mL) was added *p*-toluenesulfonic acid monohydrate (47.77 g, 0.251 mol). The mixture was stirred at room temperature for 30 min. The reaction mixture was poured into saturated aqueous $NaHCO_3$ and extracted twice with Et_2O. The extracts were dried over

$MgSO_4$. After evaporation of the solvent, the residue was purified by chromatography on silica gel with hexane–EtOAc (2:1) as the eluent to give *tert*-butyl(4-hydroxybutoxy)acetate (17.02 g, 66%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 1.48 (9H, s), 1.70 (2H, t, J = 7.0 Hz), 1.73 (2H, t, J = 7.0 Hz), 3.57 (2H, t, J = 5.8 Hz), 3.68 (2H, t, J = 6.0 Hz), 3.97 (2H, s). To a solution of this alcohol (5.48 g, 27 mmol) in CH_2Cl_2 (150 mL) were added triphenylphosphine (7.74 g, 30 mmol) and carbon tetrabromide (10.22 g, 30 mmol). The mixture was stirred at room temperature for 1 h. After evaporation of the solvent, the residue was purified by chromatography on silica gel with $CHCl_3$ as the eluent to give **11** (5.18 g, 72%) as a colorless oil. FAB-MS *m*/*z* 269 $[M+2]^+$, 267 $[MH]^+$. ^{1}H NMR ($CDCl_3$) δ: 1.48 (9H, s), 1.70–1.83 (2H, m), 1.93–2.07 (2H, m), 3.47 (2H, t, J = 6.6 Hz), 3.55 (2H, t, J = 6.2 Hz), 3.95 (2H, s). Anal. Calcd for $C_{10}H_{19}BrO_3 \cdot 0.6CHCl_3$: C, 37.58; H, 5.83. Found: C, 37.31; H, 5.66.

5.1.7. General procedure for the synthesis of 5a–d and 8a–c: 9-[(5,6-Diphenylpyrazin-2-yl)(methyl)amino]nonanoic acid (5d). To a suspension of NaH (60% dispersion in oil, 42 mg, 1.05 mmol) in DMF (2 mL) was added the aminopyrazine **7a** (233 mg, 0.89 mmol) at room temperature, and the mixture was stirred at 80 °C for 30 min. The mixture was ice cooled and a solution of methyl 9-bromononanoate (209 mg, 0.87 mmol) in DMF (0.5 mL) was added dropwise. The mixture was stirred at room temperature for 2 h, diluted with ice water, and extracted with EtOAc. The extract was washed with brine and dried over $MgSO_4$. After the solvent was evaporated, the crude product was purified by silica gel column chromatography with hexane–EtOAc (9:1) as the eluent to give the corresponding ester (95 mg, 25%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ: 1.20–1.40 (8H, m), 1.50–1.75 (4H, m), 2.29 (2H, t, J = 7.5 Hz), 3.17 (3H, s), 3.60 (2H, t, J = 7.3 Hz), 3.66 (3H, s), 7.20–7.50 (10H, m), 8.01 (1H, s). To a stirred solution of this ester (95 mg, 0.22 mmol) in MeOH (2 mL) was added 1 N NaOH (1 mL), and the mixture was heated at reflux for 1 h. After evaporation of the solvent, the residue was dissolved in water and washed with Et_2O. The aqueous layer was separated, neutralized with 1 N HCl (1 mL), and extracted with EtOAc. The organic extract was dried over $MgSO_4$ and evaporated in vacuo. The residual solid was washed with a mixture of isopropyl ether and hexane (1:1) and filtered to give **5d** (79 mg, 86%) as colorless crystals. Mp 103–105 °C. FAB-MS *m*/*z* 418 $[MH]^+$. IR (KBr): 2930, 2855, 1713, 1586, 1202, 695 cm^{-1}.^{1}H NMR ($CDCl_3$) δ: 1.20–1.50 (8H, m), 1.50–1.80 (4H, m), 2.33 (2H, t, J = 7.3 Hz), 3.18 (3H, s), 3.60 (2H, t, J = 7.3 Hz), 7.20–7.50 (10H, m), 8.03 (1H, s). Anal. Calcd for $C_{26}H_{31}N_3O_2 \cdot 0.25H_2O$: C, 73.99; H, 7.52; N, 9.96. Found: C, 73.80; H, 7.55; N, 9.65.

5.1.8. 6-[(5,6-Diphenylpyrazin-2-yl)(methyl)amino]hexanoic acid (5a). Compound **5a** was prepared from **7a** and ethyl 6-bromohexanoate. Yield 19% (in two steps), pale yellow crystals. Mp 159–160 °C. FAB-MS *m*/*z* 376 $[MH]^+$. IR (KBr): 2950, 1715, 1586, 1572, 1186, 698 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 1.30–1.50 (2H, m), 1.60–1.80 (4H, m), 2.36 (2H, t, J = 7.3 Hz), 3.17 (3H, s), 3.63 (2H, t, J = 7.4 Hz), 7.15–7.50 (10H, m), 8.03 (1H, s). Anal. Calcd for $C_{23}H_{25}N_3O_2 \cdot 0.1H_2O$: C, 73.22; H, 6.73; N, 11.14. Found: C, 73.16; H, 6.82; N, 11.01.

5.1.9. 7-[(5,6-Diphenylpyrazin-2-yl)(methyl)amino]heptanoic acid (5b). Compound **5b** was prepared from **7a** and ethyl 7-bromoheptanoate. Yield 8% (in two steps), colorless crystals. Mp 114–118 °C. FAB-MS *m*/*z* 390 $[MH]^+$. IR (KBr): 2950, 1717, 1583, 1568, 1208, 698 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 1.30–1.80 (8H, m), 2.32 (2H, t, J = 7.3 Hz), 3.17 (3H, s), 3.61 (2H, t, J = 7.1 Hz), 4.30 (1H, br), 7.20–7.50 (10H, m), 8.02 (1H, s). Anal. Calcd for $C_{24}H_{27}N_3O_2 \cdot 0.2H_2O$: C, 73.33; H, 7.03; N, 10.69. Found: C, 73.32; H, 7.06; N, 10.41.

5.1.10. 8-[(5,6-Diphenylpyrazin-2-yl)(methyl)amino]octanoic acid (5c). Compound **5c** was prepared from **7a** and methyl 8-bromooctanoate. Yield 14% (in two steps), colorless crystals. Mp 116–117 °C. FAB-MS *m*/*z* 404 $[MH]^+$. IR (KBr): 2934, 1714, 1584, 1566, 1395, 1184, 698 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 1.30–1.80 (10H, m), 2.33 (2H, t, J = 7.4 Hz), 3.17 (3H, s), 3.60 (2H, t, J = 7.5 Hz), 4.00 (1H, br), 7.20–7.50 (10H, m), 8.03 (1H, s). Anal. Calcd for $C_{25}H_{29}N_3O_2$: C, 74.41; H, 7.24; N, 10.41. Found: C, 74.16; H, 7.25; N, 10.29.

