Regular Article

*Highlighted Paper selected by Editor-in-Chief***Novel Non-carboxylate Benzoylsulfonamide-Based Protein Tyrosine Phosphatase 1B Inhibitors with Non-competitive Actions**Ko Morishita,^a Yoshimichi Shoji,^a Shunkichi Tanaka,^a Masaki Fukui,^a Yuma Ito,^a Tatsuya Kitao,^a Shin-ichiro Ozawa,^b Shuichi Hirono,^b and Hiroaki Shirahase^{*,a}^aDrug Discovery Research Department, Kyoto Pharmaceutical Industries, Ltd.; 38 Nishinokyo Tsukinowa-cho, Nakagyo-ku, Kyoto 604–8444, Japan; and ^bSchool of Pharmacy, Kitasato University; 5–9–1 Shirokane, Minato-ku, Tokyo 108–8641, Japan.

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A novel series of benzoylsulfonamide derivatives were synthesized and biologically evaluated. Among them, 4-(biphenyl-4-ylmethylsulfanylmethyl)-*N*-(hexane-1-sulfonyl)benzamide (compound 18K) was identified as a protein tyrosine phosphatase 1B (PTP1B) inhibitor with potent and selective inhibitory activity against PTP1B ($IC_{50}=0.25\mu M$). Compound 18K functioned as a non-competitive inhibitor and bound to the allosteric site of PTP1B. It also showed high oral absorption in mice (the maximum drug concentration (C_{max})= $45.5\mu M$ at 30 mg/kg), rats (C_{max} = $53.6\mu M$ at 30 mg/kg), and beagles (C_{max} = $37.8\mu M$ at 10 mg/kg), and significantly reduced plasma glucose levels at 30 mg/kg/d (*per os* (*p.o.*)) for one week with no side effects in *db/db* mice. In conclusion, the substituted benzoylsulfonamide was shown to be a novel scaffold of a non-competitive and allosteric PTP1B inhibitor, and compound 18K has potential as an efficacious and safe anti-diabetic drug as well as a useful tool for investigations of the physiological and pathophysiological effects of allosteric PTP1B inhibition.

Key words benzoylsulfonamide; protein tyrosine phosphatase 1B; allosteric inhibitor; non-competitive inhibitor; *db/db* mouse; Sprague-Dawley rat

In various cellular signaling pathways, protein tyrosine kinases (PTKs) mediate the phosphorylation of tyrosine residues in a number of proteins, while protein tyrosine phosphatases (PTPs) de-phosphorylate phosphorylated tyrosine.^{1,2} Thus, PTPs as well as PTKs play important physiological roles and are involved in the pathogenesis of diseases such as diabetes, obesity, autoimmune disorders, cancer, and neurological disorders.^{1–3} Among PTPs, PTP1B has been shown to negatively regulate insulin signals through the de-phosphorylation of insulin receptors and insulin receptor substrate (IRS), and the leptin signal by the de-phosphorylation of Janus kinase 2 (JAK2) and signal transducer and activator of transcription 3 (STAT3).^{2,4} PTP1B knockout mice show obesity resistance and insulin hypersensitivity.⁵ A PTP1B antisense oligonucleotide normalizes glucose levels and improves insulin resistance in diabetic mice.⁶ Furthermore, PTP1B has been implicated in tumorigenesis, podocyte injury, endothelial dysfunction, and retinal light damage.^{1,2,7–9} Based on this background, PTP1B has been attracting attention as a potential therapeutic target and a large number of PTP1B inhibitors have been reported as candidates for anti-diabetic and anti-obesity drugs.^{1,10–12}

Classically, some types of vanadates have been shown to inhibit PTP1B and exert insulinomimetic actions.¹⁰ Small molecule synthetic inhibitors started from peptidyl phosphonates, which mimic the substrate, a phosphorylated tyrosine (pTyr).¹¹ Non-peptidyl compounds with two phosphonate moieties have been discovered; however, these highly charged compounds cannot permeate the cell membrane (compound I in Fig. 1).¹² Non-phosphate compounds, in which two carboxyl moieties as bioisosteres of phosphonates were introduced, have been reported to function as potent inhibitors, but were still highly charged and showed low bioavailability (compound II in Fig. 1).¹² PTP1B inhibitors with one carboxyl moiety

were also discovered and shown to exhibit potent activity and efficacy (Ertiprotatib and compound III in Fig. 1).¹¹ Ertiprotatib has peroxisome proliferator-activated receptor (PPAR) γ and PPAR α agonist activities in addition to PTP1B inhibition, and its clinical development was discontinued.¹³ We previously demonstrated that a series of 3-carboxyl tetrahydroisoquinoline derivatives exhibited PPAR γ agonist activities and weak PTP1B inhibitory activities.^{14–17} Carboxyl types of inhibitors appear to compete with the substrate at the catalytic site, resulting in relatively low PTP1B selectivity because the catalytic site is similar among PTPs.^{11,18} Although JTT-551 with a carboxyl moiety exhibited modest activity and anti-diabetic effects in diabetic mice, clinical development was suspended possibly due to low efficacy in patients.¹⁹ In order to achieve high PTP1B selectivity, allosteric inhibitors without a carboxyl moiety have been synthesized and exhibited modest activity and efficacy (Trodusquemine and compound IV in Fig. 1).^{11,20} Trodusquemine is a naturally-occurring spermine derivative and an allosteric PTP1B inhibitor.^{21,22} Although its parenteral administration of trodusquemine showed anti-diabetic and anti-obesity effects in mice, its clinical development was discontinued. More potent and orally bioavailable allosteric inhibitors based on a novel scaffold are strongly desired as excellent anti-diabetic and anti-obesity drugs. These inhibitors may be effective against not only diabetes and obesity, but also cancer, neuroinflammation, and retinal and renal failure.^{1,2,7–9} Reported allosteric inhibitors consist of two or more aromatic rings connected *via* acyl, ether, or alkyl linkers, and exhibit relatively weak activity.¹¹ In the present study, we synthesized a series of benzoylsulfonamide derivatives with a linker and aromatic ring. Structure–activity relationships were discussed and 4-(biphenyl-4-ylmethylsulfanylmethyl)-*N*-(hexane-1-sulfonyl)benzamide (compound 18K) was selected

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Fig. 1. Chemical Structures of PTP1B Inhibitors

for further evaluations. Compound **18K** exhibited potent and selective non-competitive inhibitory activity, high bioavailability in mice, rats, and beagles, and anti-diabetic activity in *db/db* mice.

Chemistry

General approaches to the synthesis of benzoylsulfonamide derivatives are outlined in Charts 1–3.

Chart 1 shows the general synthetic pathway for the preparation of oxime and amide linker derivatives. The starting material **1**, which was prepared as described previously,²³⁾ underwent condensation with sulfonamide **2A**²⁴⁾ to afford **3**. Compound **3** was treated with HClO_4 in the presence of various aldehydes (**4a**, **4b**, **4d**, **4f**, **4h**, **4i**, **4k**, **4l**, and **4n**)^{25–28)} to give the corresponding oxime linker derivatives **5a**, **5c**, **5d**, **5f**, **5h**, **5i**, **5k**, **5m**, and **5o**. The methyl ester of **5d** was hydrolyzed with aqueous LiOH to afford **5e**. Compounds **5f**, **5i** were converted to **5g**, **5j** by the deprotection of trifluoroacetyl group. Compound **3** was hydrolyzed by HClO_4 , followed by acylation with **9a** to prepare **10a**. Separately, compound **8** was synthesized from commercially available compound **6**²⁹⁾ by condensation with sulfonamide **2A** and deprotection of the *t*-butoxycarbonyl (Boc) group. The amino group of **8** was acylated with **9a** to give **11a**.

The synthesis of ether linker derivatives is outlined in Chart 2. Various alcohols (**12a** and **12p–t**)^{30,31)} were alkylated by methyl 4-(bromomethyl)benzoate **13** with NaH or Ag_2O , and then hydrolyzed with aqueous LiOH or NaOH to afford the carboxylic acids **14a**, and **14p–t**. Compounds **14a** and **14p–t** underwent condensation with sulfonamides **2A–D**, and **2F–I**^{32–34)} to give **15aA**, **15pA–tA**, **15rB**, **15rC**, and **15rF–rH**. The methyl ester of **15rD** was hydrolyzed with aqueous LiOH to afford **15rE**. The *tert*-butyldimethylsilyl (TBS) group of **15rI** was deprotected with tetrabutylammonium fluoride (TBAF) to

give **15rJ**.

Chart 3 shows the synthetic procedures for thioether linker derivatives. Compound **16**, which was prepared as described previously,³⁵⁾ was deacetylated and alkylated with methyl 4-(bromomethyl)benzoate **13**, followed by hydrolysis with aqueous LiOH to afford **17**. Compound **17** was converted to **18H**, **18K** and **18L** in a similar manner to the synthesis of **15aA**, **15pA–tA**, **15rB**, **15rC**, and **15rF–rH**. Compound **17** was converted to urea **23** by the Curtius rearrangement. Separately, compound **16** was hydrolyzed and alkylated with 1-bromo-4-bromomethylbenzene in the presence of sodium methoxide, and then acylated with diethyl oxalate to give **20**. Compound **20** was hydrolyzed and underwent condensation with sulfonamide **2K**³⁴⁾ to give **22**. Compound **19** was synthesized from compound **17** by the Curtius rearrangement, deprotection of the Boc group with trifluoroacetic acid (TFA), and acylation with ethyl chloroglyoxylate. Compound **19** was converted to **21** as described in the synthesis of **22**.

Results and Discussion

Since PTP1B was discovered and shown to negatively regulate insulin and leptin signals, extensive efforts have been made to identify excellent PTP1B inhibitors that are useful for the treatment of diabetes and obesity by academia and pharmaceutical industries.^{2,4)} Inhibitors with two phosphonate or carboxyl substituents exhibit potent PTP1B inhibitory activity, but have low membrane permeability, resulting in poor bioavailability.¹¹⁾ Some inhibitors with a monocarboxyl moiety exhibit modest PTP1B inhibitory activity and anti-diabetic effects in mice.¹¹⁾ Most of the inhibitors with an acid moiety are considered to compete with a substrate, pTyr at the catalytic site, and, thus, may have low PTP1B selectivity and be directly influenced by the concentrations of substrates. Non-competitive inhibitors without a carboxyl or phosphonate group have recently been reported to interact with the allosteric site of PTP1B.^{11,20)} Among them, compound IV in Fig. 1 has a structure consisting of three aromatic rings, which line up *via* sulfonamide and amide linkers, and had modest activity and *in vivo* efficacy.^{11,20)} In the present study, we employed acylsulfonamide structure, which has been used in various enzyme inhibitors,³⁶⁾ to synthesize novel compounds and to find a novel allosteric PTP1B inhibitor with good bioavailability and *in vivo* efficacy. The chemical structures, molecular weights, calculated $\log P$ ($c\log P$), calculated $\log D_{7.4}$ ($c\log D_{7.4}$), and PTP1B inhibitory activities of the compounds synthesized were shown in Tables 1–3.

We synthesized compound **5a**, the structure of which may resemble compound IV, an allosteric inhibitor (Table 1). Compound **5a** has benzoylsulfonamide instead of sulfonamide in compound IV and an oxime structure instead of amide. Compound **5a** exhibited weak PTP1B inhibitory activity. This activity was markedly decreased following the replacement of an oxime linker (**5a**) with an amide methyl linker and a hydroxamate linker (**10a**, **11a**): the electron-rich property of carbonyl or less linear structure may not be preferable for PTP1B inhibitory activity. Changes from isopropylphenyl to indoline (**5j**, **5k**) and indole (**5m**) failed to increase PTP1B inhibitory activity: benzimidazole (**5o**) markedly reduced activity. Phenolic, carboxyl, and anilinic moieties also markedly reduced this activity (**5c**, **5e**, **5g**). Therefore, a charged moiety introduced at R_1 is considered to disturb the interaction be-

Chart 1. Synthesis of Substituted Benzoylsulfonamide Derivatives with an Oxime and Amide Linker

Chart 2. Synthesis of Substituted Benzoylsulfonamide Derivatives with an Ether Linker

Reagents and conditions: (i) methyl 4-(bromomethyl)benzoate **13**, MeONa in MeOH, MeOH; (ii) aqueous LiOH, THF, MeOH; (iii) CDI, DBU, DMF; (iv) DPPA, Et₃N, toluene then **2K**, DBU; (v) 1-bromo-4-bromomethylbenzene, MeONa in MeOH, MeOH; (vi) diethyl oxalate, *n*-BuLi in *n*-hexane, THF; (vii) DPPA, Et₃N, *t*-BuOH, toluene; (viii) TFA, CH₂Cl₂; (ix) ethyl chloroglyoxylate, Et₃N, CH₂Cl₂; (x) aqueous LiOH, THF, MeOH; (xi) aqueous NaOH, THF, MeOH; (xii) **2K**, CDI, DBU, DMF.

