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# Mono- and disalicylic acid derivatives: PTP1B inhibitors as potential anti-obesity drugs

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**Abstract**—A series of compounds containing one or two salicylic acid moieties were synthesized, and their efficacy to inhibit the phosphohydrolase activity of PTP1B examined. Some of the methylenedisalicylic acid derivatives were potent inhibitors of PTP1B. Of those derivatives, **3c** exhibited about a 14-fold selectivity against TC-PTP, and this compound was tested in a mouse model for its efficacy to prevent diet-induced obesity. It effectively suppressed the increases in body weight and adipose mass, without any noticeable toxic effect. The compound also prevented increases in the plasma triglyceride, cholesterol, and nonesterified fatty acid concentrations; thus, expanding its therapeutic potential to other related metabolic diseases, such as hyperlipidemia and hypercholesterolemia.


## 1. Introduction

Obesity is a serious health-threatening factor in an urbanized society, which increases the risk of other diseases, such as type 2 diabetes, cardiovascular disease, hypertension, and some cancers.[1] High energy intake and low energy expenditure, accompanied by changing lifestyles, have increased the prevalence of obesity and this increase is expected to continue. Despite the urgent need for safe and efficient therapeutics and the potential size of the market for anti-obesity drugs, the current status for the development of such drugs is still unsatisfactory.

Until recently, only two drugs, orlistat and sibutramine, have been accepted for long-term use in the United States, with a further three drugs, diethylpropion, phendimetrazine, and phentermine, approved for short-term use.[2] In 2006, rimonabant was approved in Europe as a novel medicine antagonizing CB1 endocannabinoid receptor to decrease appetite. All these drugs exert their anti-obesity effect by decreasing energy intake. Orlistat prevents the absorption of fat by lipase inhibition in the gastrointestinal tract. Sibutramine inhibits presynaptic reuptake of the neurotransmitters, norepinephrine and serotonin; thus, decreasing appetite.[3] Unlike the earlier amphetamine-like drugs, sibutramine is not associated with primary pulmonary hypertension or valvular heart diseases. However, as a central nervous system stimulator, sibutramine increases the heart rate and blood pressure, which have raised concerns about the unidentified complications following long-term treatment.[4] The drugs for short-term use also suppress appetite via a central mechanism, but also exhibit side effects, such as palpitations, tachycardia, isomnia, hypertension, dry mouth, and constipation.[2b] The adverse effects and risks of these drugs have limited their therapeutic potential.

Aside from the side effects, current therapies target the short-term machineries that regulate appetite or an individual meal. The physiological system for the long-term regulation of fat mass or energy balance includes leptin and insulin, but leptin has a more important role in the central nervous system (CNS) control of body weight.[5] Leptin is secreted from adipocytes and its plasma level is proportional to the mass of adipose tissues in the body.[6] The binding of leptin to the leptin receptors in the hypothalamus is known to initiate a cascade, which inhibits food intake and promotes energy expenditure.[7] PTP1B was recently identified as a negative regulator of hypothalamic leptin signaling.[8] PTP1B inhibition or

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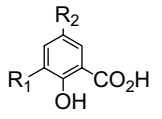
a reduction of the cellular abundance of PTP1B in mice resulted in increased sensitivities to leptin and insulin, and has also exhibited a protective effect against diet-induced obesity.[9] These observations provided the basis for PTP1B as a therapeutic target for obesity, which is the topic of this study.

In the design of PTPase inhibitors, the primary concern is the construction of a structure that mimics the phosphotyrosine residue, and recent research has produced various scaffolds, including phosphonates, 2-(oxalylamino)-benzoic acids, and *O*-carboxymethyl salicylic acids, to list but a few.[10] High dose of salicylic acid has been used, more than a century ago, to reduce the symptoms related to type II diabetes.[11] Similar effects have also been observed with aspirin treatment.[12] More recently, our and another laboratories have reported that polymeric substances, containing multiple salicylic acid moieties, are potent inhibitors of PTPases.[13] Prompted by these observations, salicylate moiety was examined as a pharmacophore for the inhibition of PTP1B, with a methylenedisalicylic acid derivative reported to have an anti-obesity effect in a mouse model.[14] In this study, more diverse compounds were synthesized, containing one or two salicylic acid moieties, and their inhibitory potency against PTP1B evaluated. The most potent PTP1B inhibitor was tested in an animal model for its efficacy in preventing the obesity induced by a high fat diet.

## 2. Results

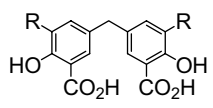
### 2.1. Chemical synthesis

The syntheses of compounds **1a-k** (Fig. 1) exemplifies the Fries rearrangement-haloform reaction approach (Scheme 1). For the syntheses of monosubstituted salicylic acids (**1a** and **1c–e**), appropriately substituted phenolic compounds were converted to 2′-hydroxyacetophenone derivatives, which were in turn O-methylated and subjected to a haloform reaction. The phenolic OH group was methylated for purification, even though the haloform reaction can be performed without protection of this group. The hydroxyacetophenone derivatives, with an aromatic substituent at C-3′ or C-5′ (**7** or **7′**), were brominated at the C-5′ or C-3′ to obtain the **8** or **8′** derivatives, respectively. For the syntheses of **1b**, **1f**, and **1g**, the haloform reaction was performed with **8** or **8′**. For the generation of **1h–j**, **8** or **8′** was subjected to a Suzuki coupling reaction, using the pinacol ester of phenylboronic acid and a palladium catalyst. The phenolic OH was protected before the coupling reaction. Resulting compounds, **9** and **9′**, were then subjected to haloform reaction to obtain compounds **1h–j**. For the dibenzyl derivative, **1k**, a second benzyl group was introduced by benzoylation of 4-benzylphenol, followed by a Wolff–Kishner reduction of the carbonyl group. For the generation of **1l**, the aldehyde (**13**) was protected following bromination, with subsequent Stille coupling. Compound **1m** was prepared from 2-bromophenol. The syntheses of 3,3′-methylenebis(2-hydroxybenzoic acid) derivatives started with the condensation of methyl 5-bromosalicylate (**19**) and formaldehyde (Scheme 2). The condensation product (**20**) served as a precursor for the syntheses of **3b–e**. Hydrolysis of the ester, **20**, produced **3b**. The treatment of **20** or **21** with the pinacol ester of 2-thiophenyl or phenyl boronic acid, in the presence of $Pd(PPh_3)_4$ catalyst, introduced 2-thiophenyl or phenyl group, yielding **3e** and **3c**, respectively. The basic reaction conditions resulted in concomitant hydrolysis of the ester. To synthesize **3d**, a fully protected compound (**21**) was subjected to a Stille coupling reaction with 2-(tributylstannyl)furan and palladium



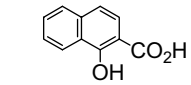

**1a-p**

| | $R_1$ | $R_2$ |
|---|---|---|
| **1a** | H | Ph |
| **1b** | Br | Ph |
| **1c** | Ph | H |
| **1d** | H | Bz |
| **1e** | Bz | H |
| **1f** | Bz | Br |
| **1g** | Br | Bz |
| **1h** | Bz | Ph |
| **1i** | Ph | Bz |
| **1j** | Ph | Ph |
| **1k** | Bz | Bz |
| **1l** | 2-furyl | CHO |
| **1m** | Br | H |
| **1n** | H | F |
| **1o** | H | CHO |
| **1p** | H | $SO_3H$ |

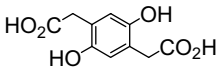



**2f-g**

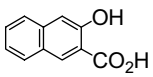
| | R |
|---|---|
| **2f** | Ph |
| **2g** | Bz |



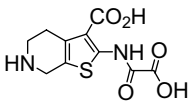

**1q**



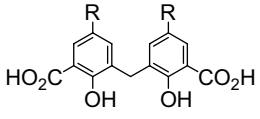

**1r**




**1s**

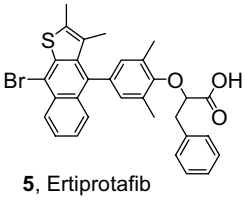



**4**



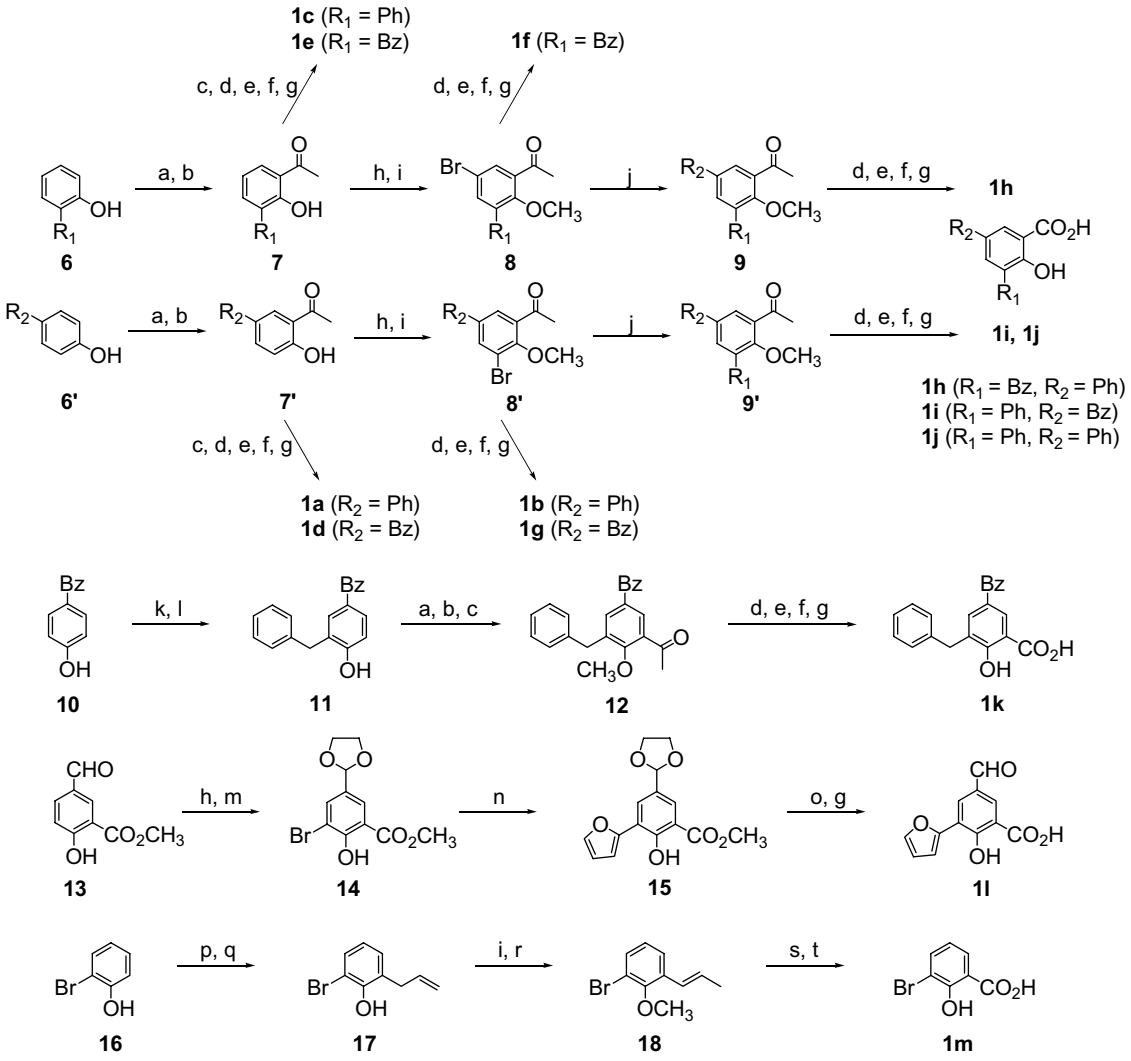

**3a-e**

| | R |
|---|---|
| **3a** | F |
| **3b** | Br |
| **3c** | Ph |
| **3d** |  |
| **3e** |  |