5.1.11. {4-[(5,6-Diphenylpyrazin-2-yl)(ethyl)amino]butoxy}acetic acid (8a). Compound **8a** was prepared from the aminopyrazine **7b** and the bromide **11**. Yield 26% (in two steps), colorless oil. ^{1}H NMR ($CDCl_3$) δ: 1.25 (3H, t, J = 7.1 Hz), 1.60–1.90 (4H, m), 3.45–3.73 (6H, m), 4.05 (2H, s), 5.43 (1H, br), 7.15–7.50 (10H, m), 8.07 (1H, s). The analytical sample was obtained as the sodium salt as follows. To a solution of **8a** in MeOH was added 1 N NaOH (1.0 equiv), and the reaction mixture was evaporated in vacuo. The residual water was removed by co-distillation with EtOH in vacuo. The sodium salt of **8a** was collected by filtration, and after an Et_2O rinse it was obtained as a colorless amorphous powder. FAB-MS *m*/*z* 450 $[M+Na]^+$. ^{1}H NMR ($CDCl_3$) δ: 1.05 (3H, t), 1.53 (4H, br), 2.93 (2H, br), 3.35 (4H, br), 3.79 (2H, s), 6.90–7.50 (10H, m), 7.89 (1H, s). Anal. Calcd for $C_{24}H_{26}N_3O_3Na \cdot H_2O$: C, 64.71; H, 6.34; N, 9.43. Found: C, 64.88; H, 6.25; N, 9.16.

5.1.12. {4-[(Allyl)(5,6-diphenylpyrazin-2-yl)amino]butoxy}acetic acid (8b). Compound **8b** was prepared from the aminopyrazine **7c** and **11**. Yield 26% (in two steps), colorless oil. ^{1}H NMR ($CDCl_3$) δ: 1.60–1.90 (4H, m), 3.61 (2H, t, J = 5.8 Hz), 3.64 (2H, t, J = 7.4 Hz), 4.06 (2H, s), 4.21 (2H, d, J = 5.0 Hz), 5.21 (1H, dd, J = 10.6, 1.4 Hz), 5.22 (1H, dd, J = 17.1, 1.4 Hz), 5.90 (1H, ddt, J = 17.1, 10.6, 5.0 Hz), 7.15–7.50 (10H, m), 8.06 (1H, s). The analytical sample was obtained as the sodium salt in the form of a colorless amorphous powder in a manner similar to that described for **8a**. FAB-MS *m*/*z* 462 $[M+Na]^+$. ^{1}H NMR (DMSO-d_6) δ: 1.47 (9H, s), 1.40–1.90 (4H, m), 3.00–3.90 (6H, overlapping with solvent signal), 4.23

(2H, br), 5.00–5.40 (2H, m), 5.70–6.10 (1H, m), 7.10–7.65 (10H, m), 8.13 (1H, s). Anal. Calcd for $C_{25}H_{26}N_3O_3Na\cdot0.7H_2O$: C, 66.42; H, 6.11; N, 9.29. Found: C, 66.31; H, 5.97; N, 8.98.

5.1.13. {4-[(Cyclopropyl)(5,6-diphenylpyrazin-2-yl)amino]-butoxy}acetic acid (8c). Compound **8c** was prepared from the aminopyrazine **7d** and **11**. Yield 24% (in two steps), pale green crystals. Mp 108–109 °C. FAB-MS *m/z* 418 $[MH]^+$. IR (KBr): 2942, 1725, 1565, 1364, 1221, 1144, 698 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.70–0.82 (2H, m), 0.94–1.05 (2H, m), 1.58–1.88 (4H, m), 2.60–2.72 (1H, m), 3.57 (2H, t, $J = 6.2$ Hz), 3.75 (2H, t, $J = 6.9$ Hz), 4.01 (2H, s), 5.96 (1H, br), 7.10–7.50 (10H, m), 8.48 (1H, s). Anal. Calcd for $C_{25}H_{27}N_3O_3$: C, 71.92; H, 6.52; N, 10.06. Found: C, 71.77; H, 6.56; N, 10.04.

5.2. Preparation of 15a–d

5.2.1. 4-(Methylamino)-1-butanol (13a). To a stirred solution of 4-amino-1-butanol **12** (10.00 g, 112 mmol) in EtOH (100 mL) was added ethyl formate (13.6 mL, 168 mmol), and the mixture was heated at reflux for 18 h. The volatiles were evaporated in vacuo and the resulting crude *N*-(4-hydroxybutyl)formamide was used for the next step without further purification. To a suspension of $LiAlH_4$ (6.36 g, 168 mmol) in THF (100 mL) was added a solution of the crude product (13.29 g) in THF (50 mL) at a rate compatible with slow reflux. After the mixture was refluxed for 1.5 h, the reaction was quenched with water (6.3 mL), aqueous 15% NaOH (6.3 mL), and water (18.9 mL), in that order, with ice cooling. The mixture was stirred at room temperature for 30 min, the insoluble precipitate was filtered off, and the filtrate was evaporated in vacuo. The residue was dissolved in $CHCl_3$ and the solution was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by distillation to afford **13a** (6.73 g, 58% in two steps) as a colorless oil. Bp 84–85 °C (16 mmHg). FAB-MS *m/z* 104 $[MH]^+$. ^1H NMR ($CDCl_3$) δ: 1.50–1.75 (4H, m), 2.43 (3H, s), 2.62 (2H, t, $J = 5.5$ Hz), 3.57 (2H, t, $J = 5.1$ Hz), 3.74 (2H, br).

5.2.2. 4-(Isopropylamino)-1-butanol (13b). A mixture of **12** (100.40 g, 1.13 mol), platinum (IV) oxide (2.10 g, 9 mmol), and acetone (108 mL, 1.47 mol) in EtOH (160 mL) was stirred under an atmosphere of hydrogen (2–3 atm) at room temperature for 48 h. The catalyst was filtered through a pad of Celite and the cake was washed with EtOH. The filtrate was concentrated in vacuo to give **13b** (147.64 g, quant.) as a colorless oil, which was used for the next step without further purification. FAB-MS *m/z* 132 $[MH]^+$. ^1H NMR ($CDCl_3$) δ: 1.09 (6H, d, $J = 6.2$ Hz), 1.52–1.76 (4H, m), 2.64 (2H, t, $J = 5.5$ Hz), 2.80 (1H, qn, $J = 6.2$ Hz), 2.90–4.40 (2H, br), 3.57 (2H, t, $J = 4.9$ Hz).

5.2.3. 4-(Cyclopentylamino)-1-butanol (13c). Compound **13c** was prepared from cyclopentanone (1 equiv) in a manner similar to that described for **13b**. Yield quant., pale yellow oil. FAB-MS *m/z* 158 $[MH]^+$. ^1H NMR ($CDCl_3$) δ: 1.25–1.90 (12H, m), 2.63 (2H, t, $J = 5.1$ Hz), 2.90–4.80 (2H, br), 3.07 (1H, qn, $J = 6.5$ Hz), 3.57 (2H, t, $J = 4.8$ Hz).