Chart 3. Synthesis of a Benzoylsulfonamide Derivative and Other Sulfonamide Derivatives with a Thioether Linker

tween an inhibitor and enzyme. Competitive inhibitors with a pTyr mimic structure, such as compounds I¹²⁾ and II,¹¹⁾ are negatively charged and bind to the phosphate-binding loop (P-loop, residues 214–221) and WPD-loop (residues 177–185) of the active site. In these competitive inhibitors, their carboxyl or phosphonate moiety also interacts with Arg24 and Arg254 of an additional non-catalytic aryl phosphate binding site (B-site), which is located close to the catalytic site. In contrast, the activities of our synthesized benzoylsulfonamide derivatives were markedly reduced by anionic and cationic substituents, suggesting that they bind to the allosteric site, but not to the catalytic and/or active site.

We then replaced the oxime with an ether linker and changed isopropyl phenyl to another substituted phenyl (Table 1). Compound **15aA** with an ether linker exhibited similar activity to that of **5a** with an oxime linker. Compound **15pA** with 4-(4-methylpent-1-enyl)benzyl, **15rA** with 4-biphenyl, and **15tA** with 4-phenylethynylbenzyl exhibited more potent activity than **5a**. Among them, **15pA** and **15rA** showed similar activities and had smaller molecular weights than the others. However, the maximum drug concentration ($C_{\max}$) of **15rA** after its oral administration at 10 mg/kg to male Sprague-Dawley (SD) was higher than that of **15pA**; the $C_{\max}$ values of **15rA** and **15pA** were 7.0 $\mu\text{g/mL}$ (13.2 μM) and 1.1 $\mu\text{g/mL}$ (2.0 μM), respectively, while their area under the plasma concentration time curve (AUC) values were 81.7 $\mu\text{g}\cdot\text{h/mL}$ (155 $\mu\text{M}\cdot\text{h}$) and 7.4 $\mu\text{g}\cdot\text{h/mL}$ (14 $\mu\text{M}\cdot\text{h}$), respectively.

In Table 2, the dichlorophenyl moiety of **15rA** was changed to another substituted phenyl and aliphatic chain in order to

improve activity and bioavailability. Unfortunately, substituted phenyl, biphenyl, and fluorenyl moieties introduced at R_2 did not increase activity. Especially, benzoic acid markedly decreased activity, suggesting a disturbance in the inhibitor–enzyme interaction by the charged moiety at R_2 . Compound **15rH** with an octyl chain showed similar activity to that of **15rA**. Compound **15rJ** with pentanol showed lower activity than **15rA**, and this may have been due to its weaker lipophilicity or hydrophilic moiety, and **18H**, in which an ether linker of **15rH** was changed to a thioether linker, exhibited markedly higher activity and bioavailability than **15rH**. Compound **18K** with a hexyl chain showed lower activity than **18H**, but still higher activity than **15rH**. Compound **18K** was selected for further evaluations because of its smaller molecular weight and lower lipophilicity than **18H**.

In Table 3, the benzoylsulfonamide structure was changed to other sulfonamide structures. Compound **21** had higher activity than that of **18K**, but showed lower $C_{\max}$ and AUC values than those of **18K** at 10 mg/kg in male SD rat: $C_{\max}$ of 1.5 $\mu\text{g/mL}$ (2.9 μM) and AUC of 10.6 $\mu\text{g}\cdot\text{h/mL}$ (20.2 $\mu\text{M}\cdot\text{h}$) in Compound **21**, and $C_{\max}$ of 7.2 $\mu\text{g/mL}$ (14.9 μM) and AUC of 63.4 $\mu\text{g}\cdot\text{h/mL}$ (132 $\mu\text{M}\cdot\text{h}$) in Compound **18K**, respectively. Among the sulfonamide derivatives, a benzoylsulfonamide derivative was considered to be the most suitable as a potent drug-like inhibitor.

In order to evaluate the inhibitory mode of compound **18K**, a kinetic study using the synthetic substrate *p*-nitrophenylphosphate (*p*-NPP) was performed. Lineweaver–Burk plots demonstrated that **18K** inhibited PTPIB activity in a non-competitive manner with a K_i value of 0.35 μM , suggest-

Table 1. Chemical Structures, Molecular Weights, $c\log P$, $c\log D_{7.4}$, and PTP1B Inhibitory Activities of Benzoylsulfonamide Derivatives with Oxime, Amide, and Ether Linkers

Compound	R ₁	Mol.Wt. ^{a)}	$c\log P$	$c\log D_{7.4}$	PTP1B IC ₅₀ (μM) ^{b)}
5a		491.39	4.82	2.83	1.49
10a		505.41	4.35	2.35	19.9
11a		507.39	4.77	2.77	23.6
5c		465.31	3.47	1.47	8.11
5e		492.32	4.04	-0.08	>10
5g^{d)}		464.32	3.10	1.11	18.52
5h		483.75	4.55	2.56	>10
5j^{d)}		490.36	4.02	2.03	1.25
5k		518.41	4.37	2.36	1.28
5m		488.34	3.65	1.66	2.74
5o		489.33	3.21	1.21	>10
15aA^{c)}		514.40	6.03	4.03	1.89
15pA^{c)}		532.48	6.74	4.74	0.94
15qA^{c)}		546.51	7.17	5.17	1.44
15rA		526.44	6.40	4.40	0.91
15sA^{c)}		552.47	7.03	5.03	1.36
15tA^{c)}		550.45	6.81	4.80	0.75
Ertiprotafib		559.51	9.79	5.77	1.01

a) Molecular weight as the free form. b) $n=2$. c) Sodium salt. d) Hydrochloride salt.

Table 2. Chemical Structures, Molecular Weights, $c \log P$, $c \log D_{7.4}$, and PTP1B Inhibitory Activities of Benzoylsulfonamide Derivatives with Ether and Thioether Linkers

Compound	R ₂	X	Mol.Wt. ^{a)}	$c \log P$	$c \log D_{7.4}$	PTP1B IC ₅₀ (μM) ^{b)}
15rA		O	526.43	6.40	4.40	0.91
15rB		O	526.43	6.26	4.26	1.97
15rC		O	482.55	4.69	2.70	0.57
15rE		O	501.55	5.18	1.05	6.05
15rF		O	533.64	6.71	4.79	0.78
15rG		O	545.65	6.90	4.99	1.00
15rH		O	493.66	7.48	5.49	0.79
15rJ		O	467.58	3.58	1.59	14.7
18H		S	509.73	8.06	6.07	0.097
18K		S	481.67	6.76	4.77	0.25

a) Molecular weight as the free form. b) $n=2$.

ing that it bound to the allosteric site, but not to the catalytic site. Several X-ray crystal structure analyses and computational simulations recently revealed the binding sites of allosteric inhibitors and the inhibitory mechanisms in which a small molecule binds to allosteric sites and stabilizes the conformation associated with the inactive state of PTP1B.^{37–44)} Based on the molecular similarities between our compound **18K** and an allosteric inhibitor bound to PTP1B (Fig. 2a), we constructed a structural model of the **18K**–PTP1B complex by molecular alignment of the compounds, followed by validation and refinement of the structure obtained using a molecular dynamic (MD) simulation. Details are shown in Experimental. Our molecular overlay program, SUPERPOSE,^{45,46)} provided almost two patterns for the alignment between **18K** and the allosteric inhibitor. The top-ranked conformations for each pattern were depicted in Fig. 2a. Of the two conformations, conformation 2 was subsequently rejected because **18K** was immediately released from PTP1B during the MD simulation. The representative structure of the **18K**–PTP1B complex derived from the MD simulation was depicted in Fig. 2b. In this structure, **18K** formed a U-shaped conformation and bound to the allosteric site of PTP1B with the formation of a hydrogen bond and salt bridge around the benzoylsulfonamide structure.

Table 3. Chemical Structures, Molecular Weights, $c \log P$, $c \log D_{7.4}$, and PTP1B Inhibitory Activities of a Benzoylsulfonamide Derivative and Other Sulfonamide Derivatives

Compound	R ₃	Mol.Wt. ^{a)}	$c \log P$	$c \log D_{7.4}$	PTP1B IC ₅₀ (μM) ^{b)}
18K		481.67	6.76	4.77	0.25
18L		496.69	6.48	4.50	0.38
21		524.70	6.08	4.08	0.16
22		509.68	6.36	4.36	0.26
23		496.69	6.68	4.71	1.47

a) Molecular weight as the free form. b) $n=2$.

Fig. 2. Structural Models of **18K**-PTP1B and **18K**-TCPTP Complexes

(a) SUPERPOSE alignments between the allosteric inhibitor (PDB: 1T4J; upper panel) and **18K** (lower panels). The functional atoms corresponding to aromatic rings (ARs), hydrophobic atoms (HPs), hydrogen-bonding donors (HDs), and hydrogen-bonding acceptors (HAs) are colored yellow, gray, blue, and red, respectively. (b) Representative structure of the **18K**-PTP1B complex derived from the MD simulation. **18K** (green) and PTP1B residues within 4 Å of **18K** (gray) are shown as balls and sticks, with the Connolly surface of PTP1B transparently depicted. Hydrogen bonds, salt bridges, and aromatic interactions are shown as dotted lines, and colored cyan, magenta, and orange, respectively. (c) Structural alignment between PTP1B and TCPTP. PTP1B (PDB: 1T4J) and TCPTP (PDB: 1L8K) are colored gray and magenta, respectively, and the allosteric inhibitor bound to PTP1B is colored green. The amino acids at the allosteric site are highly conserved, except for Phe280 of PTP1B. (d) Representative structure of the **18K**-TCPTP complex derived from the MD simulation. **18K** (green) and TCPTP residues within 4 Å of **18K** (gray) are shown as balls and sticks, with the Connolly surface of TCPTP transparently depicted. Hydrogen bonds, salt bridges, and aromatic interactions are shown as dotted lines, and colored cyan, magenta, and orange, respectively.

Table 4. Effects of the Repeated Administration of **18K** at 30mg/kg/d for One Week in Male *db/db* Mice

Compound	Glucose (mg/dL)	TG (mg/dL)	HOMA value
Control	280.0 $\pm$ 38.9	151.7 $\pm$ 8.9	2613.7 $\pm$ 685.3
18K	182.8 $\pm$ 7.2*	115.8 $\pm$ 4.8**	894.1 $\pm$ 117.2*

Mean $\pm$ S.E. (*n*=6). **p*<0.05, ***p*<0.01 vs. Control, the Student's *t*-test.

In addition, the U-shaped conformation of **18K** appeared to be preferable for the formation of aromatic and van der Waals interactions around Phe196 and Phe280 of PTP1B.

Our structural model also explained the difference in the compound's selectivity for PTP1B and T-cell protein tyrosine phosphatase (TCPTP), which is the most homologous phosphatase (sequence similarity of nearly 70%) to PTP1B (Fig. 2c). The structural model of the **18K**-TCPTP complex based on that of the **18K**-PTP1B complex was depicted in Fig. 2d. Compound **18K** may not interact as strongly with TCPTP because Phe280 of PTP1B was replaced with Cys278 in TCPTP, causing the disappearance of aromatic and van der Waals interactions with **18K**. It is important to note that the hexyl chain of **18K** was less ordered during the MD simulation in the **18K**-TCPTP complex, and this may have been due to the

loss of interactions with Phe280. The IC₅₀ values of compound **18K** were 0.25 μM for PTP1B, 1.24 μM for TCPTP, >100 μM for CD45, and >100 μM for leukocyte common antigen-related protein tyrosine phosphatase (LAR).

The *C*_{max} value of **18K** reached 21.9 μg/mL (45.5 μM) in male ICR mice at 30mg/kg, 25.8 μg/mL (53.6 μM) in male SD rats at 30mg/kg, and 18.2 μg/mL (37.8 μM) in male beagles at 10mg/kg, all of which exceeded its IC₅₀ value for PTP1B inhibition. Furthermore, compound **18K** lowered plasma glucose, triglyceride (TG), and homeostasis model assessment (HOMA) levels at 30mg/kg/d (*per os* (*p.o.*)) for one week in *db/db* mice (Table 4).

In conclusion, a series of benzoylsulfonamide derivatives were synthesized and a substituted benzoylsulfonamide structure was shown to be a novel scaffold for a non-competitive allosteric PTP1B inhibitor. Among the derivatives synthesized, compound **18K** is a potential candidate for anti-diabetic drug as well as a useful tool for investigations of the physiological and pathophysiological effects of allosteric PTP1B inhibition.

Experimental

General Melting points were measured on a melting point apparatus (Yamato MP-21; Yamato Scientific Co., Ltd., Tokyo, Japan) and were uncorrected. ¹H-NMR spectra were obtained

on a nuclear magnetic resonance spectrometer at 400 MHz (JNM-AL400; JEOL Ltd., Tokyo, Japan) using tetramethylsilane (TMS) as an internal standard. IR spectra were recorded with an infrared spectrometer (FT-IR8200PC; Shimadzu Corporation, Kyoto, Japan). Mass spectra were obtained on a QTRAP LC/MS/MS system (API2000; Thermo Fisher Scientific Inc., Foster, U.S.A.). Column chromatography was performed on silica gel (Daisogel No.1001W; Daiso Co., Ltd., Osaka, Japan). Reactions were monitored by TLC (TLC silica gel 60F254, Merck KGaA, Darmstadt, Germany).