**5**, Ertiprotafib

**Figure 1.** Compounds used or referred in this study.




**Scheme 1.** Synthetic strategy for the syntheses of **1a**–**m**. Reagents and conditions: (a) $CH_3COCl$, $Et_3N$, ether; (b) $AlCl_3$, heat; (c) $(CH_3)_2SO_4$, NaOH, EtOH, reflux; (d) $Br_2$, aq KOH; (e) $CH_3OH$, HCl, reflux; (f) $BBr_3$; (g) aq NaOH, heat; (h) $Br_2$; (i) $CH_3I$, $K_2CO_3$, acetone, reflux; (j) $ArB(OR)_2$, $Pd(PPh_3)_4$, aq $K_3PO_4$, DMF, heat; (k) benzoyl chloride, $AlCl_3$, $CS_2$, 50 °C; 20% NaOH, 1,4-dioxane, 70 °C; (l) $H_2NNH_2$, KOH, $(CH_2OH)_2$, 120 °C–reflux; (m) $(CH_2OH)_2$, pTsOH, reflux; (n) 2-(tributylstannyl)furan, $Pd(PPh_3)_2Cl_2$, 1,4-dioxane, 100 °C; (o) 1 M HCl, $CHCl_3$, reflux; (p) allyl bromide, $K_2CO_3$, acetone, reflux; (q) 200 °C, microwave; (r) aq KOH, EtOH, reflux; (s) $KMnO_4$, acetone; aq $NaHSO_3$; aq HCl; (t) PhSH, $K_2CO_3$, NMP, 190 °C.

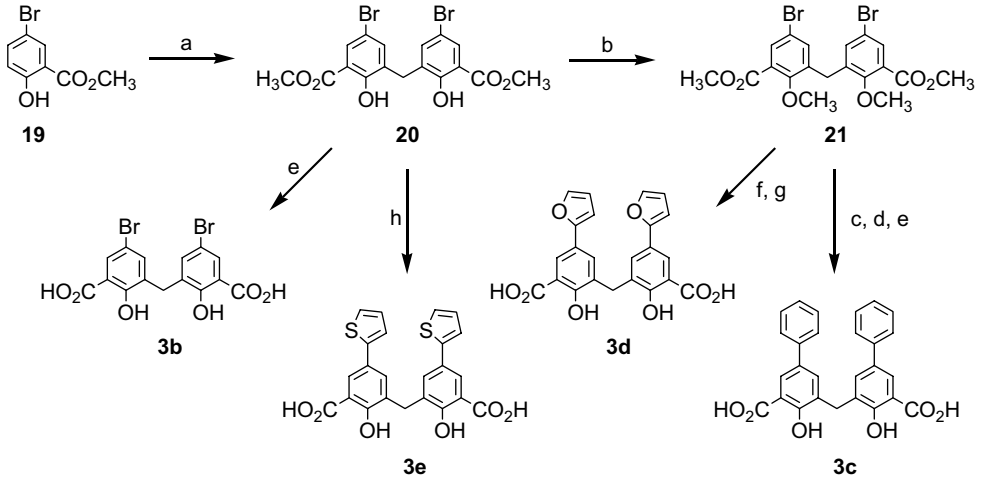
catalyst. The resulting furanylated compound was then treated with thiophenol and $K_2CO_3$ to remove the protecting groups. A similar condensation reaction of 5-flourosalicylic acid and paraformaldehyde yielded **3a**.

### 2.2. In vitro enzyme assays

The series of compounds synthesized in this study were tested for their ability to inhibit PTP1B, using *p*-nitrophenyl phosphate (*p*NPP) as the substrate. The enzyme and compounds were preincubated for 10 min prior to the initiation of the enzyme reaction by addition of the substrate. The $IC_{50}$ values for the compounds determined under these conditions are shown in Table 1. $K_i$ values were also determined for selected compounds.

As presented in Table 1, the disalicylic acid derivatives were generally found to be more potent than the monosalicylates, the **1** series. Compounds **1a**, **1c**, and **1d**, with a single phenyl or benzyl substitution of the salicylic acid moiety, did not improve the inhibitory potency. Bromine substitution resulted in a slightly increased potency, as with compounds **1b**, **1f**, and **1g**. Compounds **1h**–**k**, with double substitutions at the C-3 and C-5 positions, exhibited lower $IC_{50}$values. No significant difference was observed between the phenyl and benzyl substitutions.

In the series of compounds **3a**–**e**, two salicylic acid moieties were flanked by a methylene group. The halogenated derivatives, **3a** and **3b**, were not good inhibitors of PTP1B ($IC_{50} > 100$ μM). Compound **3c** proved to be the most potent inhibitor of PTP1B ($K_i = 6.5$ μM). In **3d** and **3e**, where the phenyl group in **3c** was replaced with heterocyclic furan or thiophene moieties, respectively, 1.5–3.5-fold decreases in the inhibitory potencies were observed. Similar results were previously obtained for the **2** series of compounds.[14]




**Scheme 2.** Synthetic strategy for the syntheses of methylenedisalicylic acid derivatives, **3**. Reagents and conditions: (a) 37% aq $CH_2O$, $H_2SO_4$, $CH_3OH$, $H_2O$; (b) $(CH_3)_2SO_4$, $K_2CO_3$, acetone, reflux; (c) (4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzene, $Pd(PPh_3)_4$, $K_3PO_4$, DMF, 80 °C; (d) $BBr_3$, $CH_2Cl_2$; (e) 20% NaOH, 1,4-dioxane, 80 °C; (f) 2-(tributylstannyl)furan, $Pd(PPh_3)_2Cl_2$, 1,4-dioxane, 100 °C; (g) PhSH, $K_2CO_3$, NMP, 190 °C; (h) 2-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)thiophene, $Pd(PPh_3)_4$, $K_3PO_4$, DMF, 80 °C.

**Table 1.** Inhibitory effect of the compounds on PTP1B

| Compound | $IC_{50}$ (μM)[a] | $K_i$ (μM)[b] |
|---|---|---|
| **1a** | 1279 ± 320 | |
| **1b** | 351 ± 56 | |
| **1c** | 859 ± 30 | |
| **1d** | 1180 ± 343 | |
| **1e** | 403 ± 63 | |
| **1f** | 148 ± 39 | |
| **1g** | 153 ± 32 | |
| **1h** | 57 ± 8 | |
| **1i** | 65 ± 9 | |
| **1j** | 84 ± 8 | |
| **1k** | 91 ± 15 | |
| **1l** | 380 ± 56 | |
| **1m** | >1000 | |
| **1n** | >1000 | |
| **1o** | >1000 | |
| **1p** | >1000 | |
| **1q** | 850 ± 150 | |
| **1r** | >1000 | |
| **1s** | >1000 | |
| **2f** | 20 ± 1[c] | 9.4[c] |
| **2g** | 15 ± 4[c] | 6.3[c] |
| **3a** | 215 ± 11 | |
| **3b** | 149 ± 45 | |
| **3c** | 19 ± 1 | 6.5 |
| **3d** | 67 ± 5 | |
| **3e** | 27 ± 5 | |
| **4** | 500 ± 100[c] | 102[c] |
| **5** (Ertiprotafib) | 1.4 ± 0.1[c] | 1.5[c] |

[a] Values are means ± standard deviations of two or more experiments.
[b] Values are from a single measurement.
[c] Data reproduced from our previous publication.[14]

Because **1i** and **3c** exhibited the lowest $IC_{50}$ values of the mono- and disalicylic acid derivatives, respectively, their inhibitory activities against other PTPases, including TC-PTP, were tested in order to verify the PTP1B specificity of these compounds, the results of which are summarized in Table 2. Compound **3c** demonstrated a 14-fold greater selectivity over TC-PTP, the most homologous with PTP1B among the human PTPases,

**Table 2.** Inhibition of PTPases by selected compounds

| Compound | $IC_{50}$ (μM)[a] ($K_i$, μM)[b] | | | | |
|---|---|---|---|---|---|
| | PTP1B | LAR-D1 | TC-PTP | SHP-1 cat | YPTP1 |
| **1i** | 65 ± 9 | >2000 | 540 ± 48 | 129 ± 15 | 271 ± 48 |
| **3c** | 19 ± 1 | >2000 | 156 ± 5 | 37 ± 3 | 86 ± 2 |
| | [6.5] | | [89] | (9.7) | |

[a] Values are means ± standard deviations of two or more experiments.
[b] Values are from a single measurement.

and excellent selectivity against LAR-D1. Less selectivity was observed over the catalytic domain of SHP-1 (SHP-1cat). YPTP, a yeast PTPase, was tested to compare the PTPases of different origin, but this exhibited only a modest selectivity.

### 2.3. In vivo experiment

Of the PTP1B inhibitors, **3c** was selected for evaluation of obesity controlling effect in mice susceptible to diet-induced obesity (C57BL/6J Jms Slc male). There is much evidence of diet-induced obese (DIO) mice being frequently used as a mice model in the determination of compound efficacy for the reduction of body weight.[15] Before feeding the test drug, mice were fed either LFD or HFD ad libitum for 8 weeks. The LFD-fed lean control mice showed much lower body weight gain compared to the HFD-fed obese control group. The HFD-fed mice were separated into two groups. Each of the groups was then given HFD or HFD plus **3c** for 4 weeks. The compound **3c** was administered as mixtures with the food (5 g **3c** per kg of diet). The daily uptake of **3c** was approximated as 13 mg/day/mouse, which is equivalent to 400 mg/day/kg of mouse weight. For lean control group, LFD was fed throughout the test period. As shown in Figure 2, the feeding of **3c** significantly reduced the body weight gain compared to that of the HFD control group ($p < 0.005$).