5.2.4. 4-[(5,6-Diphenylpyrazin-2-yl)(methyl)amino]-1-butanol (14a). A mixture of the chloropyrazine **6** (6.00 g, 22 mmol), **13a** (2.78 g, 27 mmol), and K_2CO_3 (4.67 g, 34 mmol) in DMF (22 mL) was stirred at 80 °C for 40 h. The resulting mixture was diluted with ice water and extracted with Et_2O. The extract was washed with water and brine and dried over $MgSO_4$. After the solvent was concentrated in vacuo, the residual oil was subjected to chromatography on silica gel with hexane–EtOAc (1:2) as the eluent to give **14a** (6.13 g, 82%) as colorless crystals. An analytical sample was obtained by recrystallization from isopropyl ether. Mp 97–98.5 °C. FAB-MS *m/z* 334 $[MH]^+$. ^1H NMR ($CDCl_3$) δ: 1.50–1.85 (5H, m), 3.18 (3H, s), 3.68 (4H, t, $J = 7.1$ Hz), 7.15–7.50 (10H, m), 8.03 (1H, s). Anal. Calcd for $C_{21}H_{23}N_3O$: C, 75.65; H, 6.95; N, 12.60. Found: C, 75.52; H, 6.94; N, 12.56.

5.2.5. 4-[(5,6-Diphenylpyrazin-2-yl)(isopropyl)amino]-1-butanol (14b). A mixture of **6** (30.00 g, 0.11 mol) and **13b** (131.22 g, 1.00 mol) was heated at 190 °C for 10 h. The resulting mixture was diluted with ice water and extracted with Et_2O. The extract was washed with water and brine and dried over $MgSO_4$. After the solvent was concentrated in vacuo, the residual oil was subjected to chromatography on silica gel with hexane–EtOAc (1:1) as the eluent to give **14b** (22.96 g, 56%) as colorless crystals. Mp 102–103 °C. FAB-MS *m/z* 362 $[MH]^+$. ^1H NMR ($CDCl_3$) δ: 1.28 (6H, d, $J = 6.6$ Hz), 1.40–1.88 (5H, m), 3.45 (2H, t, $J = 7.7$ Hz), 3.62–3.76 (2H, m), 4.78 (1H, qn, $J = 6.6$ Hz), 7.19–7.50 (10H, m), 8.02 (1H, s). Anal. Calcd for $C_{23}H_{27}N_3O\cdot0.5H_2O$: C, 74.56; H, 7.62; N, 11.34. Found: C, 74.39; H, 7.87; N, 11.32.

5.2.6. 4-[(Cyclopentyl)(5,6-diphenylpyrazin-2-yl)amino]-1-butanol (14c). A mixture of **6** (4.00 g, 15 mmol), **13c** (7.08 g, 45 mmol) and triisopropanolamine (8.61 g, 45 mmol) in sulfolane (16 mL) was heated at 190 °C for 22 h. The resulting mixture was diluted with ice water and extracted with Et_2O. The extract was washed with water and brine and dried over $MgSO_4$. After the solvent was concentrated in vacuo, the residual oil was subjected to chromatography on silica gel with hexane–EtOAc (1:1) as the eluent to give **14c** (3.47 g, 60%) as pale yellow crystals. The analytical sample was obtained as pale yellow crystals after washing with isopropyl ether. Mp 94–96 °C. FAB-MS *m/z* 388 $[MH]^+$. ^1H NMR ($CDCl_3$) δ: 1.50–2.20 (12H, m), 1.54 (1H, t, $J = 5.7$ Hz) 3.48 (2H, t, $J = 7.7$ Hz), 3.69 (2H, q, $J = 5.7$ Hz), 4.56–4.80 (1H, m), 7.10–7.50 (10H, m), 8.04 (1H, s). Anal. Calcd for $C_{25}H_{29}N_3O\cdot0.5H_2O$: C, 77.13; H, 7.56; N, 10.79. Found: C, 76.97; H, 7.66; N, 10.80.

5.2.7. 4-[(Benzyl)(5,6-diphenylpyrazin-2-yl)amino]-1-butanol (14d). Compound **14d** was prepared from **6** and 4-(benzylamino)-1-butanol[15] in a manner similar to that described for **14c**. Yield 69%, pale yellow crystals. The analytical sample was obtained as colorless crystals after washing with isopropyl ether. Mp 96–98 °C. FAB-MS

m/z 410 $[MH]^+$. ^1H NMR ($CDCl_3$) δ: 1.48 (1H, br), 1.52–1.93 (4H, m), 3.59–3.80 (4H, m), 4.85 (2H, s), 7.15–7.55 (15H, m), 7.99 (1H, s). Anal. Calcd for $C_{27}H_{27}N_3O$: C, 79.19; H, 6.65; N, 10.26. Found: C, 79.03; H, 6.62; N, 10.27.

5.2.8. General procedure for the synthesis of 15a–d: {4-[(5,6-Diphenylpyrazin-2-yl)(methyl)amino]butoxy}acetic acid (15a). A mixture of **14a** (18.00 g, 54 mmol), tetra-*n*-butylammonium hydrogensulfate (9.16 g, 27 mmol), benzene (40 mL) and 40% aqueous KOH (40 mL) was stirred vigorously and then cooled to 5 °C. *tert*-Butyl bromoacetate (10.5 mL, 65 mmol) was added dropwise as rapidly as possible while keeping the temperature below 10 °C. After stirring had been continued for 45 min, the reaction mixture was stirred at room temperature for a further 1 h. The reaction mixture was diluted with ice water and extracted with Et_2O. The extract was then washed with water and brine and dried over $MgSO_4$. After the solvent had been concentrated in vacuo, the residual oil was subjected to chromatography on silica gel with hexane–EtOAc (2:1) as the eluent to give *tert*-butyl {4-[(5,6-diphenylpyrazin-2-yl)(methyl)amino]butoxy}acetate (16.35 g, 68%) as a pale yellow solid. The analytical sample was obtained as colorless crystals after washing with hexane. Mp 71–74 °C. FAB-MS *m/z* 448 $[MH]^+$. ^1H NMR ($CDCl_3$) δ: 1.47 (9H, s), 1.55–1.85 (4H, m), 3.18 (3H, s), 3.56 (2H, t, J = 6.0 Hz), 3.68 (2H, t, J = 6.9 Hz), 3.93 (2H, s), 7.20–7.50 (10H, m), 8.03 (1H, s). Anal. Calcd for $C_{27}H_{33}N_3O_3$: C, 72.46; H, 7.43; N, 9.39. Found: C, 72.44; H, 7.32; N, 9.41. Saponification of this ester according to the procedure described for the preparation of **5d** gave **15a** as pale yellow crystals (85% yield). Mp 102–103 °C. FAB-MS *m/z* 392 $[MH]^+$. IR (KBr): 2984, 1700, 1584, 1566, 1393, 1127, 770, 698 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.60–1.90 (4H, m), 3.18 (3H, s), 3.60 (2H, t, J = 6.0 Hz), 3.68 (2H, t, J = 6.9 Hz), 4.04 (2H, s), 4.69 (1H, br), 7.20–7.50 (10H, m), 8.09 (1H, s). Anal. Calcd for $C_{23}H_{25}N_3O_3$: C, 70.57; H, 6.44; N, 10.73. Found: C, 70.44; H, 6.42; N, 10.64.