(E)-N-(2,4-Dichlorobenzenesulfonyl)-4-(1-ethoxyethylideneaminoxy)benzamide (3) 1,1'-Carbonylbis-1*H*-imidazole (CDI) (3.38 g, 19.7 mmol) was added to a solution of **1**²³ (4.00 g, 17.9 mmol) in *N,N*-dimethylformamide (DMF) (40 mL), and the mixture was stirred at room temperature for 1 h. 2,4-Dichlorobenzenesulfonamide **2A**²⁴ (4.46 g, 19.7 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (2.95 mL, 19.7 mmol) were added to the reaction mixture, and stirred at room temperature for 5.5 h. After the addition of 10% aqueous citric acid solution, the reaction mixture was extracted with AcOEt and the organic layer was washed with saturated brine and then dried over Na₂SO₄. The solvent was removed under reduced pressure, and the residue obtained was recrystallized from AcOEt-*n*-hexane to give **3** (6.51 g, 84% yield) as a solid. ¹H-NMR (CDCl₃) δ: 1.36 (3H, t, *J*=7.1 Hz), 2.11 (3H, s), 4.19 (2H, q, *J*=7.1 Hz), 7.15–7.20 (2H, m), 7.45–7.52 (2H, m), 7.72–7.79 (2H, m), 8.30 (1H, d, *J*=8.5 Hz).

Compound **4i** was prepared from 5-(*tert*-butyldimethylsilyloxy)methylindole²⁶ via compounds **24**, **25**, and **26** as follows:

5-(tert-Butyldimethylsilyloxy)methyl-2,3-dihydro-1*H*-indole (24) NaBH₃CN (5.34 g, 84.9 mmol) was added to a solution of 5-(*tert*-butyldimethylsilyloxy)methylindole²⁶ (4.44 g, 17.0 mmol) in AcOH (31.1 mL) under ice-cooling, and the mixture was stirred at room temperature for 2.5 h. The reaction mixture was diluted with water, neutralized with K₂CO₃, and extracted with AcOEt. The organic layer was washed with water and saturated brine and then dried over Na₂SO₄. The solvent was removed under reduced pressure. The residue obtained was purified by silica gel column chromatography to give **24** (1.58 g, 35% yield) as an oil. ¹H-NMR (CDCl₃) δ: 0.08 (6H, s), 0.93 (9H, s), 3.01 (2H, t, *J*=8.3 Hz), 3.54 (2H, t, *J*=8.3 Hz), 4.61 (2H, s), 6.59 (1H, d, *J*=7.8 Hz), 6.95 (1H, d, *J*=7.8 Hz), 7.06–7.11 (1H, m).

1-[5-(tert-Butyldimethylsilyloxy)methyl]-2,2,2-trifluoroacetyl-2,3-dihydro-1*H*-indole (25) Triethylamine (Et₃N) (7.09 mL, 7.80 mmol) and trifluoroacetic anhydride (TFAA) (0.92 mL, 6.6 mmol) were added to a solution of **24** (1.58 g, 6.00 mmol) in CH₂Cl₂ (16 mL), and the mixture was stirred at room temperature for 30 min. The reaction mixture was diluted with water and neutralized with K₂CO₃, which was extracted twice with AcOEt. The organic layer was washed with saturated brine, and dried over Na₂SO₄. The solvent was removed under reduced pressure. The residue obtained was purified by silica gel column chromatography to give **25** (1.94 g, 90% yield) as an oil. ¹H-NMR (CDCl₃) δ: 0.10 (6H, s), 0.94 (9H, s), 3.25 (2H, t, *J*=8.1 Hz), 4.28 (2H, t, *J*=8.1 Hz), 4.71 (2H, s), 7.19 (1H, d, *J*=8.3 Hz), 7.25 (1H, s), 8.14 (1H, d, *J*=8.3 Hz).

1-(2,2,2-Trifluoroacetyl)-5-hydroxymethyl-2,3-dihydro-1*H*-indole (26) One molar TBAF in tetrahydro-

furan (THF) (5.8 mL, 5.8 mmol) was added to a solution of **25** (1.75 g, 4.87 mmol) in THF (35 mL), and the mixture was stirred at room temperature for 75 min. After the addition of water, the mixture was extracted twice with AcOEt, and the organic layer was washed with saturated brine and then dried over Na₂SO₄. The solvent was removed under reduced pressure, and the residue obtained was purified by silica gel column chromatography to give **26** (670 mg, 56% yield) as an oil. ¹H-NMR (CDCl₃) δ: 1.65–1.78 (1H, br), 3.26 (2H, t, *J*=8.0 Hz), 4.30 (2H, t, *J*=8.0 Hz), 4.65–4.72 (2H, m), 7.20–7.32 (2H, m), 8.17 (1H, d, *J*=8.3 Hz).

1-(2,2,2-Trifluoroacetyl)-2,3-dihydro-1*H*-indole-5-carbaldehyde (4i) Dimethyl sulfoxide (DMSO) (0.39 mL, 5.5 mmol), Et₃N (0.76 mL, 5.5 mmol), and SO₃·pyridine (869 mg, 5.46 mmol) were added to a stirred solution of **26** (670 mg, 2.73 mmol) in CH₂Cl₂ (6.7 mL), followed by stirring at room temperature for 2 h. After the addition of 10% aqueous citric acid solution, the mixture was extracted twice with AcOEt, washed with water and saturated brine, and then dried over Na₂SO₄. The solvent was removed under reduced pressure. The residue obtained was purified by silica gel column chromatography to give **4i** (340 mg, 51% yield) as a solid. ¹H-NMR (CDCl₃) δ: 3.34 (2H, t, *J*=8.3 Hz), 4.38 (2H, t, *J*=8.3 Hz), 7.77–7.84 (2H, m), 8.35 (1H, d, *J*=8.3 Hz), 9.95 (1H, s).

Compound **4k** was prepared from 2,3-dihydro-1*H*-indole via compound **27** as follows:

1-Ethyl-2,3-dihydro-1*H*-indole (27) EtI (2.42 mL, 30.2 mmol) and *i*-Pr₂NEt (5.29 mL, 37.8 mmol) were added to a solution of 2,3-dihydro-1*H*-indole (3.00 g, 25.2 mmol) in DMF (2.4 mL), which was stirred at 80°C for 14.5 h. AcOEt was added to the reaction mixture, which was washed with water and saturated brine, and then dried over Na₂SO₄. The solvent was removed under reduced pressure. The residue obtained was purified by silica gel column chromatography to give **27** (3.32 g, 90% yield) as an oil. ¹H-NMR (CDCl₃) δ: 1.20 (3H, t, *J*=7.4 Hz), 2.94 (2H, t, *J*=8.0 Hz), 3.15 (2H, q, *J*=7.4 Hz), 3.32 (2H, t, *J*=8.0 Hz), 6.45–6.52 (1H, m), 6.60–6.68 (1H, m), 7.00–7.09 (2H, m).

1-Ethyl-2,3-dihydro-1*H*-indole-5-carbaldehyde (4k) Vilsmeier reagent was prepared by adding POCl₃ (6.29 mL, 67.4 mmol) dropwise to DMF (8.5 mL, 112 mmol) under ice-cooling, and the mixture was stirred at the same temperature for 15 min. Vilsmeier reagent was added to a solution of **27** (3.31 g, 22.5 mmol) in DMF (112 mL), followed by stirring at 40°C for 45 min. The reaction mixture was poured into cold water and basified with K₂CO₃, which was extracted twice with AcOEt. The organic layer was dried over Na₂SO₄ and then evaporated under reduced pressure. The residue was purified by silica gel column chromatography to give **4k** (2.32 g, 59% yield) as an oil. ¹H-NMR (CDCl₃) δ: 1.21 (3H, t, *J*=7.3 Hz), 3.03 (2H, t, *J*=8.6 Hz), 3.28 (2H, q, *J*=7.3 Hz), 3.59 (2H, t, *J*=8.6 Hz), 6.34–6.40 (1H, m), 7.50–7.57 (2H, m), 9.64 (1H, s).

(E)-N-(2,4-Dichlorobenzenesulfonyl)-4-(4-isopropylbenzylideneaminoxy)benzamide (5a) Compound **4a** (172 mg, 1.16 mmol) and 60% aqueous HClO₄ solution (0.19 mL, 1.7 mmol) were added to a solution of **3** (500 mg, 1.16 mmol) in 1,4-dioxane (17 mL) under ice-cooling, and the mixture was stirred at room temperature for 14 h. AcOEt was added to the reaction mixture, which was washed with water and

saturated brine, and then dried over Na_2SO_4 . The solvent was removed under reduced pressure. After the addition of Et_2O (10 mL) and *n*-hexane (20 mL), the mixture was stirred at room temperature for 3 h, and the precipitate was then collected by filtration to give **5a** (502 mg, 88% yield) as a white solid, mp 90–91°C. $^1\text{H-NMR}$ (CDCl_3) δ : 1.27 (6H, d, $J=6.8\text{ Hz}$), 2.90–3.03 (1H, m), 7.30 (2H, d, $J=8.0\text{ Hz}$), 7.27–7.35 (2H, m), 7.49 (1H, dd, $J=8.6, 2.0\text{ Hz}$), 7.52 (1H, d, $J=2.0\text{ Hz}$), 7.64 (2H, d, $J=8.0\text{ Hz}$), 7.78–7.86 (2H, m), 8.32 (1H, d, $J=8.6\text{ Hz}$), 8.40 (1H, s), 8.99–9.42 (1H, br). IR (attenuated total reflectance (ATR)) cm^{-1} : 1678. MS m/z : 513 $[\text{M}+\text{Na}]^+$.

(E)-N-(2,4-Dichlorobenzenesulfonyl)-4-(4-hydroxybenzylideneaminoxy)benzamide (5c) Sixty percent aqueous HClO_4 solution (5.8 mL, 53 mmol) was added to a solution of **3** (500 mg, 1.16 mmol) and 4-acetoxylbenzaldehyde **4b** (190 mg, 1.16 mmol) in 1,4-dioxane (10 mL), and the mixture was stirred at room temperature for 14 h. After the addition of 5.0 M aqueous NaOH solution (1.2 mL, 6.0 mmol), the reaction mixture was stirred at room temperature for 2 h. The mixture was acidified by 5% citric acid solution and extracted twice with AcOEt. The organic layer was washed with saturated brine, and then dried over Na_2SO_4 . The solvent was removed under reduced pressure, and the residue obtained was purified by silica gel column chromatography to give **5c** (296 mg, 55% yield) as a pale brown solid, mp 154–157°C. $^1\text{H-NMR}$ ($\text{DMSO}-d_6$) δ : 6.84–6.88 (2H, m), 7.29–7.32 (2H, m), 7.63–7.66 (2H, m), 7.72 (1H, dd, $J=8.8, 2.2\text{ Hz}$), 7.89 (1H, d, $J=2.0\text{ Hz}$), 7.94–7.97 (2H, m), 8.16 (1H, d, $J=8.6\text{ Hz}$), 8.63 (1H, s), 10.11 (1H, s), 12.60–13.20 (1H, br). IR (ATR) cm^{-1} : 1708. MS m/z : 487 $[\text{M}+\text{Na}]^+$.

Methyl (E)-4-([4-(2,4-Dichlorobenzenesulfonylaminocarbonyl)phenoxyimino]methyl)benzoate (5d) Compound **5d** was synthesized from **3** and **4d** according to the procedure for the synthesis of **5a**. Yield 93%. $^1\text{H-NMR}$ (CDCl_3) δ : 3.95 (3H, s), 7.30–7.37 (2H, m), 7.46–7.54 (2H, m), 7.75–7.85 (4H, m), 8.07–8.14 (2H, m), 8.32 (1H, d, $J=8.6\text{ Hz}$), 8.47 (1H, s), 8.90–9.30 (1H, br).

(E)-4-([4-(2,4-Dichlorobenzenesulfonylaminocarbonyl)phenoxyimino]methyl)benzoic Acid (5e) One molar aqueous LiOH solution (3.3 mL, 3.3 mmol) was added to a solution of **5d** (550 mg, 1.08 mmol) in MeOH (3.0 mL) and THF (9.0 mL), and the mixture was stirred at room temperature for 14 h. The reaction mixture was neutralized with 2.0 M aqueous HCl solution and extracted twice with AcOEt. The organic layer was washed with water and saturated brine, and then dried over Na_2SO_4 . The solvent was removed under reduced pressure. After the addition of Et_2O , the precipitate was collected by filtration to give **5e** (440 mg, 89% yield) as a white solid, mp 208–211°C. $^1\text{H-NMR}$ ($\text{DMSO}-d_6$) δ : 7.35–7.40 (2H, m), 7.72 (1H, dd, $J=8.6, 1.7\text{ Hz}$), 7.88 (1H, d, $J=1.7\text{ Hz}$), 7.92–8.02 (4H, m), 8.04 (1H, d, $J=8.6\text{ Hz}$), 8.15–8.19 (2H, m), 8.87 (1H, s), 12.70–13.50 (2H, br). IR (ATR) cm^{-1} : 1687. MS m/z : 491 $[\text{M}-\text{H}]^-$.