A reduction of adiposity was observed by the reduction of epididymal and retroperitoneal fat pad weights of the

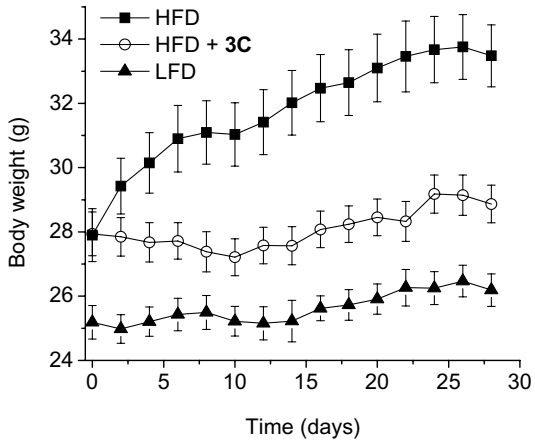



**Figure 2.** The effect of LFD, HFD, and HFD + **3c** on body weight of DIO mice. Data points represent means ± SEM; $n = 8$–9/group. $p < 0.005$ for comparisons against obese control group. Mice fed with LFD served as a reference group of lean control.

mice treated with **3c** as well as **2f** (Table 3). As depicted in Table 3, both fat pad weights were significantly lower than those of obese control mice ($p < 0.001$). A gross carcass appearance of a mouse also revealed the significantly decreased epididymal fat pad after drug treatment (Fig. 3) Differences in the liver weights, however, were not significant between any of the groups ($p > 0.05$) (Table 3). There was a modest decrease in food intake (< 8 days, $p < 0.05$) when the food was changed from HFD to HFD plus the drug (Fig. 4). After 8 days of treatment, the drug-treated mice continued to eat comparable amounts of food to that of the obese control mice ($p > 0.05$). The ratio of weight gain to consumed calories is described as the feed efficiency and reflects the metabolic efficiency. As shown in Figure 5, the drug-treated group revealed a feeding efficiency comparable to that of the lean control group, but significantly lower than that of the obese control group ($p < 0.001$). These results suggest that the significant loss of body fat and decreased body weight gain could be the result of increased energy expenditure.

Plasma was analyzed for glucose, triglyceride (TG), total cholesterol, and nonesterified fatty acids (NEFA) (Table 4). The concentrations of glucose and TG were only slightly higher in the obese control mice than those of the lean control mice ($p > 0.05$). However, HFD affects the concentrations of total cholesterol and NEFA, with the values for these parameters in the obese control group being significantly higher than those of the lean control group ($p < 0.05$). The feeding of **3c** together with the diet significantly suppressed the increases in the circulating TG, cholesterol, and NEFA ($p < 0.05$).

In addition, there was no significant change in physical activities on the administration of either compound, indicating that the treatment did not cause any behavioral effects in mice. At the end of the experiment, the obese group mice were physically distinguishable from the remaining two groups. The outer appearance of the liver showed no overt toxicity on treatment with these compounds (data not shown).

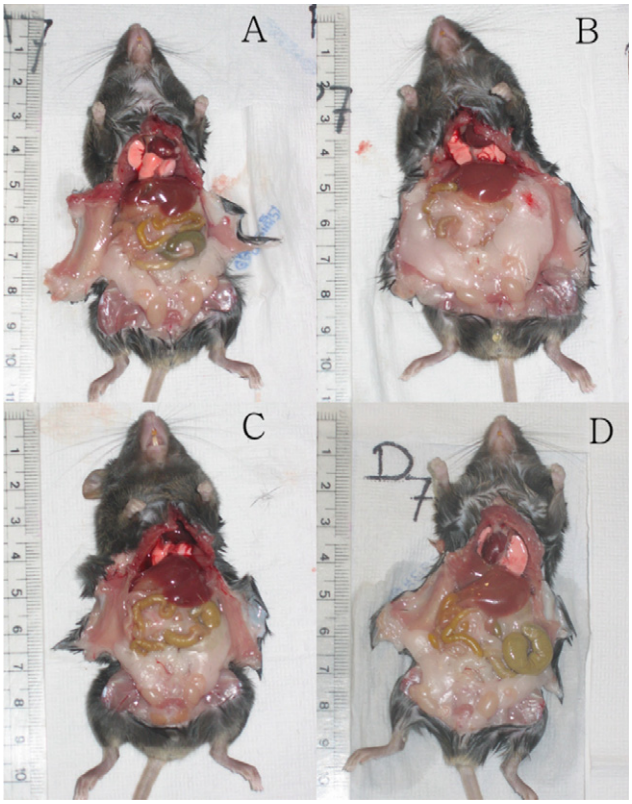



**Figure 3.** The gross carcass appearance of a mouse from each group at the end of the study. Five-week-old mice were fed LFD (A) or HFD (B–D) for 8 weeks and then treated for 4 weeks with (A) LFD, (B) HFD, (C) HFD + **3c** or (D) HFD + **2f**.

**Table 3.** Study of 28 days treatment of **2f** and **3c** on body weight, liver, and adipose tissue weight[a]

| | Body weight (g) | | Liver weight (g) | Adipose tissue weight (g) | |
|---|---|---|---|---|---|
| | Initial | Final | | Epididymal | Retroperitoneal |
| LFD | 25.2 ± 1.4 | 26.2 ± 1.4[c] | 0.99 ± 0.10 | 0.52 ± 0.09[c] | 0.15 ± 0.04[c] |
| HFD | 27.9 ± 2.4 | 33.4 ± 2.8 | 1.14 ± 0.12 | 1.80 ± 0.49 | 0.51 ± 0.11 |
| HFD + 2f | 27.9 ± 2.9[b] | 29.2 ± 2.5[b,c] | 1.03 ± 0.13 | 0.86 ± 0.24[b,c] | 0.26 ± 0.08[b,c] |
| HFD + 3c | 27.9 ± 2.0 | 28.9 ± 1.7[c] | 1.07 ± 0.09 | 0.83 ± 0.34[c] | 0.26 ± 0.07[c] |

[a] Data expressed as means ± SEM. $n = 8$ for LFD, $n = 9$ for others.
[b] Data reproduced from the previous publication in our laboratory.[14]
[c] Significantly different from the HFD ($p < 0.005$).

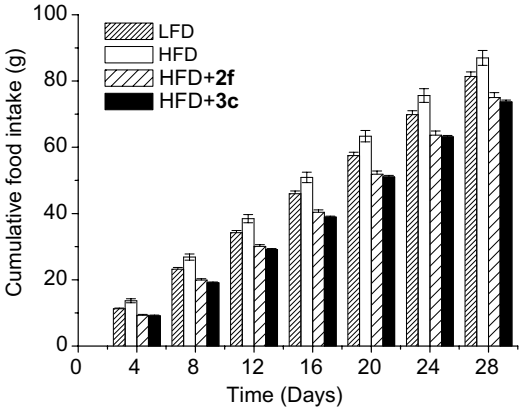



**Figure 4.** The effects of compounds **2f** and **3c** on the cumulative food intake of DIO mice. All values are means ± SEM; $n = 8$–9/group. $p < 0.05$ for comparisons against the obese control group. Mice fed with LFD served as a reference lean control group.

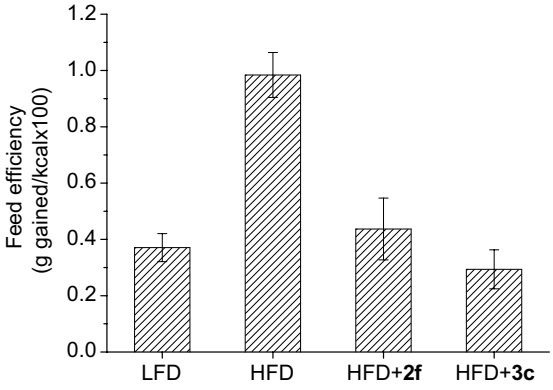



**Figure 5.** Effects of 4 week treatment with **2f** and **3c** on the feeding efficiency (body weight gain per calories consumed) of mice. All values are means ± SEM; $n = 8$–9/group. $p < 0.001$ for comparisons against obese control group. Mice fed with LFD served as a reference lean control group.

**Table 4.** Effects of PTP1B inhibitors **2f** and **3c** on plasma glucose, triglyceride, total cholesterol, and free fatty acid[a]

| | Glucose (mg/dL) | Triglyceride (mg/dL) | Total cholesterol (mg/dL) | NEFA (mEq/L) |
|---|---|---|---|---|
| LFD | 270 ± 10 | 47 ± 8 | 104 ± 11[b] | 0.23 ± 0.1[b] |
| HFD | 300 ± 40 | 63 ± 32 | 141 ± 10 | 0.35 ± 0.1 |
| HFD + **2f** | 300 ± 50 | 42 ± 7[b] | 129 ± 8[b] | 0.24 ± 0.1[b] |
| HFD + **3c** | 280 ± 40 | 48 ± 8 | 133 ± 14 | 0.14 ± 0.1[b] |

[a] Data expressed as means ± SEM. $n = 8$ for LFD, $n = 9$ for others.
[b] Significantly different from the HFD ($p < 0.05$).

## 3. Discussion

It has been reported that salicylic acid is a weak competitive inhibitor of PTP1B, with an inhibition constant of 19.4 mM.[13a] In the present study, a series of compounds containing one or two salicylic acid moieties were synthesized. Of those compounds, disalicylic acid, **3c**, was proved to be a potent inhibitor of PTP1B, and demonstrated a 14-fold greater selectivity over TC-PTP, the most homologous with PTP1B of the human PTPases. The obesity-controlling effect of the selected compound, **3c**, was determined using a DIO mouse model, with the result compared with those previously obtained for **2f**. The oral administration of **3c** (0.5% in diet w/w) significantly reduced the body weight gain compared with that of the HFD control group ($p < 0.005$). A decrease in adiposity was observed by the reduction of epididymal and retroperitoneal fat pad weights of mice treated with **3c**as well as **2f**. In contrast, there was no significant difference in the amount of food intake between the compound-treated and control groups. These results prove the efficacy of these compounds in controlling obesity. The in vivo effect of **3c**, without any noticeable toxic effect, suggests a potential therapeutic role for **3c** in the management of obesity. The experimental results also indicated that the anti-obesity effect was largely due to increased energy expenditure. The compounds also prevented the increase of the plasma levels of triglyceride, cholesterol, and nonesterified fatty acids, expanding the therapeutic potential to other related metabolic diseases, such as hyperlipidemia and hypercholesterolemia.

## 4. Experimental

### 4.1. Materials

Commercial reagents were from Aldrich Chemical Co. (Milwaukee, WI) and TCI (Tokyo, Japan). Most chemicals and solvents were of analytical grade and used without further purification. Reactions were monitored by thin-layer chromatography (TLC), using precoated silica gel plates (Silica gel 60 $F_{254}$, Merck), and spots were visualized under UV light (254 nm). Column chromatography was carried out using silica gel 60 AF-254 (0.063–0.200 mm, Merck). Melting points (uncorrected) were determined on a MEL-TEMP Electrothermal apparatus. $^{1}H$ and $^{13}C$ NMR spectra were recorded on a Varian Gemini 2000 (200 MHz) and a Varian Inova 400 (100 MHz) spectrometers, respectively. Chemical shifts ($\delta$) are expressed in parts per million relative to tetramethylsilane, which was used as an internal standard, coupling constants ($J$) are in hertz, and the signals are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br s, broad singlet. Mass spectra were obtained at the Korea Basic Science Institute, Daegu, Korea.

Compounds **1n** and **1p**–**s** were purchased from Aldrich. Compounds **1c** and **10** were purchased from TCI. The intermediates, **8** and **8′**, used for the generation of salicylic acid derivatives, were obtained by Friedel–Crafts acetylation, with subsequent controlled bromination of the commercially available phenols, as described previously.[16] A substrate, *p*NPP, for the PTPase assay was purchased from Sigma (St. Louis, USA) in the di(Tris) salt form. The absorbances were measured using a Novaspec-II spectrophotometer (Amersham Pharmacia) or a DU 650 spectrophotometer (Beckman–Coulter). The native form of PTP1B and the catalytic domain of SHP-1 (SHP-1cat) were expressed in an *Escherichia coli* expression systems and purified as described previously.[17] LAR-D1 (membrane-proximal catalytic domain

of LAR) and TC-PTP were purchased from New England Biolabs Inc. (Beverly, USA).