5.2.9. {4-[(5,6-Diphenylpyrazin-2-yl)(isopropyl)amino]butoxy}acetic acid (15b). Compound **15b** was prepared from **14b** in a manner similar to that described for **15a**. Yield 62% (in two steps), pale yellow crystals. Mp 114–116 °C. FAB-MS *m/z* 420 $[MH]^+$. IR (KBr): 2984, 1719, 1561, 1482, 1221, 1142, 772, 698 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.27 (6H, d, J = 6.8 Hz), 1.60–1.90 (4H, m), 3.44 (2H, t, J = 7.5 Hz), 3.62 (2H, t, J = 5.9 Hz), 4.07 (2H, s), 4.84 (1H, qn, J = 6.8 Hz), 7.01 (1H, br), 7.18–7.49 (10H, m), 8.09 (1H, s). Anal. Calcd for $C_{25}H_{29}N_3O_3$: C, 71.58; H, 6.97; N, 10.02. Found: C, 71.64; H, 6.96; N, 9.97.

5.2.10. {4-[(Cyclopentyl)(5,6-diphenylpyrazin-2-yl)amino]butoxy}acetic acid (15c). Compound **15c** was prepared from **14c** in a manner similar to that described for **15a**. Yield 36% (in two steps), pale yellow crystals. Mp 104–106 °C. FAB-MS *m/z* 446 $[MH]^+$. IR (KBr): 2953, 1732, 1563, 1470, 1221, 1134, 772, 698 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.50–2.15 (12H, m), 3.47 (2H, t, J = 7.5 Hz), 3.61 (2H, t, J = 6.0 Hz), 4.07 (2H, s), 4.74 (1H, qn, J = 7.7 Hz), 6.94 (1H, br), 7.10–7.50 (10H, m), 8.10 (1H, s). Anal. Calcd for $C_{27}H_{31}N_3O_3 \cdot 0.5H_2O$: C, 72.20; H, 7.05; N, 9.36. Found: C, 72.06; H, 7.02; N, 9.19.

5.2.11. {4-[(Benzyl)(5,6-diphenylpyrazin-2-yl)amino]butoxy}acetic acid (15d). Compound **15d** was prepared from **14d** in a manner similar to that described for **15a**. Yield 57% (in two steps), pale yellow amorphous powder. ^1H NMR ($CDCl_3$) δ: 1.58–1.90 (4H, m), 3.58 (2H, t, J = 5.9 Hz), 3.67 (2H, t, J = 7.3 Hz), 4.04 (2H, s), 4.86 (2H, s), 5.29 (1H, br), 7.13–7.48 (15H, m), 8.08 (1H, s). The analytical sample was obtained as the sodium salt in the form of colorless crystals in a manner similar to that described for **8a**. Mp 133–137 °C. FAB-MS *m/z* 512 $[M+Na]^+$. IR (KBr): 3440, 1601, 1557, 1482, 1210, 696 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 1.44–1.80 (4H, m), 3.47 (2H, t, J = 5.4 Hz), 3.51 (2H, s), 3.63 (2H, t, J = 7.4 Hz), 4.86 (2H, s), 7.17–7.45 (15H, m), 8.16 (1H, s). Anal. Calcd for $C_{29}H_{28}N_3O_3Na \cdot 1.7H_2O$: C, 66.96; H, 6.08; N, 8.08. Found: C, 67.17; H, 6.48; N, 8.21.

5.3. Preparation of 22a and 22b

5.3.1. 3-(Methoxymethoxy)-*N*-methylbenzylamine (17). A mixture of 3-(methoxymethoxy)benzaldehyde **16**[16] (3.00 g, 18 mmol), 5% platinum on carbon (0.10 g), a 40% solution of methylamine in MeOH (2.1 mL), and MeOH (10 mL) was stirred under an atmosphere of hydrogen (2 atm) at room temperature for 7 h. The reaction mixture was allowed to warm to 70 °C and was continuously stirred for 15 h. The catalyst was removed by filtration of the reaction mixture through a pad of Celite and the cake was washed with MeOH. The filtrate was concentrated in vacuo and the residue was purified by silica gel column chromatography with $CHCl_3$–MeOH (20:1–10:1) as the eluent to give **17** (2.51 g, 77%) as a pale yellow oil. FAB-MS *m/z* 182 $[MH]^+$. ^1H NMR ($CDCl_3$) δ: 1.39 (1H, s), 2.46 (3H, s), 3.48 (3H, s), 3.73 (2H, s), 5.18 (2H, s), 6.90–7.00 (3H, m), 7.20–7.30 (1H, m). Anal. Calcd for $C_{10}H_{15}NO_2 \cdot 0.35CHCl_3$: C, 55.74; H, 6.94; N, 6.28. Found: C, 55.62; H, 6.94; N, 6.41.

5.3.2. 2-[3-(Benzyloxy)phenyl]-*N*-methylethylamine (19). To a stirred solution of 2-[3-(benzyloxy)phenyl]ethylamine **18**[17] (8.63 g, 38 mmol) in EtOH (50 mL) was added ethyl formate (4.6 mL, 57 mmol), and the mixture was heated at reflux for 22 h. The volatiles were evaporated in vacuo and the residue was purified by silica gel column chromatography with hexane–EtOAc (1:2) as the eluent to give *N*-[3-(benzyloxy)phenethyl]formamide (4.04 g, 42%) as a pale brown oil. FAB-MS *m/z* 256 $[MH]^+$. ^1H NMR ($CDCl_3$) δ: 2.81 (2H, t, J = 6.6 Hz), 3.50–3.61 (2H, m), 5.06 (2H, s), 5.45 (1H, br), 6.70–6.90 (3H, m), 7.20–7.50 (6H, m), 8.10 (1H, s). Anal. Calcd for $C_{16}H_{17}NO_2 \cdot 0.2H_2O$: C, 74.22; H, 6.77; N, 5.41. Found: C, 74.20; H, 6.65; N, 5.44. To a suspension of $LiAlH_4$ (0.88 g, 23 mmol) in THF (20 mL) was added dropwise a solution of this formamide (3.96 g, 16 mmol) in THF (10 mL) at room temperature. After the mixture had been refluxed for 2 h, the reaction was quenched with water (0.9 mL), aqueous 15% NaOH (0.9 mL),

and water (2.7 mL), in that order, with ice cooling. The mixture was stirred at room temperature for 30 min. The insoluble precipitate was filtered off and the filtrate was evaporated in vacuo. The residue was then purified by silica gel column chromatography with EtOAc as the eluent to give **19** (3.00 g, 80%) as a pale yellow oil. FAB-MS *m/z* 242 $[MH]^+$. ^1H NMR ($CDCl_3$) δ: 1.12 (1H, br), 2.42 (3H, s), 2.70–2.90 (4H, m), 5.06 (2H, s), 6.75–6.90 (3H, m), 7.17–7.47 (6H, m). Anal. Calcd for $C_{16}H_{19}NO{\cdot}0.5H_2O$: C, 76.77; H, 8.05; N, 5.60. Found: C, 76.57; H, 7.78; N, 5.51.