(E)-N-(2,4-Dichlorobenzenesulfonyl)-4-[4-(N'-2,2,2-trifluoroacetyl amino)benzylideneaminoxy]benzamide (5f) Compound **5f** was synthesized from **3** and **4f**²⁵⁾ according to the procedure for the synthesis of **5a**. Yield quant. $^1\text{H-NMR}$ (CDCl_3) δ : 7.30–7.35 (2H, m), 7.49 (1H, dd, $J=8.6, 2.0\text{ Hz}$), 7.52 (1H, d, $J=2.0\text{ Hz}$), 7.67–7.73 (2H, m), 7.74–7.84 (4H, m), 8.07–8.17 (1H, br), 8.31 (1H, d, $J=8.6\text{ Hz}$), 8.42 (1H, s), 8.95–9.12 (1H, br).

(E)-N-[4-(4-Aminobenzylideneaminoxy)benzoyl]-2,4-dichlorobenzamide Hydrochloride Salt (5g) One molar aqueous LiOH solution (6.8 mL, 6.8 mmol) was added to a solution of **5f** (1.27 g, 2.27 mmol) in 1,4-dioxane (13 mL), and the mixture was stirred at room temperature for 2.5 h and at 40°C for 25 min. After the addition of water, the mixture was extracted three times with AcOEt. The organic layer was washed with saturated brine, and then dried over Na_2SO_4 . The solvent was removed under reduced pressure.

A total of 8.6 M HCl in *i*-PrOH (0.40 mL, 3.4 mmol) and Et_2O (20 mL) were added to a solution of the residue obtained in MeOH (2 mL) under ice-cooling, and the reaction mixture was stirred at the same temperature for 1 h. After the addition of MeOH (8 mL) and Et_2O (70 mL), the mixture was further stirred under ice-cooling for 1 h. The precipitate was collected by filtration to give **5g** (435 mg, 38% yield) as a brown solid, mp 145–150°C. $^1\text{H-NMR}$ ($\text{DMSO}-d_6$) δ : 4.35–6.01 (3H, br), 7.05–7.13 (2H, m), 7.29–7.37 (1H, m), 7.65–7.80 (3H, m), 7.87–7.93 (1H, m), 7.97 (2H, m), 8.17 (1H, d, $J=8.3\text{ Hz}$), 8.66 (1H, s). IR (ATR) cm^{-1} : 1676. MS m/z : 464 $[\text{M}+\text{H}]^+$.

(E)-N-(2,4-Dichlorobenzenesulfonyl)-4-(4-chlorobenzylideneaminoxy)benzamide (5h) Compound **5h** was synthesized from **3** and **4h** according to the procedure for the synthesis of **5a**. Yield 71%. A white solid, mp 177–179°C. $^1\text{H-NMR}$ (CDCl_3) δ : 7.28–7.37 (2H, m), 7.38–7.48 (2H, m), 7.49 (1H, dd, $J=8.5, 2.0\text{ Hz}$), 7.52 (1H, d, $J=2.0\text{ Hz}$), 7.61–7.72 (2H, m), 7.76–7.87 (2H, m), 8.32 (1H, d, $J=8.5\text{ Hz}$), 8.40 (1H, s), 8.80–9.18 (1H, br). IR (ATR) cm^{-1} : 1699. MS m/z : 505 $[\text{M}+\text{Na}]^+$.

(E)-N-(2,4-Dichlorobenzenesulfonyl)-4-[1-(2,2,2-trifluoroacetyl)-2,3-dihydro-1H-indole-5-yl]methyleaminoxy]benzamide (5i) Compound **5i** was synthesized from **3** and **4i** according to the procedure for the synthesis of **5a**. Yield 78%. $^1\text{H-NMR}$ (CDCl_3) δ : 3.33 (2H, t, $J=8.3\text{ Hz}$), 4.36 (2H, t, $J=8.3\text{ Hz}$), 7.29–7.36 (2H, m), 7.45–7.59 (3H, m), 7.72 (1H, s), 7.76–7.84 (2H, m), 8.27 (1H, d, $J=8.3\text{ Hz}$), 8.33 (1H, d, $J=8.5\text{ Hz}$), 8.41 (1H, s), 8.81–9.08 (1H, br).

(E)-N-(2,4-Dichlorobenzenesulfonyl)-4-(indolin-5-yl) methyleaminoxy]benzamide Hydrochloride Salt (5j) One molar aqueous LiOH solution (1.4 mL, 1.4 mmol) was added to a solution of **5i** (540 mg, 0.899 mmol) in 1,4-dioxane (5.4 mL), which was stirred at room temperature for 45 min. One molar aqueous LiOH solution (1.4 mL, 1.4 mmol) was then added to the reaction mixture, which was further stirred at the same temperature for 50 min. After water was added, the mixture was extracted twice with AcOEt. The organic layer was washed with saturated brine and dried over Na_2SO_4 . The solvent was removed under reduced pressure, and the residue was purified by column chromatography to give the free form of **5j**.

A total of 8.6 M HCl in *i*-PrOH (0.07 mL, 0.6 mmol) was added to a solution of the residue obtained in CHCl_3 (10 mL) and MeOH (3 mL) under ice-cooling, and the mixture was stirred at the same temperature for 10 min. After the addition of Et_2O (40 mL), the mixture was stirred under ice-cooling for 1 h. The precipitate was collected by filtration to give **5j** (14 mg, 14% yield) as a brown solid, mp 145–150°C. $^1\text{H-NMR}$ ($\text{DMSO}-d_6$) δ : 3.09 (2H, t, $J=8.3\text{ Hz}$), 3.62 (2H, t, $J=8.3\text{ Hz}$), 3.95–5.40 (3H, m), 6.92 (1H, d, $J=7.8\text{ Hz}$), 7.29–7.35 (2H, m), 7.51 (1H, d, $J=7.8\text{ Hz}$), 7.67 (1H, s), 7.73 (1H, dd, $J=8.5\text{ Hz}$, 1.7 Hz), 7.90 (1H, d, $J=1.7\text{ Hz}$), 7.93–7.99 (2H, m), 8.17 (1H,

d, $J=8.5$ Hz), 8.64 (1H, s). IR (ATR) cm^{-1} : 1687. MS m/z : 490 $[\text{M}-\text{H}]^-$.

(E)-N-(2,4-Dichlorobenzenesulfonyl)-4-(1-ethyl-2,3-dihydro-1H-indol-5-ylmethyleaminooxy)benzamide (5k) Compound **5k** was synthesized from **3** and **4k** according to the procedure for the synthesis of **5a**. Yield 65%. A white solid, mp 135–137°C. $^1\text{H-NMR}$ ($\text{DMSO}-d_6$) δ : 1.19 (3H, t, $J=7.3$ Hz), 3.02 (2H, t, $J=8.3$ Hz), 3.22 (2H, q, $J=7.3$ Hz), 3.49 (2H, t, $J=8.3$ Hz), 6.38 (1H, d, $J=8.1$ Hz), 7.25–7.30 (3H, m), 7.45–7.53 (3H, m), 7.75–7.80 (2H, m), 8.25–8.33 (2H, m), 8.95–9.17 (1H, br). IR (ATR) cm^{-1} : 1693. MS m/z : 518 $[\text{M}+\text{H}]^+$.

(E)-N-(2,4-Dichlorobenzenesulfonyl)-4-(1H-indol-5-ylmethyleaminooxy)benzamide (5m) Compound **5m** was synthesized from **3** and **4l**²⁷⁾ according to the procedure for the synthesis of **5a**. Yield 68%. A white solid, mp 172–173°C. $^1\text{H-NMR}$ ($\text{DMSO}-d_6$) δ : 6.52–6.57 (1H, m), 7.32–7.38 (2H, m), 7.41–7.47 (1H, m), 7.49 (1H, d, $J=8.6$ Hz), 7.63 (1H, dd, $J=8.6$, 1.4 Hz), 7.73 (1H, dd, $J=8.8$, 2.2 Hz), 7.90 (1H, d, $J=2.2$ Hz), 7.95–8.01 (3H, m), 8.17 (1H, d, $J=8.8$ Hz), 8.79 (1H, s), 11.39–11.47 (1H, br), 12.65–13.25 (1H, br). IR (ATR) cm^{-1} : 1693. MS m/z : 510 $[\text{M}+\text{Na}]^+$.

(E)-N-(2,4-Dichlorobenzenesulfonyl)-4-(benzoimidazol-5-ylmethyleaminooxy)benzamide (5o) Compound **5o** was synthesized from **3** and **4n**²⁸⁾ according to the procedure for the synthesis of **5a**. Yield 56%. A pale brown solid, mp 150–155°C. $^1\text{H-NMR}$ ($\text{DMSO}-d_6$) δ : 7.31–7.38 (2H, m), 7.67–7.74 (2H, m), 7.75–7.82 (1H, m), 7.85 (1H, d, $J=2.0$ Hz), 7.94–8.00 (2H, m), 8.05 (1H, s), 8.15 (1H, d, $J=8.6$ Hz), 8.49–8.53 (1H, s), 8.89 (1H, s). IR (ATR) cm^{-1} : 1598. MS m/z : 489 $[\text{M}+\text{H}]^+$.

N-(4-Aminooxybenzoyl)-2,4-dichlorobenzenesulfonamide (7) Sixty percent aqueous HClO_4 solution (0.39 mL, 3.6 mmol) was added to a solution of **3** (1.00 g, 2.32 mmol) in 1,4-dioxane (10 mL), which was stirred at room temperature for 14 h. After the addition of 5.0 M aqueous NaOH solution (0.46 mL, 2.3 mmol), the reaction mixture was extracted twice with AcOEt. The organic layer was washed with water and saturated brine, and then dried over Na_2SO_4 . The solvent was removed under reduced pressure. After the addition of *i*-Pr₂O, the precipitate that formed was collected by filtration to give **7** (890 mg, quant.) as a solid. $^1\text{H-NMR}$ (CDCl_3) δ : 4.05–4.14 (2H, m), 7.50–7.59 (2H, m), 7.69 (1H, dd, $J=8.5$, 2.2 Hz), 7.84 (1H, d, $J=2.2$ Hz), 7.90–7.94 (2H, m), 8.13 (1H, d, $J=8.5$ Hz), 8.20–8.40 (3H, br).

N-(2,4-Dichlorobenzenesulfonyl)-4-aminomethylbenzamide Hydrochloride Salt (8) CDI (948 mg, 5.84 mmol) was added to a solution of **6**²⁹⁾ (1.22 g, 4.87 mmol) in DMF (10 mL), and the mixture was stirred at room temperature for 1 h. After the addition of 2,4-dichlorobenzenesulfonamide **2A** (1.10 g, 4.87 mmol) and DBU (0.87 mL, 5.8 mmol), the reaction mixture was stirred at room temperature for 16 h. The reaction mixture was acidified with 5% aqueous citric acid solution, and extracted twice with AcOEt. The organic layer was washed with water and saturated brine, and then dried over Na_2SO_4 . The solvent was removed under reduced pressure.

The residue obtained was dissolved in HCO_2H (5.0 mL), to which 8.6 M HCl in *i*-PrOH (1.3 mL, 11 mmol) was added under ice-cooling, and this was followed by stirring at the same temperature for 10 min. The precipitate was collected by filtration to give **8** (880 mg, quant.) as a solid. $^1\text{H-NMR}$ ($\text{DMSO}-d_6$) δ : 4.05–4.12 (2H, m), 7.51–7.58 (2H, m), 7.64–7.73

(1H, m), 7.81–7.86 (1H, m), 7.89–7.95 (2H, m), 8.12–8.15 (1H, m), 8.20–8.40 (3H, m).

N-[4-(2,4-Dichlorobenzenesulfonylamino-carbonyl)-phenoxy]-4-isopropylbenzamide (10a) Compound **9a** (175 mg, 1.07 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) (241 mg, 1.26 mmol) were added to a solution of **7** in CH_2Cl_2 (2.5 mL), and the mixture was stirred at room temperature for 14 h. After the addition of 5% aqueous citric acid solution, the reaction mixture was extracted twice with AcOEt, washed with water and saturated brine, and then dried over Na_2SO_4 . The solvent was removed under reduced pressure. The residue obtained was dissolved in AcOEt and *n*-hexane was added to the solution. The precipitate was collected by filtration to give **10a** (182 mg, 37%) as a white solid, mp 208–210°C. $^1\text{H-NMR}$ ($\text{DMSO}-d_6$) δ : 1.21 (6H, d, $J=6.8$ Hz), 2.94 (1H, quintet, $J=7.1$ Hz), 4.51 (2H, d, $J=6.1$ Hz), 7.32–7.35 (2H, m), 7.37–7.42 (2H, m), 7.71 (1H, dd, $J=8.8$, 2.2 Hz), 7.79–7.83 (2H, m), 7.83–7.88 (3H, m), 8.15 (1H, d, $J=8.8$ Hz), 9.02 (1H, t, $J=6.1$ Hz). IR (ATR) cm^{-1} : 1697. MS m/z : 529 $[\text{M}+\text{Na}]^+$.