### 4.2. Chemical synthesis

#### 4.2.1. 5-Bromo-4-hydroxybiphenyl-3-carboxylic acid (1b)

**4.2.1.1. Step 1.** Bromine (0.2 mL, 3.9 mmol) was added dropwise to a stirred solution of 20% aqueous KOH (4 mL) cooled in an ice–salt bath at −5 to 0 °C. The resulting yellow solution was added to 3-bromo-2-methoxy-5-phenylacetophenone (**8′b**) (200 mg, 0.65 mmol) in 1,4-dioxane (10 mL), at 5–10 °C, over a period of 5 min, the mixture stirred below 10 °C for a further 2 h and stirred overnight at room temperature. The reaction mixture was diluted with water (20 mL), acidified with 1 M HCl (40 mL), and extracted with EtOAc (3× 20 mL). The combined organic extracts were washed successively with $Na_2S_2O_3$ (20 mL) and brine (20 mL), and dried ($Na_2SO_4$). Removal of the solvent gave a cream-colored solid, which upon recrystallization from EtOAc afforded 5-bromo-4-methoxybiphenyl-3-carboxylic acid (84 mg). The mother liquor was concentrated, and the residue subjected to column chromatography on silica gel (1% acetic acid in hexane/EtOAc, 1:1, $R_f = 0.5$) to yield the acid as white needles (92 mg, 88% overall yield): mp 193–195 °C; $^1$H NMR ($CDCl_3$, 200 MHz) $\delta$ 8.31 (d, $J$ = 2.6 Hz, 1H), 8.03 (d, $J$ = 2.6 Hz, 1H), 7.60–7.40 (m, 5H), 4.10 (s, 3H, $CH_3$).

**4.2.1.2. Step 2.** The above-obtained acid (160 mg, 0.52 mmol) was dissolved in MeOH, saturated with dry HCl (3 mL), and stirred overnight at 60 °C. The solution was further stirred at room temperature for 30 min, with the solvent evaporated to give a yellow oil, which was dissolved in EtOAc (15 mL). The EtOAc solution was washed successively with water (3× 15 mL) and 5% $NaHCO_3$(15 mL), and then dried ($Na_2SO_4$). Evaporation of solvent under reduced pressure followed by silica gel column chromatography (hexane/EtOAc 19:1, $R_f = 0.2$) afforded methyl 5-bromo-4-methoxybiphenyl-3-carboxylate as a colorless oil (142 mg, 85% yield): $^1$H NMR ($CDCl_3$, 200 MHz) $\delta$ 7.97 (d, $J$ = 2.2 Hz, 1H), 7.94 (d, $J$ = 2.6 Hz, 1H), 7.57–7.37 (m, 5H), 3.97 (s, 3H, $CO_2CH_3$), 3.96 (s, 3H, $OCH_3$).

**4.2.1.3. Step 3.** $BBr_3$ (1.0 M in $CH_2Cl_2$, 0.95 mL, 0.95 mmol) was added to a stirred solution of methyl 5-bromo-4-methoxybiphenyl-3-carboxylate (125 mg, 0.38 mmol) in $CH_2Cl_2$ (4 mL), over 5 min period, while cooling the reaction flask in a dry ice-acetone bath. The reaction mixture was stirred overnight at room temperature under nitrogen atmosphere. The reaction was quenched with the addition of 1 M HCl (2 mL) and the aqueous layer then extracted with EtOAc (2× 10 mL). The combined organic layers were washed successively with water (2× 10 mL) and brine (10 mL), and dried ($Na_2SO_4$). Removal of the solvent using a rotary evaporator gave a brown solid, which was further dissolved in 1,4-dioxane (1 mL), and then treated with 20% NaOH (0.5 mL) at 60 °C for 3 h. After cooling to room temperature, the reaction mixture was acidified with 1 M HCl (5 mL), and extracted with EtOAc (2× 10 mL). The combined organic extracts were washed successively with water (2× 10 mL) and brine (10 mL), and then dried ($Na_2SO_4$). Concentration of the solvent under reduced pressure gave a cream-colored solid, which was recrystallized from ether to give **1b** (56 mg). The mother liquor was concentrated, and the residue purified by silica gel chromatography (1% acetic acid in hexane/EtOAc, 4:1, $R_f = 0.2$), to afford **1b**as cream-colored crystals (41 mg, 87% overall yield): mp 214–216 °C; $^1$H NMR ($CDCl_3$, 200 MHz) $\delta$ 11.07 (s, 1H), 8.13 (d, $J$ = 2.6 Hz, 1H), 8.05 (d, $J$ = 2.2 Hz, 1H), 7.57–7.36 (m, 5H); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) $\delta$ 171.49 ($CO_2H$), 157.13, 137.74, 136.30, 132.26, 129.03, 127.55, 127.39, 126.35, 114.66, 111.02 ($C_{arom}$); LRMS $m/z$ 294 ($M^+$, $^{81}$Br), 292 ($M^+$, $^{79}$Br); HRMS calcd for $C_{13}H_9BrO_3$291.9735 ($M^+$, $^{79}$Br), found 291.9732.

**4.2.2. 5-Benzyl-2-methoxy-3-phenylacetophenone (9′i).** A flame-dried flask was cooled to room temperature under a nitrogen purge and charged with (4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzene (137 mg, 0.68 mmol) and tetrakis (triphenylphosphine) palladium (8 mg, 9 μmol). After 30 min nitrogen purging, 5-benzyl-3-bromo-2-methoxyacetophenone (**8′i**) (145 mg, 0.45 mmol) dissolved in dry DMF (5 mL) was added into the flask via syringe. To this mixture, 2 M $K_3PO_4$ (1.1 mL, 2.3 mmol) was added slowly and the mixture was stirred at 90 °C overnight. After cooling to room temperature, water (5 mL) and EtOAc (10 mL) were added into the reaction mixture. It was then stirred for 10 min, and the aqueous layer was extracted with EtOAc (3× 10 mL). The combined organic extracts were washed successively with water (2× 10 mL) and brine (10 mL), dried ($Na_2SO_4$), and then concentrated. The crude product was purified by column chromatography on silica gel (hexane/EtOAc, 9:1, $R_f = 0.4$) to afford **9′i** as a colorless oil (117 mg, 81% yield): $^1$H NMR ($CDCl_3$, 200 MHz) $\delta$ 7.54–7.22 (m, 12H), 3.98 (s, 2H, $CH_2$), 3.38 (s, 3H, $OCH_3$), 2.65 (s, 3H, $COCH_3$).

#### 4.2.3. 5-Benzyl-2-hydroxybiphenyl-3-carboxylic acid (1i)

**4.2.3.1. Step 1.** Bromine (0.24 mL, 4.7 mmol) was added dropwise to an aqueous KOH (20%, 5 mL) with stirring at −5 to 0 °C. The resulting yellow solution was slowly added to a solution of **9′i** (246 mg, 0.78 mmol) in 1,4-dioxane (10 mL) at 0 °C over a 5-min period. After the addition was complete, the solution was allowed to warm to 10 °C, and stirred at the same temperature for 2 h, during which the color has changed from yellow to light yellow. The mixture was further stirred at 80 °C for 1 h, at which point the solution became colorless. It was cooled, diluted with water (15 mL), acidified with 1 M HCl (10 mL), and extracted with EtOAc (3× 15 mL). The organic extracts were combined, washed successively with aqueous $Na_2S_2O_3$ (25 mL) and brine (25 mL), dried ($Na_2SO_4$), and concentrated. The crude product was taken to the next step without further purification. This crude product (307 mg, 0.96 mmol) was dissolved in MeOH saturated with dry HCl (8 mL) and stirred at 80 °C overnight. After 30 min stirring at room temperature, the solvent was evaporated and the residue was dissolved in EtOAc (20 mL). The organic solution was then washed successively with water (2× 10 mL), 5% $NaHCO_3$(10 mL),

and brine (10 mL), and dried ($Na_2SO_4$). The solvent was removed using a rotary evaporator, and the residue purified by column chromatography (hexane/EtOAc, 9:1, $R_f = 0.4$) to give methyl 5-benzyl-2-methoxybiphenyl-3-carboxylate as a colorless oil (217 mg, 84% yield): $^1H$ NMR ($CDCl_3$, 200 MHz) $\delta$ 7.59–7.19 (m, 12 H), 3.99 (s, 2H, $CH_2$), 3.91 (s, 3H, $CO_2CH_3$), 3.45 (s, 3H, $OCH_3$).

**4.2.3.2. Step 2.** $BBr_3$ (1.0 M in $CH_2Cl_2$, 2.4 mL, 2.4 mmol) was added to a stirred solution of methyl 5-benzyl-2-methoxybiphenyl-3-carboxylate (200 mg, 0.60 mmol) in dry $CH_2Cl_2$ (6 mL) under a nitrogen atmosphere over a 2-min period, while cooling the reaction flask in dry ice–acetone bath. The resulting orange to dark red solution was stirred at room temperature overnight. The reaction was quenched by addition of 1 M HCl (2 mL), and the aqueous layer was extracted with EtOAc (2× 15 mL). The organic extracts were combined and washed successively with water (2× 15 mL) and brine (15 mL), and dried ($Na_2SO_4$). After evaporation of the solvent under reduced pressure, the brown residue was dissolved in 1,4-dioxane (2 mL), and aqueous NaOH (20%, 0.85 mL) was added. The mixture was stirred at 80 °C for 5 h, quenched with 1 M HCl (6 mL), and extracted with EtOAc (2× 15 mL). The combined organic extracts were washed successively with water (2× 15 mL) and brine (15 mL), dried ($Na_2SO_4$), and concentrated. The crude product was recrystallized from EtOAc to give **1i** (94 mg), and the mother liquor was concentrated and purified by silica gel chromatography (1% acetic acid in hexane/EtOAc, 4:1, $R_f = 0.3$) to yield **1i** as a cream-colored solid (67 mg, 88% overall yield): mp 171 °C; $^1H$ NMR ($CDCl_3$, 200 MHz) $\delta$ 10.80 (s, 1H), 7.78 (d, $J = 2.2$ Hz, 1H), 7.57–7.19 (m, 11H), 3.97 (s, 2H, $CH_2$); $^{13}C$ NMR ($CDCl_3$, 100 MHz) $\delta$ 175.14 ($CO_2H$), 158.01, 140.60, 138.71, 136.89, 132.16, 130.82, 129.95, 129.33, 128.72, 128.62, 128.16, 127.50, 126.32, 111.26 ($C_{arom}$), 40.95 ($CH_2$); LRMS *m/z* 304 ($M^+$); HRMS calcd for $C_{20}H_{16}O_3$304.1099 (Mtextsuperscript+), found 304.1099.

Compounds **1a**–**j** were prepared using similar methods and conditions.