5.3.3. *N*-(5,6-Diphenylpyrazin-2-yl)-3-(methoxymethoxy)-*N*-methylbenzylamine (20a). A mixture of the chloropyrazine **6** (1.00 g, 3.7 mmol), the methylamine **17** (0.82 g, 4.5 mmol) and K_2CO_3 (0.77 g, 5.6 mmol) in DMF (4 mL) was stirred at 80 °C for 40 h. The resulting mixture was diluted with ice water and extracted with EtOAc. The extract was washed with water and brine and dried over $MgSO_4$. After the solvent was concentrated in vacuo, the resulting solid was washed with isopropyl ether–Et_2O (2:1) and dried in vacuo to give **20a** (0.93 g, 60%) as colorless crystals. Mp 109–111 °C. FAB-MS *m/z* 412 $[MH]^+$. ^1H NMR ($CDCl_3$) δ: 3.22 (3H, s), 3.46 (3H, s), 4.87 (2H, s), 5.15 (2H, s), 6.90–7.00 (3H, m), 7.20–7.50 (11H, m), 8.05 (1H, s). Anal. Calcd for $C_{26}H_{25}N_3O_2$: C, 75.89; H, 6.12; N, 10.21. Found: C, 75.81; H, 6.11; N, 10.18.

5.3.4. 2-[3-(Benzyloxy)phenyl]-*N*-(5,6-diphenylpyrazin-2-yl)-*N*-methylethylamine (20b). Compound **20b** was prepared from **6** and the methylamine **19** in a manner similar to that described for **14a**. Yield 71%, pale yellow crystals. Mp 78–78.5 °C. FAB-MS *m/z* 472 $[MH]^+$. ^1H NMR ($CDCl_3$) δ: 2.94 (2H, t, J = 7.5 Hz), 3.12 (3H, s), 3.85 (2H, t, J = 7.5 Hz), 5.02 (2H, s), 6.81–6.87 (3H, m), 7.17–7.51 (16H, m), 8.02 (1H, s). Anal. Calcd for $C_{32}H_{29}N_3O$: C, 81.50; H, 6.20; N, 8.91. Found: C, 81.39; H, 6.22; N, 8.95.

5.3.5. *N*-(5,6-Diphenylpyrazin-2-yl)-3-hydroxy-*N*-methylbenzylamine (21a). To a suspension of **20a** (0.91 g, 2.2 mmol) in MeOH (10 mL) was added an 18% solution of HCl in MeOH (2 mL) at room temperature. After the suspension had been stirred for 2 h, a further portion of 18% HCl in MeOH (1 mL) was added and the mixture was stirred continuously at the same temperature for 1 h. After evaporation of the volatiles, the residue was diluted with saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The resulting solid was washed with Et_2O and dried in vacuo to give **21a** (0.66 g, 81%) as colorless crystals. Mp 156–157 °C. FAB-MS *m/z* 368 $[MH]^+$. ^1H NMR ($CDCl_3$) δ: 3.17 (3H, s), 4.81 (2H, s), 5.92 (1H, br), 6.65–6.75 (2H, m), 6.82 (1H, d, J = 7.8 Hz), 7.10–7.50 (11H, m), 8.00 (1H, s). Anal. Calcd for $C_{24}H_{21}N_3O$: C, 78.45; H, 5.76; N, 11.44. Found: C, 78.22; H, 5.87; N, 11.34.

5.3.6. *N*-(5,6-Diphenylpyrazin-2-yl)-2-(3-hydroxyphenyl)-*N*-methylethylamine (21b). A mixture of **20b** (1.17 g, 2.5 mmol), EtOH (12 mL) and 35% aqueous HCl (6 mL) was heated at 80 °C for 17 h. The reaction mixture was neutralized with saturated aqueous $NaHCO_3$ and extracted with EtOAc. The extract was washed with water and dried over $MgSO_4$. After the solvent was concentrated in vacuo, the crude solid was washed with isopropyl ether and dried in vacuo to give **21b** (0.87 g, 92%) as pale yellow crystals. Mp 158–161 °C. FAB-MS *m/z* 382 $[MH]^+$. ^1H NMR ($CDCl_3$) δ: 2.89 (2H, t, J = 7.6 Hz), 3.12 (3H, s), 3.83 (2H, t, J = 7.6 Hz), 5.40 (1H, br), 6.62–6.67 (2H, m), 6.78 (1H, d, J = 7.6 Hz), 7.10–7.50 (11H, m), 8.00 (1H, s). Anal. Calcd for $C_{25}H_{23}N_3O$: C, 78.71; H, 6.08; N, 11.02. Found: C, 78.51; H, 6.10; N, 10.73.

5.3.7. General procedure for the synthesis of 22a and 22b: [3-{[(5,6-Diphenylpyrazin-2-yl)(methyl)amino]methyl}-phenoxy]acetic acid (22a). A mixture of **21a** (605 mg, 1.65 mmol), methyl bromoacetate (278 mg, 1.82 mmol), K_2CO_3 (273 mg, 1.98 mmol), and KI (catalytic amount) in MeCN (10 mL) was heated at reflux for 3 h. The insoluble materials were filtered off and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography with $CHCl_3$–MeOH (40:1) as the eluent, and the resulting solid was washed with Et_2O and dried in vacuo to give methyl [3-{[(5,6-diphenylpyrazin-2-yl)(methyl)amino]methyl}phenoxy]acetate (632 mg, 87%) as colorless crystals. Mp 132–134 °C. FAB-MS *m/z* 440 $[MH]^+$. ^1H NMR ($CDCl_3$) δ: 3.20 (3H, s), 3.77 (3H, s), 4.58 (2H, s), 4.87 (2H, s), 6.79 (1H, dd, J = 1.8, 8.0 Hz), 6.88 (1H, m), 6.94 (1H, d, J = 8.0 Hz), 7.20–7.50 (11H, m), 8.05 (1H, s). Anal. Calcd for $C_{27}H_{25}N_3O_3$: C, 73.78; H, 5.73; N, 9.56. Found: C, 73.76; H, 5.68; N, 9.60. Saponification of this ester according to the procedure described for the preparation of **5d** gave **22a** as pale yellow crystals (79% yield). Mp 182–187 °C. FAB-MS *m/z* 426 $[MH]^+$. IR (KBr): 3050, 1754, 1586, 1560, 1395, 1229, 770, 700 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 3.19 (3H, s), 4.62 (2H, s), 4.85 (2H, s), 6.70–7.90 (3H, m), 7.15–7.40 (11H, m), 8.21 (1H, s). Anal. Calcd for $C_{26}H_{23}N_3O_3$: C, 73.39; H, 5.45; N, 9.88. Found: C, 73.26; H, 5.47; N, 9.83.