N-[4-(2,4-Dichlorobenzenesulfonylamino-carbonyl)-benzyl]-4-isopropylbenzamide (11a) (COCl_2) (80 μL , 0.93 mmol) and 1 drop of DMF were added to a solution of **9a** (151 mg, 0.917 mmol) in CH_2Cl_2 (5.0 mL), and the mixture was stirred at room temperature for 15 min. After the addition of **8** (330 mg, 0.834 mmol) and *i*-Pr₂NEt (0.71 mL, 4.2 mmol), the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was acidified with 5% aqueous citric acid solution, which was extracted twice with AcOEt. The organic layer was washed with water and saturated brine, and then dried over Na_2SO_4 . The solvent was removed under reduced pressure. The residue obtained was dissolved in AcOEt, to which *n*-hexane was added, and the precipitate was collected by filtration to give **11a** (240 mg, 57%) as a white solid, mp 225–227°C. $^1\text{H-NMR}$ ($\text{DMSO}-d_6$) δ : 1.23 (6H, d, $J=7.1$ Hz), 2.97 (1H, quintet, $J=7.1$ Hz), 7.15–7.22 (2H, m), 7.36–7.44 (2H, m), 7.72 (1H, dd, $J=8.6$, 2.0 Hz), 7.78–7.85 (2H, m), 7.89 (1H, d, $J=2.0$ Hz), 7.90–7.96 (2H, m), 8.16 (1H, d, $J=8.6$ Hz), 12.57 (1H, s), 12.70–13.20 (1H, br). IR (ATR) cm^{-1} : 1658. MS m/z : 527 $[\text{M}+\text{Na}]^+$.

4-(4-Isopropylbenzyloxymethyl)benzoic Acid (14a) A 60% suspension of NaH in mineral oil (800 mg, 20 mmol) was added portionwise to a stirred solution of **12a** (3.00 g, 20.0 mmol) in THF (20 mL), which was stirred at 50°C for 30 min. After the addition of **13** (2.29 g, 10.0 mmol) in THF (10 mL), the reaction mixture was stirred at room temperature for 1.5 h, and 5.0 M aqueous NaOH solution (10 mL, 50 mmol) and MeOH (10 mL) were then added to the mixture, which was stirred at 50°C for 30 min. After cooling, water and Et₂O were added to the reaction mixture, the aqueous layer was then acidified with concentrated hydrochloric acid, followed by extraction with AcOEt. The organic layer was washed with water and saturated brine, and then dried over Na_2SO_4 . The solvent was removed under reduced pressure. The residue obtained was purified by silica gel column chromatography to give **14a** (1.19 g, 42% yield for 2 steps) as a solid. $^1\text{H-NMR}$ (CDCl_3) δ : 1.25 (6H, d, $J=6.8$ Hz), 2.91 (1H, septet, $J=6.8$ Hz), 4.56 (2H, s), 4.62 (2H, s), 7.21–7.32 (4H, m), 7.45–7.52 (2H, m), 8.07–8.13 (2H, m).

Compound **14p** was prepared from ethyl 4-iodobenzoate *via* compound **28** as follows:

Ethyl 4-(4-Methylpent-1-enyl)benzoate (28) A mixture of ethyl 4-iodobenzoate (7.35 g, 26.6 mmol), 4-methyl-1-pentene (3.36 g, 39.9 mmol), Pd(OAc)₂ (119 mg, 0.532 mmol), tri(*o*-tolyl)phosphine (405 mg, 1.33 mmol), and Et₃N (13.0 mL, 93.3 mmol) in toluene (43 mL) was stirred at 80°C for 16 h under a nitrogen atmosphere. The solvent was removed under reduced pressure. The residue obtained was purified by silica gel column chromatography to give **28** (6.69 g, quant.) as an oil. ¹H-NMR (CDCl₃) δ: 0.95 (6H, d, *J*=6.6 Hz), 1.26 (3H, t, *J*=7.1 Hz), 1.69–1.82 (1H, m), 2.12 (2H, t, *J*=7.1 Hz), 4.12 (2H, q, *J*=7.1 Hz), 6.35–6.43 (2H, m), 7.36–7.41 (2H, m), 7.92–7.98 (2H, m).

[4-(4-Methylpent-1-enyl)benzyloxymethyl]benzoic Acid (14p) Compound **28** (6.69 g, 28.8 mmol) in THF (20 mL) was added dropwise to the suspension of lithium aluminium hydride (LiAlH₄) (1.31 g, 34.6 mmol) in THF (134 mL), which was stirred at room temperature for 15 min. After the addition of water under ice-cooling, the precipitate was removed by filtration and washed with AcOEt. The filtrate was dried over Na₂SO₄ and then evaporated under reduced pressure to give **12p** (5.61 g, crude).

A 60% suspension of NaH in mineral oil (1.91 g, 48 mmol) was added portionwise to the crude product (3.64 g) and compound **13** (2.19 g, 9.55 mmol) in THF (44 mL) under ice-cooling, and the mixture was stirred at room temperature for 18.5 h. After the addition of 2.0 M aqueous NaOH solution (23.9 mL, 48 mmol), the reaction mixture was stirred at 50°C for 2.5 h, and water and Et₂O were then added. The aqueous layer was acidified with 10% aqueous citric acid solution, followed by extraction with AcOEt. The organic layer was washed with water and saturated brine, and then dried over Na₂SO₄. The solvent was removed under reduced pressure, and the residue obtained was recrystallized from AcOEt and *n*-hexane to give **14p** (1.61 g, 52% yield for 3 steps) as a solid. ¹H-NMR (CDCl₃) δ: 0.94 (6H, d, *J*=6.6 Hz), 1.66–1.80 (1H, m), 2.10 (2H, t, *J*=7.3 Hz), 4.57 (2H, s), 4.61 (2H, s), 6.23 (1H, dt, *J*=15.8, 7.3 Hz), 6.36 (1H, d, *J*=15.8 Hz), 7.26–7.37 (4H, m), 7.43–7.49 (2H, m), 8.05–8.12 (2H, m).

Compound **14q** was prepared from ethyl 4-iodobenzoate *via* compound **29** as follows:

Ethyl 4-(5-Methyl-1-hexenyl)benzoate (29) Compound **29** was synthesized according to the procedure for the synthesis of **28**. Yield quant. ¹H-NMR (CDCl₃) δ: 0.92 (6H, d, *J*=6.6 Hz), 1.34–1.42 (5H, m), 1.56–1.66 (1H, m), 2.19–2.28 (2H, m), 4.36 (2H, q, *J*=7.1 Hz), 6.30–6.40 (2H, m), 7.34–7.41 (2H, m), 7.91–7.98 (2H, m).

4-[4-(5-Methyl-1-hexenyl)benzyloxymethyl]benzoic Acid (14q) Compound **14q** was synthesized from **29** according to the procedure for the synthesis of **14p**. Yield 13% for 3 steps. ¹H-NMR (CDCl₃) δ: 0.91 (6H, d, *J*=6.6 Hz), 1.31–1.40 (2H, m), 1.56–1.67 (1H, m), 2.16–2.26 (2H, m), 4.56 (2H, s), 4.60 (2H, s), 6.17–6.28 (1H, m), 6.38 (1H, d, *J*=15.8 Hz), 7.26–7.37 (4H, m), 7.43–7.49 (2H, m), 8.05–8.12 (2H, m).

4-(Biphenyl-4-ylmethoxymethyl)benzoic Acid (14r) Compound **14r** was synthesized from **12r** and **13** according to the procedure for the synthesis of **14a**. Yield 61% for 2 steps. ¹H-NMR (CDCl₃) δ: 4.64 (2H, s), 4.67 (2H, s), 7.31–7.39 (1H, m), 7.40–7.63 (10H, m), 8.08–8.15 (2H, m).

4-(4-Styrylbenzyloxymethyl)benzoic Acid (14s) Compound **14s** was synthesized from **12s**³⁰ and **13** according to the procedure for the synthesis of **14a**. Yield 61% for 2 steps.

¹H-NMR (DMSO-*d*₆) δ: 4.57 (2H, s), 4.62 (2H, s), 7.24–7.30 (3H, m), 7.34–7.41 (4H, m), 7.45–7.52 (2H, m), 7.57–7.64 (4H, m), 7.91–7.96 (2H, m), 12.60–13.15 (1H, br).

Compound **14t** was prepared from methyl 4-iodobenzoate *via* compound **30** as follows:

Methyl 4-(4-Phenylethynylbenzyloxymethyl)benzoate (30) Ag₂O (4.00 g, 17.3 mmol) was added to a solution of **12t**³¹ (1.20 g, 5.76 mmol) and **13** (1.32 g, 5.76 mmol) in toluene (20 mL), and the mixture was stirred at 60°C for 2 h under an argon atmosphere. After the removal of Ag₂O by filtration, the filtrate was concentrated under reduced pressure. The residue obtained was purified by silica gel column chromatography to give **30** (250 mg, 12%) as a solid. ¹H-NMR (CDCl₃) δ: 3.91 (3H, s), 4.58 (2H, s), 4.61 (2H, s), 7.32–7.38 (5H, m), 7.40–7.45 (2H, m), 7.50–7.56 (4H, m), 8.00–8.05 (2H, m).

4-(4-Phenylethynylbenzyloxymethyl)benzoic Acid (14t) One molar aqueous LiOH solution (3.4 mL, 3.4 mmol) was added to a solution of **30** (250 mg, 0.701 mmol) in MeOH (2.0 mL) and THF (2.0 mL), and the mixture was stirred at 60°C for 2 h. The reaction mixture was neutralized with 1.0 M aqueous HCl solution, which was concentrated under reduced pressure. After the addition of water, the precipitate was collected by filtration to give **14t** (200 mg, 83% yield) as a solid. ¹H-NMR (CDCl₃) δ: 4.59 (2H, s), 4.63 (2H, s), 7.32–7.38 (5H, m), 7.42–7.47 (2H, m), 7.50–7.56 (4H, m), 8.01–8.06 (2H, m).

9H-Fluorene-2-sulfonamide (2G) Concentrated hydrochloric acid (27 mL) was added to a solution of 9H-fluorenyl-2-amine (3.00 g, 16.6 mmol) in MeCN (38 mL) at –5°C, and the mixture was stirred at the same temperature for 10 min. NaNO₂ (1.37 g, 19.9 mmol) was dissolved in water (3.8 mL), which was added dropwise at –5°C to the above reaction mixture. After stirring for 5 min, 30% SO₂ in AcOH (42.5 mL) and 2.2 M aqueous CuCl₂ solution (3.8 mL, 8.4 mmol) were added dropwise at –5°C to the reaction mixture, followed by stirring at room temperature for 45 h. After the addition of water, the mixture was extracted twice with AcOEt. The organic layer was washed with water and saturated brine, and then dried over Na₂SO₄. The solvent was removed under reduced pressure.

The residue obtained was dissolved in THF (40 mL), to which 28% aqueous NH₃ solution (7.1 mL, 83 mmol) was added, and the mixture was stirred at room temperature for 10 min. AcOEt was added to the reaction mixture, which was washed with 10% aqueous citric acid solution, water, and saturated brine, and then dried over Na₂SO₄. The solvent was removed under reduced pressure. The residue was rinsed with Et₂O to give **2G** (2.47 g, 61% yield for 2 steps) as a solid. ¹H-NMR (DMSO-*d*₆) δ: 4.04 (2H, s), 7.32–7.36 (2H, br), 7.37–7.46 (2H, m), 7.63–7.65 (1H, m), 7.84–7.88 (1H, m), 7.98–8.01 (1H, m), 8.02–8.03 (1H, m), 8.06–8.09 (1H, m).

Compound **2I** was prepared from 5-benzyloxypentylbromide *via* compounds **31**, **32**, **33**, and **2J** as follows:

S-(5-Benzyloxypentyl)thioacetate (31) AcSK (2.23 g, 19.5 mmol) was added to a stirred solution of 5-benzyloxypentylbromide (2.51 g, 9.76 mmol) in acetone (25 mL), and the reaction mixture was stirred at room temperature for 19 h. After the addition of water, the mixture was extracted twice with AcOEt, washed with saturated brine, and then dried over Na₂SO₄. The solvent was removed under reduced pressure. The residue obtained was purified by silica gel column chromatography to give **31** (2.40 g, 97% yield) as an oil. ¹H-NMR

(CDCl₃) δ : 1.38–1.50 (2H, m), 1.55–1.68 (4H, m), 2.32 (3H, s), 2.87 (2H, t, J =7.3 Hz), 3.46 (2H, t, J =6.6 Hz), 4.49 (2H, s), 7.26–7.37 (5H, m).

5-(2-Chlorobenzyloxy)pentane-1-sulfonamide (32) and 5-Benzyloxypentane-1-sulfonamide (33) AcONa (7.80 g, 95.1 mmol) was added to a stirred solution of **31** (2.40 g, 9.51 mmol) in AcOH (35 mL) and water (7 mL), and the mixture was bubbled with Cl₂ gas under ice-cooling for 30 min. The reaction mixture was degassed by purging with argon, which was diluted with water and extracted with CHCl₃. The organic layer was washed with 10% aqueous Na₂S₂O₃ solution and saturated aqueous NaHCO₃ solution, and then dried over Na₂SO₄. The solvent was removed under reduced pressure.

The residue obtained was dissolved in THF (60 mL), to which 28% NH₃ solution (3.3 mL, 54 mmol) was added, followed by stirring at room temperature for 14 h. After the addition of 10% aqueous citric acid solution, the reaction mixture was extracted with AcOEt and the organic layer was washed with saturated brine, and then dried over Na₂SO₄. The solvent was removed under reduced pressure, and the residue obtained was purified by silica gel column chromatography to give **32** (370 mg, 12% yield) as an oil and **33** (270 mg, 9.7% yield) as an oil.