**4.2.4. 4-Hydroxybiphenyl-3-carboxylic acid (1a).** Friedel–Crafts acetylation product of 4-phenyl phenol was subjected to haloform reaction, and proctection and deprotection as for **1b** to obtain **1a** as a white solid. mp 214 °C; $^1H$ NMR (DMSO-$d_6$, 200 MHz) $\delta$ 8.04 (d, $J = 2.2$ Hz, 1H), 7.87–7.81 (dd, 8.8 and 2.4 Hz, 1H), 7.64–7.34 (m, 5H), 7.09 (d, $J$=8.8 Hz, 1H); $^{13}C$ NMR (DMSO-$d_6$, 100 MHz) $\delta$ 171.74 ($CO_2H$), 160.55, 139.00, 133.89, 131.29, 128.97, 127.93, 127.07, 126.16, 117.81, 113.33 ($C_{arom}$); LRMS *m/z* 214 ($M^+$); HRMS calcd for $C_{13}H_{10}O_3$214.0630 ($M^+$), found 214.0632.

**4.2.5. 5-Benzyl-2-hydroxybenzoic acid (1d).** In the same way as **1a**, from 4-benzyl phenol, **1d** was obtained as cream-colored recrystallized needles. Mp 139–140 °C; $^1H$ NMR ($CDCl_3$, 200 MHz) $\delta$ 10.25 (s, 1H), 7.75 (d, $J =$ 2.2 Hz, 1H), 7.37–7.15 (m, 6H), 6.96 (d, $J = 8.4$ Hz, 1H), 3.94 (s, 2H, $CH_2$); $^{13}C$ NMR ($CDCl_3$, 100 MHz) $\delta$ 174.79 ($CO_2H$), 160.68, 140.64, 137.86, 132.37, 130.57, 128.75, 128.59, 126.30, 117.99, 110.95 ($C_{arom}$), 40.81 ($CH_2$); LRMS *m/z* 228 ($M^+$); HRMS calcd for $C_{14}H_{12}O_3$228.0786 ($M^+$), found 228.0789.

**4.2.6. 3-Benzyl-2-hydroxybenzoic acid (1e).** From 3-benzyl phenol, **1e** was obtained as a cream-colored solid in the same way as **1a**. Mp 129–131 °C; $^1H$ NMR ($CDCl_3$, 200 MHz) $\delta$ 10.70 (s, 1 H), 7.83–7.78 (dd, $J =$ 7.6 Hz, 1.8 Hz, 1H), 7.35–7.19 (m, 6H), 6.89 (t, 1 H), 4.03 (s, 2 H, $CH_2$); $^{13}C$ NMR ($CDCl_3$, 100 MHz) $\delta$ 174.95 ($CO_2H$), 160.28, 140.06, 137.50, 129.99, 129.06, 128.93, 128.41, 125.12, 119.11, 110.87 ($C_{arom}$), 35.37 ($CH_2$); LRMS *m/z* 228 ($M^+$); HRMS calcd for $C_{14}H_{12}O_3$228.0786 ($M^+$), found 228.0789.

**4.2.7. 3-Benzyl-5-bromo-2-hydroxybenzoic acid (1f).** The Friedel–Crafts acetylated and brominated product of 3-benzyl phenol was subjected to the reaction steps (haloform reaction, protection, and deprotection steps) as for **1b** to obtain **1f** as a cream-colored solid. mp 204 °C; $^1H$ NMR ($CDCl_3$, 200 MHz) $\delta$ 10.68 (s, 1 H), 7.91 (d, $J = 2.2$, Hz, 1H), 7.41 (d, $J = 2.6$ Hz, 1H), 7.30–7.18 (m, 5H), 3.99 (s, 2H, $CH_2$); $^{13}C$ NMR (DMSO-$d_6$, 100 MHz) $\delta$ 171.20 ($CO_2H$), 158.40, 139.65, 137.95, 132.23, 130.06, 128.68, 128.41, 126.13, 114.40, 109.56 ($C_{arom}$), 34.24 ($CH_2$); LRMS *m/z* 308 ($M^+$, $^{81}Br$), 306 ($M^+$, $^{79}Br$); HRMS calcd for $C_{14}H_{11}BrO_3$305.9892 ($M^+$, $^{79}Br$), found 305.9892.

**4.2.8. 5-Benzyl-3-Bromo-2-hydroxybenzoic acid (1g).** In the same way as for **1f**, **1g** was obtained from 4-benzyl phenol as a cream-colored solid. Mp 166 °C; $^1H$ NMR ($CDCl_3$, 200 MHz) $\delta$ 10.90 (s, 1H), 7.72 (d, $J = 2.2$ Hz, 1H), 7.62 (d, $J = 2.2$ Hz, 1H), 7.35–7.15 (m, 5H), 3.92 (s, 2H, $CH_2$); $^{13}C$ NMR ($CDCl_3$, 100 MHz) $\delta$ 174.14 ($CO_2H$), 157.06, 140.79, 139.83, 133.53, 130.02, 128.74, 126.58, 111.99, 111.52 ($C_{arom}$), 40.54 ($CH_2$); LRMS *m/z* 308 ($M^+$, $^{81}Br$), 306 ($M^+$, $^{79}Br$); HRMS calcd for $C_{14}H_{11}BrO_3$305.9892 ($M^+$, $^{79}Br$), found 305.9889.

**4.2.9. 5-Benzyl-4-hydroxybiphenyl-3-carboxylic acid (1h).** Compound **1h** was obtained as a white solid following the same reaction steps as for **1i**. Mp 205 °C; $^1H$ NMR ($CDCl_3$, 200 MHz) $\delta$ 10.87 (s, 1H), 8.04 (d, $J = 2.2$ Hz, 1H), 7.59–7.20 (m, 11H), 4.10 (s, 2H, $CH_2$); $^{13}C$ NMR (DMSO-$d_6$, 100 MHz): $\delta$ 172.38 ($CO_2H$), 158.82, 140.33, 139.10, 134.47, 129.88, 128.95, 128.66, 128.31, 127.04, 126.15, 125.94, 111.81 ($C_{arom}$), 34.80 ($CH_2$); LRMS *m/z* 304 ($M^+$); HRMS calcd for $C_{20}H_{16}O_3$304.1099 ($M^+$), found 304.1101.

**4.2.10. 2-Hydroxy-3,5-diphenylbenzoic acid (1j).** The similar reaction steps for **1i** were performed to 2-phenylphenol to obtain **1j** as cream-colored crystals. Mp 219–221 °C; $^1H$ NMR (DMSO-$d_6$, 200 MHz) $\delta$ 8.08 (d, $J = 2.4$ Hz, 1H), 7.84 (d, $J = 2.2$ Hz, 1H), 7.73–7.35 (m, 10H); $^{13}C$ NMR (DMSO-$d_6$, 100 MHz) $\delta$ 172.49 ($CO_2H$), 158.12, 138.98, 136.77, 134.45, 131.19, 130.02, 129.32, 128.97, 128.12, 127.37, 127.29, 127.17, 126.37, 113.44 ($C_{arom}$).

**4.2.11. 2,4-Dibenzyl phenol (11)**

**4.2.11.1. Step 1.** A mixture of 4-benzyl phenol (3.00 g, 16.3 mmol) and benzoyl chloride (9.5 mL, 82 mmol) in

$CS_2$ (12 mL) was added into ice-cooled suspension of $AlCl_3$ (6.5 g, 49 mmol) in $CS_2$ (20 mL) over a 15-min period. After stirring at 50 °C for 8 h, the flask was cooled in an ice bath and 1 M HCl (30 mL) was added (white precipitate formed). It was then extracted with EtOAc (3× 50 mL), and the organic layers were combined, washed successively with 10% $NaHCO_3$(50 mL), water (2× 50 mL), and brine (50 mL), dried ($MgSO_4$), and concentrated. The crude product was dissolved in 1,4-dioxane (80 mL), treated with 20% NaOH (40 mL), and allowed to stir at 70 °C for 2 h. The reaction was cooled, quenched by acidifying with 1 M HCl (230 mL), and aqueous layer was extracted with EtOAc (3× 60 mL). The organic extracts were combined, washed successively with 5% NaOH (60 mL), water (2× 60 mL), and brine (60 mL), and dried ($MgSO_4$). Evaporation of solvent and purification by silica gel column chromatography (hexane/EtOAc, 2:1, $R_f = 0.4$) afforded C-benzoylated intermediate as a white solid (4.044 g, 86% yield): mp 133 °C; $^1$H NMR ($CDCl_3$, 200 MHz) $\delta$ 7.81–7.72 (m, 4H), 7.58–7.43 (m, 4H), 7.30 (s, 1H), 7.09 (d, $J = 8.4$ Hz, 2H), 6.81–6.77 (m, 2H), 3.99 (s, 2H, $CH_2$).

**4.2.11.2. Step 2.** Hydrazine hydrate (2.8 mL, 90 mmol) and KOH (780 mg, 14 mmol) were added to the intermediate (2.00 g, 6.94 mmol) in ethylene glycol (6 mL). The reaction mixture was stirred at 120 °C for 2 h and allowed to reflux overnight. After cooling the mixture to room temperature, it was diluted with benzene (70 mL), washed with water (2× 80 mL), and then dried ($MgSO_4$). The benzene solution was concentrated under reduced pressure and the purification of resulting residue by silica gel column chromatography (hexane/EtOAc, 4:1, $R_f = 0.3$) gave **11** as white shiny crystals (1.38 g, 73% yield): mp 89 °C; $^1$H NMR ($CDCl_3$, 200 MHz) $\delta$ 7.25–7.02 (m, 11H), 6.76 (d, $J = 8.4$ Hz, 2H), 4.67 (s, 1H, OH), 3.94 (s, 2H, $CH_2$), 3.87 (s, 2H, $CH_2$).

**4.2.12. 3,5-Dibenzyl-2-methoxyacetophenone (12).** Acetylation of **11** (2.45 g, 8.93 mmol) with acetyl chloride (2.5 mL, 36 mmol) and $Et_3N$ (7.5 mL, 54 mmol) in dry ether (50 mL) gave 2,4-dibenzyl O-acetylated intermediate as a white solid (2.426 g, 89% yield). A mixture of this intermediate (500 mg, 1.58 mmol) and acetyl chloride (0.34 mL, 4.7 mmol) in $CS_2$ (1.5 mL) was added into ice-cold suspension of $AlCl_3$ (420 mg, 3.2 mmol) in $CS_2$ (2 mL) over a 5-min period. The reaction mixture was stirred at 60 °C for 5 h. After cooling with an ice bath, the reaction was quenched by addition of 1 M HCl (8 mL, yellow precipitate). It was then extracted with EtOAc (2× 20 mL) and the combined organic extracts were washed successively with 10% $NaHCO_3$(20 mL), water (2× 20 mL) and brine (20 mL), dried ($MgSO_4$), and then concentrated to yield a yellow solid. The residue was dissolved in 1,4-dioxane (4 mL), treated with 20% NaOH (1 mL), and stirred at 70 °C for 2 h. It was cooled and acidified with 1 M HCl (8 mL, brown precipitate), and extracted with EtOAc (3× 20 mL). The organic extracts were combined, washed successively with water (2× 20 mL) and brine (20 mL), dried ($MgSO_4$), and concentrated. The residue was recrystallized from EtOAc to give dibenzyl C-acetylated intermediate (298 mg). The mother liquor was evaporated, and the residue purified by silica gel column chromatography (benzene/EtOAc, 9:1, $R_f = 0.3$), to obtain a yellow solid (58 mg). Overall, 356 mg (71% yield) of solid product was obtained. Methylation of this acetylated intermediate (219 mg, 0.69 mmol) in EtOH (4 mL), 10 M NaOH (0.26 mL), and dimethyl sulfate (0.19 mL, 2.1 mmol) gave **12** as white crystals (214 mg, 94% yield): mp 79 °C; $^1$H NMR ($CDCl_3$, 200 MHz) $\delta$ 7.90 (s, 1H), 7.85 (s, 1H), 7.29–7.25 (m, 3H), 7.11–7.07 (m, 5H), 6.84 (d, $J = 8.8$ Hz, 2H), 3.99 (s, 2H, $CH_2$), 3.89 (s, 2H, $CH_2$), 3.78 (s, 3H, $OCH_3$), 2.57 (s, 3H, $COCH_3$).