5.3.8. [3-{[(5,6-Diphenylpyrazin-2-yl)(methyl)amino]ethyl}phenoxy]acetic acid (22b). Compound **22b** was prepared from **21b** in a manner similar to that described for **22a**. Yield 51% (in two steps), pale yellow crystals. Mp 174–176 °C. FAB-MS *m/z* 440 $[MH]^+$. IR (KBr): 2900, 1709, 1586, 1570, 1262, 1248, 1177, 696 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 2.94 (2H, t, J = 7.6 Hz), 3.13 (3H, s), 3.85 (2H, t, J = 7.6 Hz), 4.57 (2H, s), 6.77–6.89 (3H, m), 7.17–7.45 (11H, m), 8.03 (1H, s). Anal. Calcd for $C_{27}H_{25}N_3O_3$: C, 73.78; H, 5.73; N, 9.56. Found: C, 73.43; H, 5.79; N, 9.32.

5.4. Preparation of 25

5.4.1. 5-(5,6-Diphenylpyrazin-2-yl)-4-pentyn-1-ol (23). A mixture of **6** (1.58 g, 5.9 mmol), 4-pentyn-1-ol (0.60 g, 7.1 mmol), dichlorobis(triphenylphosphine)palladium (II) (210 mg, 0.3 mmol), and copper (I) iodide (57 mg, 0.3 mmol) in triethylamine (10 mL) was stirred under an argon atmosphere at 80 °C for 7 h. The resulting mixture was diluted with Et_2O and the insoluble materials

were filtered off. The filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography with hexane–EtOAc (1:1) as the eluent, and the resulting solid was washed with isopropyl ether and dried in vacuo to give **23** (1.12 g, 60%) as pale yellow crystals. Mp 97–99 °C. FAB-MS *m/z* 315 $[MH]^+$. ^1H NMR ($CDCl_3$) δ: 1.62 (1H, t, $J = 5.2$ Hz), 1.92 (2H, qn, $J = 6.6$ Hz), 2.64 (2H, t, $J = 7.2$ Hz), 3.83 (2H, t, $J = 5.7$ Hz), 7.20–7.60 (10H, m), 8.62 (1H, s). Anal. Calcd for $C_{21}H_{18}N_2O$: C, 80.23; H, 5.77; N, 8.91. Found: C, 80.27; H, 5.73; N, 8.88.

5.4.2. 5-(5,6-Diphenylpyrazin-2-yl)-1-pentanol (24). A mixture of **23** (409 mg, 1.3 mmol) and 5% palladium on carbon (40 mg) in MeOH (20 mL) was stirred under an atmosphere of hydrogen (3 atm) at room temperature for 4 h. The catalyst was filtered through a pad of Celite and the cake was washed with MeOH. After the filtrate was concentrated in vacuo, the residual oil was subjected to chromatography on silica gel with hexane–EtOAc (1:1) as the eluent to give **24** (409 mg, 99%) as a pale yellow oil. FAB-MS *m/z* 319 $[MH]^+$. ^1H NMR ($CDCl_3$) δ: 1.30–1.80 (5H, m), 1.80–2.00 (2H, m), 2.93 (2H, t, $J = 7.7$ Hz), 3.68 (2H, t, $J = 6.0$ Hz), 7.20–7.50 (10H, m), 8.47 (1H, s). Anal. Calcd for $C_{21}H_{22}N_2O \cdot 0.3C_4H_8O_2$: C, 77.32; H, 7.13; N, 8.12. Found: C, 77.30; H, 6.75; N, 8.48.

5.4.3. [5-(5,6-Diphenylpyrazin-2-yl)pentyloxy]acetic acid (25). Compound **25** was prepared from **24** in a manner similar to that described for **15a**. Yield 25% (in two steps), pale yellow oil. ^1H NMR ($CDCl_3$) δ: 1.40–2.00 (6H, m), 2.93 (2H, t, $J = 7.7$ Hz), 3.58 (2H, t, $J = 6.4$ Hz), 4.09 (2H, s), 7.20–7.50 (10H, m), 8.50 (1H, s), 9.05 (1H, br). The analytical sample was obtained as the sodium salt in the form of a pale brown amorphous powder in a manner similar to that described for **8a**. FAB-MS *m/z* 421 $[M+Na]^+$. ^1H NMR ($CDCl_3$) δ: 1.00–2.00 (6H, m), 2.74 (2H, br), 3.40 (2H, br), 3.80 (2H, s), 6.90–7.50 (10H, m), 8.32 (1H, s). Anal. Calcd for $C_{23}H_{23}N_2O_3Na \cdot 0.5H_2O$: C, 67.80; H, 5.94; N, 6.88. Found: C, 68.03; H, 6.22; N, 6.48.

5.5. Preparation of 28a–d

5.5.1. *tert*-Butyl[4-(5,6-diphenylpyrazin-2-yloxy)butoxy]acetate (27a). To a suspension of NaH (60% dispersion in oil, 96 mg, 2.4 mmol) in DMF (4 mL) was added **26a**[1] (500 mg, 2.0 mmol) by portions at room temperature. The mixture was stirred for 30 min and then ice cooled. A solution of the bromide **11** (641 mg, 2.4 mmol) in DMF (1 mL) was added dropwise, and the resulting mixture was allowed to warm to room temperature and stirred for 18 h. The mixture was diluted with ice water and extracted with EtOAc. The extract was washed with water and dried over $MgSO_4$. After the solvent was evaporated, the crude product was purified by silica gel column chromatography with hexane–EtOAc (6:1) as the eluent to give **27a** (474 mg, 54%) as a colorless oil. The alternative *N*-linked regioisomer was almost undetectable by TLC. FAB-MS *m/z* 435 $[MH]^+$. ^1H NMR ($CDCl_3$) δ: 1.48 (9H, s), 1.72–2.05 (4H, m), 3.61 (2H, t, $J = 6.0$ Hz), 3.97 (2H, s), 4.48 (2H, t, $J = 6.0$ Hz), 7.20–7.50 (10H, m), 8.23 (1H, s). Anal. Calcd for $C_{26}H_{30}N_2O_4 \cdot 0.2H_2O$: C, 71.28; H, 6.99; N, 6.39. Found: C, 71.00; H, 6.86; N, 6.26.