Compound **32**: ¹H-NMR (CDCl₃) δ : 1.52–1.63 (2H, m), 1.65–1.75 (2H, m), 1.85–1.95 (2H, m), 3.08–3.17 (2H, m), 3.56 (2H, t, J =5.9 Hz), 4.48–4.58 (2H, br), 4.58 (2H, s), 7.17–7.38 (3H, m), 7.42–7.49 (1H, m).

Compound **33**: ¹H-NMR (CDCl₃) δ : 1.49–1.60 (2H, m), 1.61–1.72 (2H, m), 1.83–1.95 (2H, m), 3.06–3.18 (2H, m), 3.47 (2H, t, J =6.1 Hz), 4.45 (2H, s), 4.51–4.58 (2H, br), 7.21–7.38 (5H, m).

5-(tert-Butyldimethylsilanyloxy)pentane-1-sulfonamide (2I) and 5-Hydroxypentane-1-sulfonamide (2J) A solution of **32** (370 mg, 1.27 mmol) in MeOH (7.5 mL) was hydrogenated at 0.4 MPa in the presence of Pd–C (40 mg) at room temperature for 22 h. After removal of the catalyst by filtration, the filtrate was evaporated under reduced pressure to give 5-hydroxypentane-1-sulfonamide **2J** (150 mg) as an oil.

Compound **33** (270 mg, 1.05 mmol) was hydrogenated as described above to give 5-hydroxypentane-1-sulfonamide **2J** (220 mg) as an oil.

Imidazole (196 mg, 2.87 mmol) and *tert*-butylchlorodimethylsilane (TBSCl) (350 mg, 2.32 mmol) were added to a solution of 5-hydroxypentane-1-sulfonamide (370 mg, 2.21 mmol) in CH₂Cl₂ (4.0 mL), and the mixture was stirred at room temperature for 3 h. After the addition of 10% aqueous citric acid solution, the reaction mixture was extracted with AcOEt and the organic layer was washed with saturated brine, and then dried over Na₂SO₄. The solvent was removed under reduced pressure, and the residue obtained was purified by silica gel column chromatography to give **2I** (490 mg, 75% yield for 2 steps) as an oil. ¹H-NMR (CDCl₃) δ : 0.05 (6H, s), 0.89 (9H, s), 1.45–1.65 (4H, m), 1.83–1.97 (2H, m), 3.09–3.22 (2H, m), 3.62 (2H, t, J =5.9 Hz), 4.47–4.66 (2H, br).

(E)-N-(2,4-Dichlorobenzenesulfonyl)-4-(4-isopropylbenzyloxymethyl)benzamide Sodium Salt (15aA) CDI (116 mg, 0.715 mmol) was added to a solution of **14a** (170 mg, 0.570 mmol) in DMF (2.0 mL), and the mixture was stirred at room temperature for 1 h. After the addition of 2,4-dichlorobenzenesulfonamide **2A** (162 mg, 0.716 mmol) and DBU (0.11 mL, 0.72 mmol), the reaction mixture was stirred at

room temperature for 14 h. The mixture was acidified with 10% aqueous citric acid solution, followed by extraction with AcOEt and the organic layer was washed with saturated aqueous NaHCO₃ solution and saturated brine, and then dried over Na₂SO₄. The solvent was removed under reduced pressure. After the addition of Et₂O, the precipitate was collected by filtration to give **15aA** (125 mg, 43% yield) as a white solid, mp 185–190°C. ¹H-NMR (DMSO-*d*₆) δ : 1.18 (6H, d, J =6.8 Hz), 2.82–2.92 (1H, m), 4.46 (2H, s), 4.51 (2H, s), 7.18–7.30 (6H, m), 7.82–7.88 (2H, m), 7.45 (1H, dd, J =8.6, 2.0 Hz), 7.50 (1H, d, J =2.0 Hz), 7.98 (1H, d, J =8.6 Hz). IR (ATR) cm^{−1}: 1593. MS *m/z*: 514 [M+Na]⁺.

(E)-N-(2,4-Dichlorobenzenesulfonyl)-4-[4-(4-methylpent-1-enyl)benzyloxymethyl]benzamide Sodium Salt (15pA) CDI (275 mg, 1.69 mmol) was added to a solution of **14p** (500 mg, 1.54 mmol) in DMF (5.0 mL), and the mixture was stirred at room temperature for 1 h. 2,4-Dichlorobenzenesulfonamide **2A** (382 mg, 1.69 mmol) and DBU (0.25 mL, 1.7 mmol) were added to the reaction mixture, followed by stirring at room temperature for 13.5 h. After the addition of 10% aqueous citric acid solution, the reaction mixture was extracted with AcOEt and the organic layer was washed with water and saturated brine, and then dried over Na₂SO₄. The solvent was removed under reduced pressure. The residue obtained was purified by silica gel column chromatography to give the free form of **15pA**.

Five molar MeONa in MeOH (0.31 mL, 0.15 mmol) was added to a solution of the residue obtained in THF (10 mL) under ice-cooling, and the mixture was stirred at the same temperature for 5 min. Et₂O (50 mL) and *n*-hexane (20 mL) were added to the mixture and the precipitate was collected by filtration to give **15pA** (450 mg, 53% yield for 2 steps) as a white solid, mp 190–191°C. ¹H-NMR (DMSO-*d*₆) δ : 0.91 (6H, d, J =6.6 Hz), 1.62–1.79 (1H, m), 2.04–2.11 (2H, m), 4.49 (2H, s), 4.52 (2H, s), 6.27 (1H, dt, J =15.8, 6.8 Hz), 6.38 (1H, d, J =15.8 Hz), 7.25–7.31 (4H, m), 7.35–7.40 (2H, m), 7.46 (1H, dd, J =8.5, 2.2 Hz), 7.51 (1H, d, J =2.2 Hz), 7.83–7.88 (2H, m), 7.99 (1H, d, J =8.5 Hz). IR (ATR) cm^{−1}: 1593, 1554, 1332. MS *m/z*: 554 [M+Na]⁺.

N-(2,4-Dichlorobenzenesulfonyl)-4-[4-(5-methyl-1-hexenyl)benzyloxymethyl]benzamide Sodium Salt (15qA) Compound **15qA** was synthesized from **14q** and **2A** according to the procedure for the synthesis of **15aA**. Yield 87% for 2 steps. A white solid, mp 165–175°C. ¹H-NMR (DMSO-*d*₆) δ : 0.85 (6H, d, J =6.6 Hz), 1.25–1.32 (2H, m), 1.48–1.58 (1H, m), 2.10–2.19 (2H, m), 4.44 (2H, s), 4.82 (2H, s), 6.23 (1H, dt, J =16.1, 6.8 Hz), 6.35 (1H, d, J =16.1 Hz), 7.21–7.28 (4H, m), 7.30–7.35 (2H, m), 7.44 (1H, dd, J =8.6, 2.2 Hz), 7.49 (1H, d, J =2.2 Hz), 7.80–7.86 (2H, m), 7.96 (1H, d, J =8.6 Hz). IR (ATR) cm^{−1}: 1655. MS *m/z*: 568 [M+Na]⁺.

N-(2,4-Dichlorobenzenesulfonyl)-4-(biphenyl-4-ylmethoxymethyl)benzamide (15rA) Compound **15rA** was synthesized from **14r** and **2A** according to the procedure for the synthesis of **15aA**. Yield 69%. A white solid, mp 114–115°C. ¹H-NMR (CDCl₃) δ : 4.56–4.65 (4H, m), 7.31–7.55 (9H, m), 7.55–7.63 (4H, m), 7.76–7.83 (2H, m), 8.31 (1H, d, J =8.6 Hz), 8.80–9.50 (1H, br). IR (ATR) cm^{−1}: 1693, 1610. MS *m/z*: 548 [M+Na]⁺. Anal. Calcd for C₂₇H₂₁Cl₂NO₄S: C, 61.60; H, 4.02; N, 2.66. Found: C, 61.53; H, 4.06; N, 2.49.

N-(2,4-Dichlorobenzenesulfonyl)-4-(4-styrylbenzyloxymethyl)benzamide Sodium Salt (15sA) Compound **15sA**

was synthesized from **14s** and **2A** according to the procedure for the synthesis of **15pA**. Yield 65% for 2 steps. A white solid, mp 171–173°C. $^1\text{H-NMR}$ ($\text{DMSO-}d_6$) δ : 4.53 (2H, s), 4.55 (2H, s), 7.24–7.32 (3H, m), 7.33–7.40 (5H, m), 7.43–7.52 (2H, m), 7.56–7.64 (4H, m), 7.85–7.92 (3H, m), 7.97–8.01 (1H, m). IR (ATR) cm^{-1} : 1670. MS m/z : 574 $[\text{M}+\text{Na}]^+$.

***N*-(2,4-Dichlorobenzenesulfonyl)-4-(4-phenylethynylbenzyloxymethyl)benzamide Sodium Salt (15tA)** Compound **15tA** was synthesized from **14t** and **2A** according to the procedure for the synthesis of **15pA**. Yield 41% for 2 steps. A white solid, mp 160–170°C. $^1\text{H-NMR}$ ($\text{DMSO-}d_6$) δ : 4.55 (4H, s), 7.27–7.32 (2H, m), 7.38–7.44 (5H, m), 7.46 (1H, dd, $J=8.3$, 2.2 Hz), 7.51 (1H, d, $J=2.2$ Hz), 7.52–7.58 (4H, m), 7.85–7.90 (2H, m), 7.98 (1H, d, $J=8.3$ Hz). IR (ATR) cm^{-1} : 1591. MS m/z : 572 $[\text{M}+\text{Na}]^+$.

***N*-(2,6-Dichlorobenzenesulfonyl)-4-(biphenyl-4-ylmethoxymethyl)benzamide (15rB)** Compound **15rB** was synthesized from **14r** and **2B**³² according to the procedure for the synthesis of **15aA**. Yield 24%. A white solid, mp 127–128°C. $^1\text{H-NMR}$ (CDCl_3) δ : 4.60–4.66 (4H, m), 7.32–7.63 (14H, m), 7.77–7.85 (2H, m), 9.02–9.33 (1H, br). IR (ATR) cm^{-1} : 1668, 1610. MS m/z : 548 $[\text{M}+\text{Na}]^+$.

***N*-(2-Cyanobenzenesulfonyl)-4-(biphenyl-4-ylmethoxymethyl)benzamide (15rC)** Compound **15rC** was synthesized from **14r** and **2C** according to the procedure for the synthesis of **15aA**. Yield 47%. A white solid, mp 184–186°C. $^1\text{H-NMR}$ ($\text{DMSO-}d_6$) δ : 4.64 (2H, s), 4.70 (2H, s), 7.33–7.39 (1H, m), 7.44–7.52 (4H, m), 7.57–7.64 (2H, m), 7.65–7.72 (4H, m), 7.86–7.96 (2H, m), 7.98–8.04 (2H, m), 8.12–8.18 (1H, m), 8.48–8.56 (1H, m), 11.64–11.75 (1H, br). IR (ATR) cm^{-1} : 1698. MS m/z : 505 $[\text{M}+\text{Na}]^+$.

Methyl 2-[4-(Biphenyl-4-ylmethoxymethyl)benzoylsulfamoyl]benzoate (15rD) Compound **15rD** was synthesized from **14r** and **2D** according to the procedure for the synthesis of **15aA**. Yield 50%. $^1\text{H-NMR}$ (CDCl_3) δ : 3.99 (3H, s), 4.61 (2H, s), 4.62 (2H, s), 7.31–7.52 (7H, m), 7.55–7.63 (4H, m), 7.64–7.75 (2H, m), 7.77–7.89 (3H, m), 8.41–8.48 (1H, m), 9.30–9.45 (1H, br).

2-[4-(Biphenyl-4-ylmethoxymethyl)benzoylsulfamoyl]benzoic Acid (15rE) Five molar aqueous LiOH solution (1.24 mL, 6.29 mmol) was added to a solution of **15rD** (640 mg, 1.24 mmol) in MeOH (5.0 mL) and THF (10 mL), and the mixture was stirred at 50°C for 4 h. The reaction mixture was neutralized with 6.0 M aqueous HCl solution and extracted twice with AcOEt. The organic layer was washed with water and saturated brine, and then dried over Na_2SO_4 . The solvent was removed under reduced pressure. After the addition of Et_2O , the precipitate was collected by filtration to give **15rE** (183 mg, 29% yield) as a white solid, mp 97–99°C. $^1\text{H-NMR}$ ($\text{DMSO-}d_6$) δ : 4.60 (2H, s), 4.63 (2H, s), 7.33–7.40 (1H, m), 7.42–7.52 (6H, m), 7.63–7.80 (7H, m), 7.88–7.95 (2H, m), 8.10–8.17 (1H, m), 11.50–14.50 (2H, br). IR (ATR) cm^{-1} : 1724, 1687. MS m/z : 524 $[\text{M}+\text{Na}]^+$.