**4.2.13. 3,5-Dibenzyl-2-hydroxybenzoic acid (1k).** Compound **1k** was obtained as a cream-colored solid in 90% yield from 3,5-dibenzyl-2-methoxyacetophenone (**12**) following the similar steps as for **1b**. Mp 208–210 °C; $^1$H NMR ($CDCl_3$, 200 MHz) $\delta$ 9.16 (s, 1H), 7.86 (s, 1H), 7.82 (s, 1H), 7.34 (s, 1H), 7.30 (s, 1H), 7.11 (s, 4H), 6.99 (s, 1H), 6.96 (s, 1H), 6.67 (d, $J = 8.4$ Hz, 2H), 3.95 (s, 2H, $CH_2$), 3.76 (s, 2H, $CH_2$); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) $\delta$ 167.19 ($CO_2H$), 155.47, 146.65, 139.82, 137.87, 131.38, 129.53, 129.50, 128.79, 128.74, 128.67, 128.51, 115.11 ($C_{arom}$), 40.13, 38.88 ($CH_2$); LRMS *m*/*z* 318 ($M^+$); HRMS calcd for $C_{21}H_{18}O_3$318.1256 ($M^+$), found 318.1255.

**4.2.14. Methyl 3-bromo-5-(1,3-dioxolan-2-yl)-2-hydroxybenzoate (14)**

**4.2.14.1. Step 1.** Bromine (1 M in $CH_2Cl_2$, 55 mL, 55 mmol) was added dropwise over a 3-h period into the ice-cooled solution of methyl 5-formylsalicylate (4.95 g, 27.47 mmol) in $CH_2Cl_2$ (200 mL) with occasional TLC check of the reaction progress. After the reaction was complete, saturated $NaHCO_3$ (200 mL) was added to the reaction mixture and it was allowed to stir for 30 min. Aqueous layer was extracted with $CH_2Cl_2$ (100 mL). The combined organic layers were washed successively with saturated $NaHCO_3$ (100 mL), water (2× 100 mL) and brine (100 mL), dried ($Na_2SO_4$), and then concentrated. The crude product was dissolved in EtOAc (80 mL) and adsorbed on 7 g of silica. Removal of EtOAc under reduced pressure gave a powder which, after purification on silica gel (hexane/EtOAc, 4:1, $R_f = 0.3$), gave methyl 3-bromo-5-formyl-2-hydroxybenzoate as a light yellow solid (4.72 g, 67% yield): mp 114 °C; $^1$H NMR ($CDCl_3$, 200 MHz): $\delta$ 12.09 (s, 1H), 9.85 (s, 1H), 8.37 (d, $J = 2.2$ Hz, 1H), 8.28 (d, $J = 1.8$ Hz, 1H), 4.05 (s, 3H).

**4.2.14.2. Step 2.** Ethylene glycol (2.53 mL, 45.5 mmol) was added to a solution of methyl 3-bromo-5-formyl-2-hydroxybenzoate (2.35 g, 9.1 mmol), *p*-toluenesulfonic acid (94 mg, 5 mol%), and anhydrous copper sulfate (5.8 g) in benzene (47 mL) and the resulting mixture was refluxed overnight. After cooling to ambient temperature, the reaction mixture was filtered, the residue was washed with EtOAc, and the filtrate was treated with 1 M NaOH (20 mL). The organic solution was washed with water (2× 100 mL) and brine (100 mL), dried ($Na_2SO_4$), and concentrated. The crude product was subjected to silica gel column chromatography (hexane/

EtOAc, 4:1, $R_f = 0.3$) to give 14 as a light yellow solid (1.60 g, 58% yield): mp 72–73 °C; $^1$H NMR ($CDCl_3$, 200 MHz): $\delta$ 11.54 (s, 1H), 7.92 (d, $J = 2.2$ Hz, 1H), 7.86 (d, $J = 2.2$ Hz, 1H), 5.72 (s, 1H), 4.12 (m, 2H), 4.05 (m, 2H), 3.98 (s, 3H).

**4.2.15. Methyl 5-(1,3-dioxolan-2-yl)-3-(furan-2-yl)-2-hydroxybenzoate (15).** A flame-dried flask was cooled to room temperature under a nitrogen purge and charged with dichlorobis(triphenylphosphine)palladium (104 mg, 3 mol%), 1,4-dioxane (3 mL), and **14** (1.5 g, 4.97 mmol) in 1,4-dioxane (5 mL) successively. After nitrogen purging for 10 min, 2-(tributylstannyl)furan (2.34 mL, 7.45 mmol) was added via syringe and the reaction mixture was stirred at 100 °C for 16 h. The reaction mixture was then cooled to room temperature and quenched by addition of 10% ammonium hydroxide (25 mL). It was extracted with EtOAc (3× 100 mL) and the combined organic solution was washed successively with water (2× 100 mL) and brine (100 mL), dried ($Na_2SO_4$), and concentrated. The brown oil was subjected to silica gel column chromatography (hexane/EtOAc, 2:1, $R_f = 0.5$) to give **15** as a white solid (1.14 g, 79% yield): mp 105–108 °C; $^1$H NMR ($CDCl_3$, 200 MHz): $\delta$ 11.73 (s, 1H), 8.15 (d, $J = 2.2$ Hz, 1H), 7.90 (d, $J = 2.2$ Hz, 1H), 7.48 (d, $J = 1.4$ Hz, 1H), 7.15 (d, $J = 3.4$ Hz, 1H), 6.53 (m, 1H), 5.79 (s, 1H), 4.18 (m, 2H), 4.08 (m, 2H), 3.98 (s, 3H).

**4.2.16. 5-Formyl-3-(furan-2-yl)-2-hydroxybenzoic acid (1l)**

**4.2.16.1. Step 1.** HCl (1M, 27 mL) was added to a solution of compound 15 (1.11 g, 3.83 mmol) in chloroform (27 mL). The mixture was heated to reflux for 18 h. The organic layer was separated and the aqueous solution was extracted with $CHCl_3$ (2× 50 mL). The combined organic layers were washed successively with 5% $NaHCO_3$ (3× 50 mL), water (2× 50 mL), and brine (50 mL), and dried over $Na_2SO_4$. The solvent was evaporated and the residue was recrystallized from EtOAc to provide methyl 5-formyl-3-(furan-2-yl)-2-hydroxybenzoate as brown needles (902 mg, 96% yield): mp 160–161 °C; $^1$H NMR ($CDCl_3$, 200 MHz): $\delta$ 12.25 (s, 1H), 9.93 (s, 1H), 8.54 (d, $J = 2.2$ Hz, 1H), 8.32 (d, $J = 2.2$ Hz, 1H), 7.53 (d, $J = 1.4$ Hz, 1H), 7.19 (d, $J = 3.6$ Hz, 1H), 6.57 (m, 1H), 4.03 (s, 3H).

**4.2.16.2. Step 2.** Methyl 5-formyl-3-(furan-2-yl)-2-hydroxybenzoate (190 mg, 0.77 mmol) was dissolved in methanol (15 mL) and mixed with 1 M NaOH (2 mL). The mixture was heated to reflux for 1 day. After cooling to room temperature, the reaction mixture was acidified with 1 M HCl (2.5 mL), and solvent was evaporated to remove methanol. The residue was dissolved in EtOAc (50 mL), and washed successively with water (2× 30 mL) and brine (30 mL), and dried ($Na_2SO_4$). The solvent was evaporated under reduced pressure to give brownish solid (153 mg, 85% yield). It could be further purified by recrystallization from EtOAc to provide 1l as brown needles: mp 208–210 °C (dec); $^1$H NMR (DMSO- $d_6$, 200 MHz): $\delta$ 9.96 (s, 1H), 8.42 (d, $J =$ 2.2 Hz, 1H ), 8.33 (d, $J = 2.2$ Hz, 1H), 7.86 (d, $J = 1.8$ Hz, 1H ), 7.15 (d, $J = 3.6$ Hz, 1H ), 6.68 (m, 1H); $^{13}$C NMR (DMSO-$d_6$, 100 MHz): $\delta$ 191.16 (CHO), 171.94 ($CO_2H$), 161.94, 147.60, 142.92, 131.41, 129.63, 127.63, 119.76, 113.97, 112.25, 111.34; LRMS $m/z$ 232 ($M^+$); HRMS calcd for $C_{12}H_8O_5$232.0372 ($M^+$), found 232.0368.

**4.2.17. 2-Bromo-6-(2-propenyl)phenol (17).** To a solution of 2-bromophenol (13.43 g, 77.6 mmol) in acetone (90 mL) were added $K_2CO_3$ (19.1 g, 138.15 mmol) and allyl bromide (14.83 g, 122.62 mmol). The mixture was refluxed for 4 h and cooled to ambient temperature. The inorganic salt was removed by filtration and the organic solution was evaporated to dryness. Resulting dark brown solution was passed through a cotton plug and taken to the next step. The crude product obtained above was irradiated with microwave (Samsung, 700 W) for 66 min maintaining the temperature 200 °C. Compound **17** was obtained as a dark-colored liquid (16.5 g, 100% yield): $^1$H NMR ($CDCl_3$, 200 MHz): $\delta$ 7.33 (d, $J = 8$ Hz, 1H), 7.08 (d, $J = 8$ Hz, 1H), 6.77 (t, $J = 8$ Hz, 1H), 6.05 (m, 1H), 5.59 (s, 1H), 5.13 (m, 2H), 3.44 (d, $J = 6.6$ Hz, 2H).

**4.2.18. 3-Bromo-2-methoxy-1-(1-propenyl)benzene (18).** Methyl iodide (14.5 mL, 232 mmol) was added to the mixture of **17** (16.5 g, 77.5 mmol) and $K_2CO_3$ (16 g, 116 mmol) in acetone (130 mL) and the mixture was heated to reflux for 4.5 h. After cooling to room temperature, inorganic salt was removed by filtration and the solvent was evaporated to dryness. The crude product was passed through a silica gel pad to obtain brown liquid, which was dissolved in EtOH (62 mL) and mixed with KOH (27 g, 0.48 mol) in $H_2O$ (9 mL). The resulting mixture was stirred at 90 °C for 5 h and then cooled to room temperature. EtOH was removed under reduced pressure and the brown aqueous solution was diluted with water (140 mL). It was extracted with EtOAc (2× 100 mL), and the combined extracts were washed with water (2× 100 mL) and brine (100 mL), dried ($Na_2SO_4$), and then concentrated to afford **18** as a brown oil (13.3 g, 75% yield): $^1$H NMR ($CDCl_3$, 200 MHz): $\delta$ 7.40 (d, $J = 7.6$ Hz, 2H), 6.96 (t, $J = 7.6$ Hz, 1H), 6.69 (m, 1H), 6.32 (m, 1H), 3.79 (s, 3H), 1.94 (dd, $J = 1.4$, 6.6 Hz, 3H).