5.5.2. *tert*-Butyl[4-(5,6-diphenylpyrazin-2-ylsulfanyl)butoxy]acetate (27b). To an ice-cooled mixture of 5,6-diphenyl-2-pyrazinethiol **26b**[18] (500 mg, 1.9 mmol) and Na_2CO_3 (321 mg, 3.0 mmol) in acetone (20 mL) was added dropwise a solution of **11** (556 mg, 2.1 mmol) in acetone (2 mL). The solution was allowed to warm to room temperature and stirred for 24 h. After evaporation of the solvent, the residue was diluted with water and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by chromatography on silica gel with hexane–EtOAc (7:3) as the eluent to give **27b** (701 mg, 82%) as a pale yellow oil. ^1H NMR ($CDCl_3$) δ: 1.47 (9H, s), 1.70–2.00 (4H, m), 3.31 (2H, t, $J = 7.0$ Hz), 3.55 (2H, t, $J = 6.0$ Hz), 3.92 (2H, s), 7.20–7.50 (10H, m), 8.44 (1H, s).

5.5.3. [4-(5,6-Diphenylpyrazin-2-yloxy)butoxy]acetic acid (28a). Saponification of **27a** according to the procedure described for the preparation of **5d** gave **28a** as colorless crystals. Yield 62%. Mp 65–66 °C. FAB-MS *m/z* 379 $[MH]^+$. IR (KBr): 2959, 1715, 1483, 1327, 1148, 696 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.75–2.05 (4H, m), 3.66 (2H, t, $J = 5.9$ Hz), 4.13 (2H, s), 4.48 (2H, t, $J = 5.9$ Hz), 7.15–7.55 (10H, m), 8.25 (1H, s). Anal. Calcd for $C_{22}H_{22}N_2O_4$: C, 69.83; H, 5.86; N, 7.40. Found: C, 69.84; H, 5.90; N, 7.34.

5.5.4. [4-(5,6-Diphenylpyrazin-2-ylsulfanyl)butoxy]acetic acid (28b). Saponification of **27b** according to the procedure described for the preparation of **5d** gave **28b** as pale yellow crystals. Yield 61%. Mp 86–88 °C. FAB-MS *m/z* 395 $[MH]^+$. IR (KBr): 2936, 1748, 1734, 1416, 1138, 696 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.70–2.00 (4H, m), 3.31 (2H, t, $J = 6.9$ Hz), 3.59 (2H, t, $J = 6.1$ Hz), 4.05 (2H, s), 6.00 (1H, br), 7.20–7.50 (10H, m), 8.46 (1H, s). Anal. Calcd for $C_{22}H_{22}N_2O_3S$: C, 66.98; H, 5.62; N, 7.10. Found: C, 66.77; H, 5.65; N, 7.45.

5.5.5. [4-(5,6-Diphenylpyrazin-2-ylsulfinyl)butoxy]acetic acid (28c). To an ice-cooled solution of **27b** (350 mg, 0.78 mmol) in $CHCl_3$ (5 mL) was added 3-chloroperoxybenzoic acid (purity ca. 70%; 191 mg, 0.77 mmol). The mixture was stirred at 0 °C for 2 h, diluted with 0.2 N NaOH (20 mL), and extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by chromatography on silica gel with hexane–EtOAc (4:1–1:1) as the eluent to give *tert*-butyl [4-(5,6-diphenylpyrazin-2-ylsulfinyl)butoxy]acetate (145 mg, 40%) as a pale yellow oil. ^1H NMR ($CDCl_3$) δ: 1.46 (9H, s), 1.70–2.20 (4H, m), 3.06–3.39 (2H, m), 3.56 (2H, t, $J = 5.8$ Hz), 3.92 (2H, s), 7.27–7.53 (10H, m), 9.16 (1H, s). To a solution of this oil (145 mg, 0.31 mmol) in 1,4-dioxane (2 mL) was added 4 N HCl (1 mL). The mixture was stirred at 80 °C for 30 min, diluted with ice water, neutralized with 2 N NaOH, and extracted with EtOAc. The extract was washed with brine and dried over $MgSO_4$. Evaporation of the solvent gave **28c** (125 mg, 98%) as a pale yellow

oil. ^{1}H NMR ($CDCl_3$) δ: 1.70–2.22 (4H, m), 3.11–3.41 (2H, m), 3.61 (2H, t, J = 5.8 Hz), 4.08 (2H, s), 5.60 (1H, br), 7.20–7.60 (10H, m), 9.17 (1H, s). The analytical sample was obtained as the sodium salt in the form of a pale yellow amorphous powder in a manner similar to that described for **8a**. FAB-MS m/z 455 $[M+Na]^+$. ^{1}H NMR ($CDCl_3$) δ: 1.50–2.10 (4H, m), 2.90–3.25 (2H, m), 3.43 (2H, br), 3.82 (2H, s), 7.10–7.50 (10H, m), 9.07 (1H, s). Anal. Calcd for $C_{22}H_{21}N_2O_4SNa{\cdot}0.2C_4H_{10}O{\cdot}1.4H_2O$: C, 57.96; H, 5.50; N, 5.93. Found: C, 58.30; H, 5.10; N, 5.45.

5.5.6. [4-(5,6-Diphenylpyrazin-2-ylsulfonyl)butoxy]acetic acid (28d). *tert*-Butyl[4-(5,6-diphenylpyrazin-2-ylsulfonyl)butoxy]acetate was prepared from **27b** in a manner similar to that described for **28c** except that the reaction was carried out for 19 h with 2 mol equivalents of 3-chloroperoxybenzoic acid, and it was obtained as a colorless oil (yield quant.). ^{1}H NMR ($CDCl_3$) δ: 1.45 (9H, s), 1.71–1.85 (2H, m), 1.90–2.09 (2H, m), 3.53 (2H, t, J = 6.0 Hz), 3.56 (2H, t, J = 7.6 Hz), 3.89 (2H, s), 7.27–7.53 (10H, m), 9.22 (1H, s). Compound **28d** was prepared by saponification of this oil in a manner similar to that described for **28c** except that it was purified by recrystallization from EtOAc–hexane. Yield 66%, colorless crystals. Mp 123–125 °C. FAB-MS m/z 427 $[MH]^+$. IR (KBr): 2950, 1738, 1323, 1121, 698 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 1.72–1.90 (2H, m), 1.90–2.13 (2H, m), 3.55 (2H, t, J = 7.8 Hz), 3.58 (2H, t, J = 5.8 Hz), 4.04 (2H, s), 7.20–7.60 (10H, m), 9.23 (1H, s). Anal. Calcd for $C_{22}H_{22}N_2O_5S$: C, 61.96; H, 5.20; N, 6.57. Found: C, 61.95; H, 5.25; N, 6.41.