***N*-(Biphenyl-4-sulfonyl)-4-(biphenyl-4-ylmethoxymethyl)benzamide (15rF)** Compound **15rF** was synthesized from **14r** and **2F**³³ according to the procedure for the synthesis of **15aA**. Yield 41%. A white solid, mp 181–183°C. $^1\text{H-NMR}$ (CDCl_3) δ : 4.60 (2H, s), 4.63 (2H, s), 7.32–7.38 (1H, m), 7.41–7.54 (9H, m), 7.63–7.67 (4H, m), 7.73–7.76 (2H, m), 7.87–7.90 (2H, m), 7.90–7.95 (2H, m), 8.00–8.09 (2H, m), 12.53–12.67 (1H, br). IR (ATR) cm^{-1} : 1697. MS m/z : 556

$[\text{M}+\text{Na}]^+$.

***N*-(9H-Fluorene-2-sulfonyl)-4-(biphenyl-4-ylmethoxymethyl)benzamide (15rG)** Compound **15rG** was synthesized from **14r** and **2G** according to the procedure for the synthesis of **15aA**. Yield 34%. A white solid, mp 180–182°C. $^1\text{H-NMR}$ ($\text{DMSO-}d_6$) δ : 4.08 (2H, s), 4.59 (2H, s), 4.62 (2H, s), 7.33–7.38 (1H, m), 7.40–7.48 (8H, m), 7.63–7.66 (5H, m), 7.86–7.88 (2H, m), 8.02–8.04 (2H, m), 8.14–8.16 (1H, m), 8.19–8.20 (1H, m), 12.52–12.60 (1H, br). IR (ATR) cm^{-1} : 1698. MS m/z : 568 $[\text{M}+\text{Na}]^+$.

***N*-(Octane-1-sulfonyl)-4-(biphenyl-4-ylmethoxymethyl)benzamide (15rH)** Compound **15rH** was synthesized from **14r** and **2H** according to the procedure for the synthesis of **15aA**. Yield 37%. A white solid, mp 107–109°C. $^1\text{H-NMR}$ ($\text{DMSO-}d_6$) δ : 0.80–0.86 (3H, m), 1.15–1.31 (8H, m), 1.34–1.45 (2H, m), 1.65–1.75 (2H, m), 3.46–3.55 (2H, m), 4.62 (2H, s), 4.65 (2H, s), 7.33–7.39 (1H, m), 7.43–7.55 (6H, m), 7.65–7.70 (4H, m), 7.91–7.97 (2H, m), 12.05 (1H, s). IR (ATR) cm^{-1} : 1675. MS m/z : 516 $[\text{M}+\text{Na}]^+$.

***N*-[5-(tert-Butyldimethylsilyloxy)pentane-1-sulfonyl]-4-(biphenyl-4-ylmethoxymethyl)benzamide (15rI)** Compound **15rI** was synthesized from **14r** and **2I** according to the procedure for the synthesis of **15aA**. Yield quant. $^1\text{H-NMR}$ (CDCl_3) δ : 0.03 (6H, s), 0.87 (9H, s), 1.48–1.62 (4H, m), 1.85–1.98 (2H, m), 3.55–3.68 (4H, m), 4.64 (2H, s), 4.66 (2H, s), 7.26–7.65 (11H, m), 7.78–7.85 (2H, m), 8.17–8.23 (1H, br).

***N*-(5-Hydroxypentane-1-sulfonyl)-4-(biphenyl-4-ylmethoxymethyl)benzamide (15rJ)** One molar TBAF in THF (1.9 mL, 1.9 mmol) was added to a solution of **15rI** (560 mg, 0.962 mmol) in THF (8.4 mL) under ice-cooling, and the mixture was stirred at the same temperature for 16 h. After the addition of water, the reaction mixture was extracted twice with AcOEt, washed with saturated brine, and then dried over Na_2SO_4 . The solvent was removed under reduced pressure. Et_2O (5.0 mL) was added to the residue obtained and the precipitate that formed was collected by filtration to give **15rJ** (135 mg, 31% yield for 2 steps) as a white solid, mp 113–114°C. $^1\text{H-NMR}$ (CDCl_3) δ : 1.48–1.75 (4H, m), 1.80–2.00 (2H, m), 3.55–3.68 (4H, m), 4.64 (2H, s), 4.64 (2H, s), 7.30–7.38 (1H, m), 7.39–7.47 (4H, m), 7.47–7.54 (2H, m), 7.56–7.65 (4H, m), 7.80–7.89 (2H, m), 8.45–9.10 (1H, br). IR (ATR) cm^{-1} : 1678, 1612. MS m/z : 590 $[\text{M}+\text{Na}]^+$.

Compound **17** was prepared from compound **16**³⁵ via compound **34** as follows:

Methyl 4-(Biphenyl-4-ylmethylsulfanylmethyl)benzoate (34) Five molar MeONa in MeOH (5.5 mL, 28 mmol) was added to a stirred solution of **16** (2.42 g, 10.0 mmol) in MeOH (30 mL), and the mixture was stirred at room temperature for 30 min. Methyl 4-(bromomethyl)benzoate **13** (2.29 g, 10.0 mmol) was added to the reaction mixture, and this was followed by stirring at room temperature for 30 min. After the addition of AcOEt and water to the reaction mixture, the organic layer was washed with saturated brine, and then dried over Na_2SO_4 . The solvent was removed under reduced pressure, and the residue obtained was purified by silica gel column chromatography and recrystallized from AcOEt and *n*-hexane to give **34** (3.41 g, 98% yield) as a solid. $^1\text{H-NMR}$ (CDCl_3) δ : 3.63 (2H, s), 3.66 (2H, s), 3.91 (3H, s), 7.30–7.40 (5H, m), 7.40–7.47 (2H, m), 7.51–7.61 (4H, m), 7.96–8.02 (2H, m).

4-(Biphenyl-4-ylmethylsulfanylmethyl)benzoic Acid (17)

One molar aqueous LiOH solution (5.2 mL, 5.2 mmol) was added to a solution of **34** (600 mg, 1.72 mmol) in MeOH (3.0 mL) and THF (9.0 mL), and the mixture was stirred at room temperature for 1 h. The reaction mixture was acidified by 10% citric acid solution and extracted with AcOEt. The organic layer was washed with saturated brine, and then dried over Na₂SO₄. The solvent was removed under reduced pressure. After the addition of *n*-hexane, the precipitate was collected by filtration to give **17** (410 mg, 71% yield) as a solid. ¹H-NMR (DMSO-*d*₆) δ : 3.71 (2H, s), 3.76 (2H, s), 7.33–7.49 (7H, m), 7.58–7.68 (4H, m), 7.87–7.92 (2H, m), 12.70–13.10 (1H, br).

4-(Biphenyl-4-ylmethylsulfanylmethyl)-*N*-(octane-1-sulfonyl)benzamide (18H) Compound **18H** was synthesized from **2H**³⁴) and **17** according to the procedure for the synthesis of **15aA**. Yield 30%. A white solid, mp 138–140°C. ¹H-NMR (DMSO-*d*₆) δ : 0.83 (3H, t, *J*=6.6 Hz), 1.15–1.30 (8H, m), 1.34–1.44 (2H, m), 1.65–1.75 (2H, m), 3.47–3.54 (2H, m), 3.71 (2H, s), 3.77 (2H, s), 7.32–7.39 (3H, m), 7.42–7.48 (4H, m), 7.59–7.68 (4H, m), 7.87–7.92 (2H, m), 12.01 (1H, s). IR (ATR) cm⁻¹: 1678, 1441, 1336. MS *m/z*: 532 [M+Na]⁺. Anal. Calcd for C₂₉H₃₅NO₃S₂: C, 68.33; H, 6.92; N, 2.75. Found: C, 68.30; H, 6.92; N, 2.65.

4-(Biphenyl-4-ylmethylsulfanylmethyl)-*N*-(hexane-1-sulfonyl)benzamide (18K) Compound **18K** was synthesized from **2K**³⁴) and **17** according to the procedure for the synthesis of **15aA**. Yield 36%. A white solid, mp 134–136°C. ¹H-NMR (DMSO-*d*₆) δ : 0.80–0.88 (3H, m), 1.20–1.30 (4H, m), 1.34–1.45 (2H, m), 1.69 (2H, quintet, *J*=7.6 Hz), 3.51 (2H, t, *J*=7.6 Hz), 3.71 (2H, s), 3.77 (2H, s), 7.32–7.39 (3H, m), 7.42–7.50 (4H, m), 7.58–7.68 (4H, m), 7.86–7.93 (2H, m), 11.97–12.07 (1H, br). IR (ATR) cm⁻¹: 1681. MS *m/z*: 504 [M+Na]⁺. Anal. Calcd for C₂₇H₃₁NO₃S₂: C, 67.33; H, 6.49; N, 2.91. Found: C, 67.31; H, 6.52; N, 2.85.

4-(Biphenyl-4-ylsulfanylmethyl)-*N'*-(hexane-1-aminosulfonyl)benzamide (18L) Compound **18L** was synthesized from **2L**⁴⁷) and **17** according to the procedure for the synthesis of **15aA**. Yield 60%. A white solid, mp 136–137°C. ¹H-NMR (DMSO-*d*₆) δ : 0.77–0.84 (3H, m), 1.12–1.29 (6H, m), 1.39–1.49 (2H, m), 2.87–2.95 (2H, m), 3.71 (2H, s), 3.76 (2H, s), 7.32–7.40 (3H, m), 7.40–7.49 (4H, m), 7.58–7.68 (4H, m), 7.74–7.80 (1H, m), 7.85–7.91 (2H, m), 11.72 (1H, s). IR (ATR) cm⁻¹: 1687, 1604. MS *m/z*: 547 [M+H]⁺.

Compound **19** was prepared from compound **17** via compound **35** as follows:

***tert*-Butyl *N*-[4-(Biphenyl-4-ylmethylsulfanylmethyl)-phenyl]carbamate (35)** Diphenylphosphoryl azide (DPPA) (3.9 mL, 18 mmol), Et₃N (2.7 mL, 19 mmol), and *t*-BuOH (33 mL), which were stirred at 100°C for 3 h, were added to a stirred solution of **17** (5.01 g, 15.0 mmol) in toluene (33 mL). After cooling, water was added to the reaction mixture, followed by extraction with AcOEt. The organic layer was washed with saturated brine, and then dried over Na₂SO₄. The solvent was removed under reduced pressure. The residue obtained was purified by silica gel column chromatography to give **35** (3.66 g, 60% yield) as a solid. ¹H-NMR (CDCl₃) δ : 1.52 (9H, s), 3.59 (2H, s), 3.61 (2H, s), 6.40–6.48 (1H, br), 7.14–7.45 (9H, m), 7.50–7.56 (2H, m), 7.57–7.62 (2H, m).

Ethyl *N*-[4-(Biphenyl-4-ylmethylsulfanylmethyl)phenyl]-oxalamate (19) TFA (10 mL, 59 mmol) was added to a solution of **35** (4.20 g, 10.4 mmol) in CH₂Cl₂ (10 mL) under

ice-cooling, and the mixture was stirred at the same temperature for 50 min. The reaction mixture was basified by saturated aqueous NaHCO₃ solution, followed by extraction with CH₂Cl₂. The organic layer was washed with saturated brine, and dried over Na₂SO₄. The solvent was removed under reduced pressure.

Et₃N (1.1 mL, 7.3 mmol) and ethyl chloroglyoxylate (0.69 mL, 6.2 mmol) were added to a solution of the residue obtained (1.72 g, 5.63 mmol) in CH₂Cl₂ (15 mL) under ice-cooling, and the mixture was stirred at the same temperature for 40 min. The reaction mixture was washed with water and saturated brine, and then dried over Na₂SO₄. The solvent was removed under reduced pressure. Et₂O (2.0 mL) and *n*-hexane (4.0 mL) were added to the mixture and the precipitate was collected by filtration to give **19** (2.30 g, 55% yields for 2 steps) as a solid. ¹H-NMR (CDCl₃) δ : 1.44 (3H, t, *J*=7.1 Hz), 3.62 (2H, s), 3.63 (2H, s), 4.42 (2H, q, *J*=7.1 Hz), 7.28–7.37 (5H, m), 7.40–7.46 (2H, m), 7.52–7.62 (6H, m), 8.82–8.91 (1H, br).

Compound **20** was prepared from **16** via compound **36** as follows:

4-(4-Bromobenzylsulfanylmethyl)biphenyl (36) Compound **36** was synthesized from **16** and 1-bromo-4-bromomethylbenzene according to the procedure for the synthesis of **34**. Yield 87% for 2 steps. ¹H-NMR (CDCl₃) δ : 3.57 (2H, s), 3.63 (2H, s), 7.14–7.19 (2H, m), 7.30–7.37 (3H, m), 7.40–7.47 (4H, m), 7.51–7.62 (4H, m).