**4.2.19. 3-Bromosalicylic acid (1m)[18]**

**4.2.19.1. Step 1.** Potassium permanganate (3.45 g, 21.81 mmol) was added to the solution of 18 (1.5 g, 6.61 mmol) in acetone (80 mL) while cooling the reaction flask at 0 °C. After stirring the heterogeneous mixture at 0 °C for 3 h, solvent was removed under reduced pressure. The dark brown solid was mixed with $H_2O$ (17 mL) and $NaHSO_3$ (4 g) and the resulting mixture was stirred for 1 h at room temperature. It was filtered by passing through Celite and the filtrate was acidified with concd HCl (1 mL) and additional 1 M HCl (2 mL). The white precipitate was separated and dissolved in 2 M NaOH (15 mL). The pale yellow solution was washed with $CH_2Cl_2$ (2× 6 mL) and the organic washings were discarded. The aqueous solution was neutralized with concd HCl (1.5 mL) and acidified with 1 M HCl (5.3 mL). White precipitate was filtered, washed with 0.1 M HCl (3 mL), and dissolved in EtOAc

(30 mL). The EtOAc solution was then washed with brine (1 mL), dried ($Na_2SO_4$), and concentrated to obtain 3-bromo-2-methoxybenzoic acid as a white solid (846 mg, 57% yield): mp 119–121 °C; $^1$H NMR ($CDCl_3$, 200 MHz): $\delta$ 10.01 (br s, 1H, exchangeable with $D_2O$), 8.03 (dd, $J$ = 1.8, 8 Hz, 1H), 7.82 (dd, $J$ = 1.8, 8 Hz, 1H), 7.14 (t, $J$ = 8 Hz, 1H), 4.02 (s, 3H).

**4.2.19.2. Step 2.** A mixture of 3-bromo-2-methoxybenzoic acid (800 mg, 3.46 mmol), PhSH (0.35 ml, 3.46 mmol), and $K_2CO_3$ (29 mg, 0.207 mmol) in anhydrous NMP (3.46 mL) was heated at 190 °C for 40 min under nitrogen atmosphere. The reaction mixture was cooled, diluted with saturated aqueous $NaHCO_3$solution (35 mL), and extracted with EtOAc (3× 30 mL, discarded). The aqueous layer was acidified with 6 M HCl (10 mL) and extracted with EtOAc (3× 30 mL). The combined organics were washed with water (2× 10 mL) and brine (20 mL), dried ($Na_2SO_4$), and concentrated to afford a yellow oil. The crude product was subjected to silica gel column chromatography 2% AcOH in hexane/EtOAc, 2:1, to obtain pure 1m as a white solid (480 mg, 64% yield): mp 179–182 °C; $^1$H NMR ($CDCl_3$, 200 MHz): $\delta$ 11.01 (s, 1H), 7.94 (d, $J$ = 8 Hz, 1H), 7.81 (d, $J$ = 8 Hz, 1H), 6.86 (t, $J$ = 8 Hz, 1H); $^{13}$C NMR (Acetone-$d_6$, 100 MHz) $\delta$ 172.47 ($CO_2H$), 159.64, 140.03, 130.83, 121.04, 114.68, 111.45, ($C_{arom}$); LRMS $m/z$ 218 ($M^+$, $^{81}$Br), 216 ($M^+$, $^{79}$Br); HRMS calcd for $C_7H_5BrO_3$215.9422 ($M^+$, $^{79}$Br), found 215.9421.

**4.2.20. 3,3′-Dicarboxy-5,5′-difluoro-2,2′-dihydroxydiphenylmethane (3a).**[19] To a suspension of 5-fluorosalicylic acid (500 mg, 3.20 mmol) in 1,4-dioxane (1 mL) was added conc. $H_2SO_4$ (3 mL) dropwise while cooling the reaction flask in water bath. Paraformaldehyde (97 mg) was added and the mixture was vigorously stirred at 65 °C for 9 h. The reaction mixture was poured on crushed ice (18 g). The precipitate was filtered and subjected to column chromatography (3% AcOH in hexane/EtOAc, 1:1, $R_f$ = 0.3) to give **3a** as a white solid (300 mg, 58% yield). ): mp >300 °C (dec); $^1$H NMR (Acetone-$d_6$, 200 MHz) $\delta$ 11.57 (br s, 2 H), 7.49 (dd, $J$ = 8.8 Hz, 3.4 Hz, 2H), 7.27 (dd, $J$ = 9.0 Hz, 3.4 Hz, 2H), 4.03 (s, 2H); $^{13}$C NMR (Acetone-$d_6$, 100 MHz) $\delta$ 172.27 ($CO_2H$), 157.60, 154.36, 130.83, 124.74, 114.44, 113.14, 30.49 ($CH_2$); LRMS $m/z$ 324 ($M^+$); HRMS calcd for $C_{15}H_{10}F_2O_6$324.0445 ($M^+$), found 324.0449.

**4.2.21. 5,5′-Dibromo-3,3′-dicarbomethoxy-2,2′-dihydroxydiphenylmethane (20).** [20] To a stirred suspension of methyl 5-bromosalicylate (725 mg, 3.14 mmol) in water (0.31 mL) was added concentrated $H_2SO_4$ (12.5 mL) over a 20-min period while the temperature was maintained between −5 and 0 °C. Then 1:1 mixture of 37% aqueous HCHO and MeOH (3.1 mL) was added to the reaction mixture over a 15-min period. The reaction mixture was then stirred at 0 °C for 2 h and at room temperature for 22 h, and poured into crushed ice (100 g). White precipitate was filtered, washed with water, and dried to give a brown solid. The crude product was column chromatographed on silica gel ($CHCl_3$) to obtain **20** as a white solid (304 mg, 41% yield): mp 233 °C; $^1$H NMR ($CDCl_3$, 200 MHz) $\delta$ 11.04 (s, 2H), 7.86 (d, $J$ = 2.6 Hz, 2H), 7.47 (d, $J$ = 1.8 Hz, 2H), 3.97 (s, 2H, $CH_2$), 3.95 (s, 6H, $CH_3$); $^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$ 169.83 ($CO_2Me$), 158.73, 138.93, 130.53, 130.16, 113.49, 110.54, ($C_{arom}$), 52.62 ($CH_3$), 28.80 ($CH_2$).

**4.2.22. 5,5′-Dibromo-3,3′-dicarboxy-2,2′-dihydroxydiphenylmethane (3b).**[20] To a solution of **20** (100 mg, 0.21 mmol) in 1,4-dioxane (1 mL) was added 10% aqueous NaOH (2.0 mL) and the resulting solution was stirred at 80 °C for 40 min. The reaction mixture was cooled to room temperature and acidified by addition of 1 M HCl (3 mL). The white precipitate was dissolved in EtOAc (10 mL) and aqueous layer was extracted with EtOAc (2× 10 mL). The combined organic extracts were washed with water (2× 10 mL) and brine (10 mL), dried ($Na_2SO_4$), and concentrated under reduced pressure to give **3b** as a white solid (91 mg, 97% yield): mp 281–286 °C (dec), rep 290–291 °C; $^1$H NMR ($CDCl_3$, 200 MHz) $\delta$ 11.17 (s, 2H), 7.88 (d, $J$ = 2.6 Hz, 2H), 7.56 (d, $J$ = 2.4 Hz, 2H), 4.01 (s, 2H, $CH_2$); LRMS $m/z$ 446 ($M^+$, $^{81}$Br), 444 ($M^+$, $^{79}$Br); HRMS calcd for $C_{15}H_{10}Br_2O_6$443.8844 ($M^+$, $^{79}$Br), found 443.8848.

**4.2.23. 5,5′-Dibromo-3,3′-dicarbomethoxy-2,2′-dimethoxydiphenylmethane (21).** In a 250-mL flask fitted with a reflux condenser and $CaCl_2$ tube was added **20** (2.28 g, 4.81 mmol) in anhydrous acetone (100 mL), $K_2CO_3$ (2.0 g, 14 mmol), and dimethyl sulfate (2.3 mL, 24 mmol). After refluxing overnight, the solvent was evaporated under reduced pressure, and the solid was partitioned between water (100 mL) and EtOAc (100 mL). The organic layer was separated to save and the aqueous layer was extracted with EtOAc (2× 100 mL). The combined organic extracts were washed successively with water (2× 50 mL) and brine (50 mL), and dried ($Na_2SO_4$). After concentration, the crude product was column chromatographed (hexane/EtOAc, 9:1) to give **21** as a white solid (2.35 g, 97% yield): mp 81 °C; $^1$H NMR ($CDCl_3$, 200 MHz) $\delta$ 7.85 (d, $J$ = 2.6 Hz, 2H), 7.35 (d, $J$ = 2.6 Hz, 2H), 4.02 (s, 2H, $CH_2$), 3.93 (s, 6H, $CO_2CH_3$), 3.78 (s, 6H, $OCH_3$).

**4.2.24. 3,3′-Dicarboxy-2,2′-dihydroxy-5,5′-diphenyldiphenylmethane (3c)**

**4.2.24.1. Step 1.** A flame-dried flask was cooled to room temperature under a nitrogen purge and charged with (4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzene (463 mg, 2.27 mmol) and tetrakis(triphenylphosphine)palladium (34 mg, 0.03 mmol). After nitrogen purging for 20 min, compound **22** (245 mg, 0.77 mmol) dissolved in dry DMF (16 mL) was added into the flask via syringe. Then 2 M $K_3PO_4$ (3.7 mL, 7.6 mmol) was slowly added and the mixture was stirred at 80 °C overnight. The reaction mixture was allowed to cool to room temperature, diluted with water (20 mL) and EtOAc (20 mL), and stirred for 10 min. The aqueous layer was extracted with EtOAc (2× 50 mL), and the combined organic layers were washed successively with water (2× 50 mL) and brine (50 mL), dried ($Na_2SO_4$), and concentrated. The black oil was purified by silica gel column chromatography (hexane/EtOAc, 2:1,

$R_f = 0.5$) to obtain 3,3′-dicarbomethoxy-2,2′-dimethoxy-5,5′-diphenyldiphenylmethane as a pale yellow solid (125 mg, 33% yield): mp 147 °C; $^1$H NMR ($CDCl_3$, 200 MHz) $\delta$ 7.94 (d, $J = 1.6$ Hz, 2H), 7.52–7.31 (m, 12H), 4.23 (s, 2H, $CH_2$), 3.95 (s, 6H, $CO_2CH_3$), 3.84 (s, 6H, $OCH_3$).