5.6. Preparation of 30

5.6.1. 2-{4-[(5,6-Diphenylpyrazin-2-yl)(isopropyl)amino]butoxy}acetonitrile (29). Compound **29** was prepared from the alcohol **14b** and bromoacetonitrile in a manner similar to that described for **10**. Yield 28%, yellow oil. FAB-MS m/z 401 $[MH]^+$. ^{1}H NMR ($CDCl_3$) δ: 1.28 (6H, d, J = 6.6 Hz), 1.60–1.90 (4H, m), 3.44 (2H, t, J = 7.7 Hz), 3.64 (2H, t, J = 5.8 Hz), 4.19 (2H, s), 4.75 (1H, qn, J = 6.6 Hz), 7.10–7.50 (10H, m), 8.01 (1H, s). Anal. Calcd for $C_{25}H_{28}N_4O{\cdot}0.2C_4H_8O_2$: C, 74.11; H, 7.14; N, 13.40. Found: C, 73.87; H, 6.86; N, 13.57.

5.6.2. 5-{4-[(5,6-Diphenylpyrazin-2-yl)(isopropyl)amino]butoxymethyl}-1*H*-tetrazole (30). A mixture of **29** (0.80 g, 2.0 mmol), sodium azide (1.04 g, 16.0 mmol), and ammonium chloride (0.75 g, 14 mmol) in DMF (7 mL) was stirred at 120 °C under an argon atmosphere for 1.5 h. The reaction mixture was poured into ice water and extracted with EtOAc. The extract was washed with water and dried over $MgSO_4$. After the solvent was concentrated in vacuo, the residue was purified by chromatography on silica gel with $CHCl_3$–MeOH (50:1) as the eluent to give **30** (0.55 g, 62%) as a pale yellow amorphous powder. FAB-MS m/z 444 $[MH]^+$. IR (KBr): 2973, 1557, 1447, 1372, 1235, 1125, 772, 698 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 1.25 (6H, d, J = 6.6 Hz), 1.67–2.00 (4H, m), 3.39 (2H, t, J = 7.9 Hz), 3.71 (2H, t, J = 5.1 Hz), 4.90 (2H, s), 5.08 (1H, qn, J = 6.6 Hz), 7.20–7.53 (10H, m), 8.33 (1H, s). Anal. Calcd for $C_{25}H_{29}N_7O{\cdot}0.25CHCl_3$: C, 64.06; H, 6.23; N, 20.71. Found: C, 64.23; H, 6.31; N, 20.74.

5.7. Preparation of 31

5.7.1. 2-{4-[(5,6-Diphenylpyrazin-2-yl)(isopropyl)amino]butoxy}-*N*-(methylsulfonyl)acetamide (31). Compound **31** was prepared from the carboxylic acid **15b** by a method similar to one described in the literature.[11] A mixture of **15b** (300 mg, 0.72 mmol) and 1,1′-carbonyldiimidazole (128 mg, 0.79 mmol) in THF (5 mL) was stirred at room temperature under an argon atmosphere for 30 min and at reflux for a further 30 min. After cooling, methanesulfonamide (69 mg, 0.73 mmol) was added and the mixture was stirred at room temperature for 10 min. 1,8-Diazabicyclo[5.4.0]-7-undecene (DBU) (0.11 mL, 0.74 mmol) was then added dropwise to the mixture. After being stirred at room temperature for 12 h, the reaction mixture was poured into 1 N HCl and extracted with Et_2O. The extract was washed with water and dried over $MgSO_4$. After the solvent was concentrated in vacuo, the residue was subjected to chromatography on silica gel to give **31** (272 mg, 77%) as a pale yellow solid. FAB-MS m/z 497 $[MH]^+$. ^{1}H NMR ($CDCl_3$) δ: 1.29 (6H, d, J = 6.6 Hz), 1.60–1.90 (4H, m), 3.29 (3H, s), 3.46 (2H, t, J = 7.3 Hz), 3.59 (2H, t, J = 6.0 Hz), 3.97 (2H, s), 4.72 (1H, qn, J = 6.6 Hz), 7.15–7.50 (10H, m), 8.02 (1H, s). Anal. Calcd for $C_{26}H_{32}N_4O_4S$: C, 62.88; H, 6.49; N, 11.28. Found: C, 63.06; H, 6.53; N, 10.98.

5.8. Inhibition of platelet aggregation

Blood samples freshly collected from volunteers were mixed with 1/10th volume of 3.8% trisodium citrate solution. Platelet-rich plasma was prepared by centrifugation of blood samples at 150*g* for 10 min at room temperature. Platelet aggregation induced by ADP (10 μM) was monitored at 37 °C with an aggregometer (model PAM-8C, Mebanix, Tokyo, Japan) in the presence or absence of the test compound by a literature method.[24]

5.9. Pharmacokinetic studies in animals

Male Sprague–Dawley rats (7 weeks old; Japan SLC, Shizuoka, Japan), male beagle dogs weighing 10–12 kg (Nosan Corporation, Tokyo), and male cynomolgus monkeys weighing 4–6 kg (Japan SLC) were used. The animals were acclimatized to the laboratory conditions for more than 1 week (rats) or more than 3 weeks (dogs and monkeys) before dosing at a room temperature of 21–25 °C and 45–65% humidity. The rats, dogs, and monkeys were fed diet F-2 (Funabashi Farms, Funabashi, Japan), diet TC-1 (Aixia, Tokyo), and diet PS (Oriental Yeast Co., Tokyo), respectively. None of the animals were fasted before the experiments. Compound **15b** was intravenously or orally administered to groups of three animals. Blood samples were collected at various times (before administration and 5, 15, and 30 min and 1, 2, 4, 6, 8, 10, and 24 h after administration) by venous withdrawal. Each sample was taken into a heparinized tube and plasma was prepared by centrifugation (3000 rpm, 15 min, 4 °C). The plasma concentrations of test compounds after administration were measured by

liquid chromatography mass spectrometry (LC/MS) or liquid chromatography tandem mass spectrometry (LC/MS/MS). Briefly, plasma was mixed with an equal volume of 0.2 N HCl and a small amount of MeOH containing the internal standard. The compound was extracted by liquid–liquid extraction or solid-phase extraction. The extracted sample was then assayed by LC/MS(/MS). For LC/MS(/MS), a Series 1050 (Agilent, Palo Alto, CA, USA) or Alliance 2795 (Waters, Milford, MA, USA) liquid chromatograph was used with a Quattro micro API tandem quadrupole mass spectrometer (Waters) equipped with a field-desorption apparatus (M-2500; Hitachi, Tokyo). The ionization mode was atmospheric pressure chemical ionization positive or electrospray ionization positive. The peak intensities of characteristic ions (*m*/*z*) were measured in the selected-ion-monitoring mode or the selected-reaction-monitoring mode. HPLC was carried out on a SymmetryShield RP_8 (4.6×20 mm, Waters) or Xterra MS_{18}IS (3.5 μm, 2.1×20 mm, Waters) column. The mobile phase was MeCN/0.1% formic acid (80/20) and the column was operated at a flow rate of 1 or 0.3 mL/min and a temperature of 40 °C.

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