Ethyl [4-(Biphenyl-4-ylmethylsulfanylmethyl)phenyl]oxoacetate (20) A total of 1.6 M *n*-BuLi in *n*-hexane (3.3 mL, 5.3 mmol) was added dropwise at –78°C to a solution of **36** (1.62 g, 4.39 mmol) in THF (10 mL), and the mixture was stirred at the same temperature for 30 min. Diethyl oxalate (0.71 mL, 5.3 mmol) was added to the reaction mixture, followed by stirring at room temperature for 2 h. After the addition of 10% aqueous citric acid solution, the reaction mixture was extracted with AcOEt and the organic layer was washed with saturated brine, and then dried over Na₂SO₄. The solvent was removed under reduced pressure. The residue obtained was purified by column chromatography to give **20** (500 mg, 29% yield) as an oil. ¹H-NMR (CDCl₃) δ : 1.43 (3H, t, *J*=7.1 Hz), 3.64 (2H, s), 3.67 (2H, s), 4.45 (2H, q, *J*=7.1 Hz), 7.30–7.37 (3H, m), 7.41–7.47 (4H, m), 7.52–7.62 (4H, m), 7.96–8.00 (2H, m).

***N*-[4-(Biphenyl-4-ylmethylsulfanylmethyl)phenyl]-2-(hexane-1-sulfonylamino)-2-oxoacetamide (21)** One molar aqueous LiOH solution (14 mL, 14 mmol) was added to a solution of **19** (2.30 g, 5.67 mmol) in MeOH (14 mL) and THF (28 mL), and the mixture was stirred at room temperature for 30 min. The reaction mixture was acidified with 2.0 M aqueous HCl solution, which was extracted with AcOEt. The organic layer was washed with saturated brine, and then dried over Na₂SO₄. The solvent was removed under reduced pressure.

CDI (1.83 g, 11.3 mmol) was added to a solution of the residue obtained in DMF (15 mL), and the reaction mixture was stirred at room temperature for 30 min. After the addition of **2K**³⁴) (930 mg, 5.63 mmol) and DBU (2.5 mL, 17 mmol), the mixture was stirred at 50°C for 14 h. The reaction mixture was acidified with 2.0 M aqueous HCl solution, and the precipitate was then collected by filtration. CHCl₃ (2.0 mL) and MeOH (1.0 mL) were added to the solid obtained, and the precipitate was then collected by filtration to give **21** (250 mg,

8.5% yield for 2 steps) as a white solid, mp 199–201°C. ¹H-NMR (DMSO-*d*₆) δ : 0.82–0.88 (3H, m), 1.22–1.30 (4H, m), 1.36–1.44 (2H, m), 1.64–1.74 (2H, m), 3.40–3.50 (2H, m), 3.67 (2H, s), 3.70 (2H, s), 7.26–7.33 (2H, m), 7.37–7.40 (3H, m), 7.41–7.48 (2H, m), 7.58–7.67 (5H, m), 7.68–7.74 (2H, m), 10.87–10.90 (1H, br). IR (ATR) cm⁻¹: 1676. MS *m/z*: 519 [M+Na]⁺.

{2-[4-(Biphenyl-4-ylmethylsulfanylmethyl)phenyl]-*N*-(hexane-1-sulfonyl)-2-oxoacetamide (22)} Compound **22** was synthesized from **20** and **2K**³⁴) according to the procedure for the synthesis of **21**. Yield 12% for 2 steps. A white solid, mp 135–137°C. ¹H-NMR (DMSO-*d*₆) δ : 0.85–0.92 (3H, m), 1.26–1.34 (4H, m), 1.39–1.48 (2H, m), 1.68–1.74 (2H, m), 3.07–3.17 (2H, m), 3.73 (2H, s), 3.80 (2H, s), 7.20–7.32 (3H, m), 7.20–7.32 (2H, m), 7.52–7.58 (2H, m), 7.20–7.32 (4H, m), 7.84–7.89 (2H, m). IR (ATR) cm⁻¹: 1670. MS *m/z*: 519 [M+Na]⁺.

***N*-[4-(Biphenyl-4-ylmethylsulfanylmethyl)]-*N'*-(hexane-1-sulfonyl)urea (23)** DPPA (0.45 mL, 2.1 mmol) and Et₃N (0.31 mL, 4.2 mmol) were added to a stirred suspension of **17** (670 mg, 2.00 mmol) in toluene (6.7 mL), and the mixture was stirred at 100°C for 25 min. Compound **2K**³⁴) (500 mg, 3.03 mmol) and DBU (0.45 mL, 3.0 mmol) were added to the reaction mixture, followed by stirring at 100°C for 30 min. After cooling, 1.0 M aqueous HCl solution and Et₂O were added to the reaction mixture, and the precipitate was then collected by filtration and washed with water, MeOH, and AcOEt. The solid obtained was dissolved in DMF (2.0 mL), to which AcOEt (20 mL) was added, and the mixture was stirred at room temperature for 1 h. The precipitate was removed by filtration and washed with AcOEt and Et₂O. The filtrate was evaporated under reduced pressure, and the residue obtained was washed with Et₂O to give **23** (310 mg, 50% yield for 2 steps) as a white solid, mp 140–141°C. ¹H-NMR (DMSO-*d*₆) δ : 0.77–0.84 (3H, m), 1.15–1.30 (6H, m), 1.40–1.50 (2H, m), 2.88–2.94 (2H, m), 3.71 (2H, s), 3.76 (2H, s), 7.32–7.50 (7H, m), 7.58–7.68 (4H, m), 7.75–7.81 (1H, m), 7.85–7.91 (2H, m), 11.72 (1H, s). IR (ATR) cm⁻¹: 1687, 1604, 1546. MS *m/z*: 532 [M+Na]⁺.

PTP1B Inhibitory Activity and Enzyme Selectivity Inhibitory activity against recombinant human PTP1B (Enzo Life Sciences, Inc., Farmingdale, NY, U.S.A.), recombinant human T-cell PTP (TCPTP) (CALBIOCHEM, EMD Biosciences, Darmstadt, Germany), recombinant human CD45 PTP (CD45) (Enzo Life Sciences, Inc.) and recombinant human leucocyte common antigen-related (LAR) PTP (Enzo Life Sciences, Inc.) was measured using *p*-NPP as a substrate. Briefly, PTP1B inhibitory activities were assessed in the absence and presence of the test compounds in 100 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (pH 7.2) containing the enzyme, 1 mM dithiothreitol (DTT), 1 mM ethylenediaminetetraacetic acid (EDTA), and 0.0005% Triton-X. The reaction was started by the addition of *p*-NPP and stopped by the addition of 1 M NaOH after a 30-min incubation at 37°C. The absorbance of *p*-nitrophenol produced was measured at 405 nm.

PTP1B Inhibitory Mode The inhibitory mode was examined using recombinant human PTP1B (Enzo Life Sciences, Inc.) and *p*-NPP as the substrate. In order to clarify the inhibitory mode, phosphatase activities were measured at a fixed PTP1B concentration, while concentrations of the substrate

and inhibitor varied.

The enzyme (23.3 ng/mL final) solved in 100 mM HEPES buffer (pH 7.2) containing 1 mM DTT, 1 mM EDTA 0.0005% Triton-X, and various concentrations of **18K** was added to the wells of a 96-well plate. The reaction was started by the addition of *p*-NPP (0.15, 0.25, 0.5, 1, 3, 10, 30 μ M) and stopped by the addition of 1 M NaOH after a 30-min incubation at 37°C, and the absorbance of *p*-nitrophenol produced was then measured at 405 nm.

The 1/*s* and 1/*v* values, which were obtained from substrate concentration (*s*) and enzyme activity (*v*) at 0, 0.1, 0.2, and 0.5 μ M of **18K**, were plotted on the *x*-axis and *y*-axis, respectively. The inhibitory mode was evaluated from the intersection characteristics of the approximation straight lines obtained.

Plasma Concentrations after Oral Administration to Male ICR Mice, Male SD Rats, and Male Beagles Male ICR mice (8 weeks old; Japan SLC, Inc.), male SD rats (7 weeks old; Japan SLC, Inc.), and male beagles (45 months old; Narc Corporation, Chiba, Japan) were used. All animal experiments in the present study were conducted according to the guidelines for animal experiments of our institute and the guidelines for animal experimentation approved by the Japanese Association of Laboratory Animal Science. Test compounds suspended in 0.5% methylcellulose solution were orally administered at 30 mg/kg to mice and rats, and at 10 mg/kg to beagles. Blood samples were taken from the tail vein in mice, and from the jugular vein in rats and beagles 0.25, 0.5, 1, 3, 5, and 8 h after administration. Plasma concentrations of the test compounds were assessed using a COSMOSIL 5C18-MS-II column (4.6 $\times$ 150 mm; Nacalai Tesque) and Hitachi Elite LaChrom HPLC system (Hitachi High Technologies, Tokyo, Japan) consisting of a pump (L-2130), autosampler (L-2200), column oven (L-2300), and UV detector (L-2400) in mice and rats, and a COSMOSIL ODS-MS-II column (4.6 mm $\times$ 150 mm; Nacalai Tesque) and HPLC system consisting of a pump (LC-10ATvp, Shimadzu Techno-Research, Kyoto, Japan), autosampler (SIL-HTc, Shimadzu Techno-Research), column oven (CTO-10Avp, Shimadzu Techno-Research), degasser (DGU-14A, Shimadzu Techno-research), and UV detector (SPD-10Avp, Shimadzu Techno-Research) in beagles.

Anti-diabetic Effects in Male *db/db* Mice Male *db/db* diabetic mice (9 weeks old; Institute for Animal Reproduction, Japan) were allocated to vehicle-treated and **18K**-treated groups (*n*=6). Compound **18K** suspended in 0.5% methylcellulose solution was orally administered at 30 mg/kg/d to mice for one week. On day 7, blood samples were taken from the tail vein in fasted mice 50 min after the final administration and plasma glucose and triglyceride levels were measured. In order to estimate insulin resistance, the HOMA value was

Table 5. Scoring Matrix for the SUPERPOSE Analysis

	HP	AR	HD	HA	DA
HP	+3	+3	-2	-2	-2
AR	+3	+4 ^{a)}	-2	-2	-2
HD	-2	-2	+2	-2	+1
HA	-2	-2	-2	+2	+1
DA	-2	-2	+1	+1	+1

^{a)} If the planes of two rings cannot be superposed upon each other, a score of +3 is given.

calculated.⁴⁸⁾

Protein Structure Preparation for *in Silico* Analyses

The crystal structures of the allosteric inhibitor–PTP1B complex (residue 1–298, but no coordinates from 283 to the last; PDB: 1T4J)³⁷⁾ and TCPTP (residue 1–314, but no coordinates from 278 to the last; PDB: 1L8K)⁴⁹⁾ were used. All crystallographic water molecules were removed. The missing amino acids of TCPTP (Cys278, Ile279, and Lys280, corresponding to Phe280, Ile281, and Met282 of PTP1B) were compensated for using the Prime program from Schrödinger Suite 2012. Structures were minimized using force-field OPLS_2005.

Structural Model of the 18K–PTP1B Complex The initial superposition between compound **18K** and the allosteric inhibitor bound to PTP1B was performed using our molecular overlay program SUPERPOSE.^{45,46)} This program was developed based on the premise that compounds binding to the same site of a protein possess common 3D physicochemical features. Two molecules are superposed based on their respective physicochemical properties using a pseudo-molecule consisting of functional atoms instead of a real molecule. The properties of the functional atoms are divided into five types, comprising aromatic rings (ARs), hydrophobic atoms (HPs), hydrogen-bonding donors (HDs), hydrogen-bonding acceptors (HAs), and hydrogen-bonding donors/acceptors (DAs), each of which is represented as a sphere with a predefined radius (1.0 Å). After molecular superposition, overlaps of the spheres are scored using the scoring matrix (Table 5). In this study, 220 conformers of compound **18K**, generated by the CAMDAS program,⁵⁰⁾ were superposed on the conformation of the allosteric inhibitor bound to PTP1B, and the top-ranked conformations were then validated and refined by the MD simulation described below.

The MD simulation presented here used the force-field OPLS_2005 with an explicit solvent, and was run using the default parameters in the Desmond program from Schrödinger Suite 2012. The SPC water model was used. The systems were neutralized with Na⁺ or Cl[−] ions, and 0.15 M NaCl was also added. Periodic boundary conditions and a 9.0-Å cut-off for non-bonding interactions were used, with electrostatic interactions treated using the particle mesh Ewald method with a tolerance of 10^{−9}. A Desmond default relaxation protocol was employed prior to the MD simulation. After relaxation, a 9.0-ns simulation in a normal pressure and temperature ensemble [temperature=300 K, thermostat relaxation time=1.0 ps, pressure=1 atm, and barostat relaxation time=2.0 ps] was performed for each system using a Nose–Hoover thermostat and Martyna–Tobias–Klein barostat. Trajectory atomic coordinate data were recorded every 20 ps. Based on the MD trajectories obtained for the ligand-binding residues (within 4 Å of each ligand), conformations between 6.0 and 9.0 ns were clustered using the distance matrix of the Maestro clustering program from Schrödinger Suite 2012, and a representative structure was then selected as the closest to the average root-mean-square value in the cluster area.

Structural Model of the 18K–TCPTP Complex The protein backbone heavy atoms of the prepared TCPTP structure and representative structure of the **18K**–PTP1B complex were aligned using the Maestro protein structure alignment tool from Schrödinger Suite 2012. The PTP1B protein structure was removed, and the remaining structure of the **18K**–TCPTP complex was then used as the initial structure for the MD

simulation. The MD simulation and selection of a representative structure were performed as described above.

Conflict of Interest The authors declare no conflict of interest.

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