**4.2.24.2. Step 2.** $BBr_3$ (1.0 M in $CH_2Cl_2$, 2.0 mL, 2.0 mmol) was added to a stirred solution of 3,3′-dicarbomethoxy-2,2′-dimethoxy-5,5′-diphenyldiphenylmethane (125 mg, 0.20 mmol) in dry $CH_2Cl_2$ (5 mL) under a nitrogen atmosphere over 2 min period with cooling the reaction mixture in dry-ice acetone base. The resulting orange to dark red solution was stirred at room temperature overnight. The reaction was quenched by addition of 1 M HCl (4 mL) and the aqueous layer was extracted with EtOAc (3× 20 mL). The organic extracts were combined, washed successively with water (2× 20 mL) and brine (20 mL), and then dried ($Na_2SO_4$). After evaporation of the solvent under reduced pressure, the brown residue was dissolved in 1,4-dioxane (4 mL) and mixed with 20% aqueous NaOH (0.74 mL). The reaction mixture was stirred at 80 °C for 4 h. After evaporation of the solvent, the brown solution was diluted with water (2 mL) and washed with EtOAc (2× 4 mL). The aqueous layer was acidified with 1 M HCl (4 mL), and the precipitate formed was dissolved in 100 mL EtOAc. The organic layer was washed successively with water (2× 20 mL) and brine (30 mL), and then dried ($Na_2SO_4$). Evaporation of the solvent under reduced pressure gave **3c** as a yellowish brown solid (86 mg, 98% yield): mp >300 °C (dec); $^1$H NMR (DMSO-$d_6$, 200 MHz) $\delta$ 11.82 (s, 2H, OH), 7.93 (d, $J = 2$ Hz, 2H), 7.73 (d, $J = 2$ Hz, 2H), 7.57–7.32 (m, 10H), 4.09 (s, 2H, $CH_2$); $^{13}$C NMR (DMSO-$d_6$, 100 MHz): $\delta$ 172.37 ($CO_2H$), 159.06, 139.23, 134.46, 130.65, 129.01, 128.33, 127.04, 126.14, 112.75, ($C_{arom}$), 40.14 ($CH_2$); LRMS *m*/*z* 440 ($M^+$); HRMS calcd for $C_{27}H_{20}O_6$440.1260 ($M^+$), found 440.1259.

### 4.2.25. 3,3′-Dicarboxy-5,5′-difuran-2-yl-2,2′-dihydroxy diphenylmethane (3d)

**4.2.25.1. Step 1.** A flame-dried flask was cooled to room temperature under a nitrogen purge and charged with dichlorobis(triphenylphosphine)palladium (51 mg, 0.07 mmol) and 1,4-dioxane (0.5 mL). Compound **21** (455 mg, 0.91 mmol) in 1,4-dioxane (10 mL) was then added. After 10 min nitrogen purging, 2-(tributylstannyl)furan (1.14 mL, 3.62 mmol) was added via syringe and the reaction mixture was stirred at 100 °C for 7 h. The reaction mixture was then cooled to room temperature and quenched by addition of 10% ammonium hydroxide (40 mL). It was extracted with EtOAc (3× 50 mL) and the combined organic solution was washed successively with water (2× 50 mL) and brine (50 mL), dried ($Na_2SO_4$), and concentrated. The brown solid was subjected to silica gel column chromatography to give 3,3′-dicarbomethoxy-5,5′-difuran-2-yl-2,2′-dimethoxydiphenylmethane as a yellow solid (248 mg, 58% yield): mp 107 °C; $^1$H NMR ($CDCl_3$, 200 MHz) $\delta$ 8.01 (d, $J = 2.2$ Hz, 2H), 7.57 (d, $J = 2.2$ Hz, 2H), 7.43 (d, $J = 1.8$ Hz, 2H), 6.58 (d, $J = 3.2$ Hz, 2H), 6.45 (m, 2H), 4.15 (s, 2H, $CH_2$), 3.95 (s, 6H, $CO_2CH_3$), 3.79 (s, 6H, $OCH_3$).

**4.2.25.2. Step 2.** A mixture of 3,3′-dicarbomethoxy-5,5′-difuran-2-yl-2,2′-dimethoxydiphenylmethane (248 mg, 0.52 mmol), PhSH (0.21 ml, 2.1 mmol), and $K_2CO_3$ (140 mg, 1.0 mmol) in anhydrous NMP (20 mL) was heated at 190 °C for 40 min under nitrogen atmosphere. The reaction mixture was cooled and saturated aqueous $NaHCO_3$ (25 mL) was added. It was extracted with EtOAc (3× 100 mL) and the organic layers discarded. The aqueous layer was acidified with 1 M HCl (40 mL) and extracted with EtOAc (3× 50 mL). Combined organic extracts were washed with water (2× 50 mL) and brine (50 mL), dried ($Na_2SO_4$), and concentrated to afford yellow solid (215 mg). It was washed with EtOAc to obtain pure **3d** as a yellow solid (165 mg, 75% yield): mp >300 °C (dec);$^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ 7.98 (d, $J = 2.4$ Hz, 2H), 7.68 (d, $J = 2.4$ Hz, 2H), 7.65 (d, $J = 2$ Hz, 2H), 6.77(d, $J = 3.2$ Hz, 2H), 6.52 (m, 2H), 4.01 (s, 2H); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) $\delta$ 172.18 ($CO_2H$), 158.84, 152.17, 142.42, 131.03, 128.31, 123.16, 121.47, 112.76, 111.99, 104.57, 38.89 ($CH_2$); LRMS *m*/*z* 420 ($M^+$); HRMS calcd for $C_{23}H_{16}O_8$420.0845 ($M^+$), found 420.0844.

### 4.2.26. 3,3′-Dicarboxy-2,2′-dihydroxy-5,5′-dithiophen-2-yl-diphenylmethane (3e).

In a similar way with the synthesis of **3c**, **3e** was obtained from **20** (300 mg, 0.63 mmol), tetrakis(triphenylphosphine)palladium (29 mg), 2.0 M $K_3PO_4$ (3.16 mL, 6.33 mmol), 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiophene (332 mg, 1.58 mmol), and DMF (9 mL). Compound **3e** was obtained as a white solid (72 mg, 25% yield) along with the monosubstituted product (37 mg, 13% yield): mp >300 °C (dec); $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ 7.89 (d, $J = 2.4$ Hz, 2H), 7.67 (d, $J = 2.4$ Hz, 2H), 7.45 (m, 2H), 7.32 (m, 2H), 7.08 (m, 2H), 4.01 (s, 2H, $CH_2$); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) $\delta$ 172.13 ($CO_2H$), 158.98, 142.44, 133.15, 128.52, 128.41, 124.98, 124.83, 124.65, 122.93, 112.82, 38.88 ($CH_2$); LRMS *m*/*z* 452 ($M^+$); HRMS calcd for $C_{23}H_{16}O_6S_2$452.0388 ($M^+$), found 452.0387.

## 4.3. $IC_{50}$ determination

The PTPase activities were assayed in buffer A (100 mM Hepes, 5 mM EDTA, pH 7.0) at 37 °C in a final *p*NPP concentration of 2 mM. For this assay, the enzyme was diluted with enzyme dilution buffer (25 mM Hepes, 5 mM EDTA, 1 mM DTT, and 1 mg/mL bovine serum albumin, pH 7.3). The absorbance at 405 nm was measured to determine the amount of *p*-nitrophenol released. The $IC_{50}$ values of the inhibitors were determined by measuring the PTPase activity with a range of different inhibitor concentrations. A typical 50-μL reaction system contained 5 μL *p*NPP, 5 μL enzyme, 5 μL inhibitor dissolved in DMSO, 10 μL 5× buffer A, and 25 μL $H_2O$. After the mixture, without *p*NPP, had been incubated at 37 °C for 10 min, the enzyme reaction was initiated by the addition of *p*NPP. After 3 min at 37 °C, the reaction was quenched by the addition of 0.5 M NaOH (0.95 mL), with the absorbance at 405 nm measured to quantify the *p*-nitrophenol produced. The concentrations of PTPases in the assay mixtures were 2.1 μg/mL for PTP1B, 5 μg/mL for SHP-1cat,

30 ng/mL for YPTP1 and 33 U (manufacturer's definition)/mL for LAR and TC-PTP. The kinetic data were analyzed using the GraFit 5.0 program (Erithacus Software).

### 4.4. Mouse experiment for obesity

Twenty-six C57BL/6J Jms Slc mice (4-week-old, male, 17–19 g) were purchased from Japan SLC Inc., Haruno Breeding branch. The mice were individually housed and maintained in a 12-h light/dark cycle at 22 ± 2 °C. Food and water were available ad libitum. The experimental diets, high fat diet (HFD, D12451) and low fat diet (LFD, D10012G) containing 45% and 16% of the calories from fat, respectively, were obtained from Research Diets (New Brunswick, NJ). The diets were either in pellet or powder form.

All mice were acclimatized for 1 week (LFD), with 18 mice fed HFD for first 8 weeks of the study for the development of obesity; the remaining eight were fed LFD. The mice assigned to the LFD group were maintained on this diet throughout the study, as a lean control group. At week 8, all the HFD-fed mice were assigned to one of two groups containing nine mice each. The first group remained on HFD throughout the study, as an obese control group. The remaining group was fed HFD containing the PTP1B inhibitor, **3c**, for 4 weeks. The concentration of **3c** in the diets was 5 g/kg of diet (0.5% w/w).

LFD was provided in pellet form throughout the experiment. Conversely, the obese control and inhibitor-treated mice groups were fed with HFD in pellet form for 8 weeks of the obesity developing period, and then the powdered form during the inhibitor treatment period. Because the inhibitor is acidic, 2.5 g (5.7 mmol) of **3c** was dissolved in 11.4 mL of 1.0 M NaOH (2 equiv) and 50 mL of water. The solution was then added to 500 g of the HFD powder and kneaded to form a dough. Similarly, the HFD powder was kneaded with the addition of same volume of distilled water in 500 g of HFD powder.

The body weight and food intake were recorded every other day throughout the study. On completion of the experiment, after 8 h of fasting, the mice were anesthetized with secobarbital by an ip injection (40 mg/kg body weight), with blood samples taken by cardiac puncture into EDTA tubes and immediately placed on ice. Blood samples were spun (5000*g*, 10 min, 4 °C), with the plasma removed and frozen until further analysis.

Plasma was analyzed for glucose, triglyceride, total cholesterol, and free fatty acids using diagnostic kits (Glucose C2, TG E, T-Cho E, and NEFA C, respectively, from Wako Pure Chemical Industries Ltd. Osaka, Japan) following the manufacturer's protocol. A mixture of 10 μL of plasma sample and color reagent (1 mL for cholesterol and 1.5 mL for glucose and TG) was incubated at 37 °C for 5 min, and then $A_{600}$ for cholesterol and TG, and $A_{505}$ for glucose, measured. To determine the NEFA concentration, 25 μL of plasma sample and 1.5 mL of NEFA C reagent were incubated at 37 °C for 20 min, and then $A_{550}$ measured.

Liver, epididymal fat depots, and retroperitoneal fat depots were excised immediately following blood collection, washed in cold physiological (isotonic) saline, gently blotted, and then weighed. Tissues were immediately frozen in liquid nitrogen and stored at −75 °C for further analysis.

### 4.5. Statistical analysis

The data for the mice were expressed as means ± SEM. Data were analyzed using a 1-way ANOVA with the SPSS version 11.5 statistical package for windows (SPSS Inc., Chicago, Illinois). Differences were considered significant at $p < 0.05$.

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