糖尿病治療薬を指向した
縮合環アルカン酸系 GPR40 作動薬の
創薬研究

2013

根　来　伸　行

目次

略語表

AcOEt	ethyl acetate
AcOH	acetic acid
ADDP	1,1′-(azodicarbonyl)dipiperidine
ADME-Tox	absorption, distribution, metabolism, excretion and toxicology
AIBN	2,2′-azobis(isobutyronitrile)
aq.	aqueous
Arg	arginine
AUC	area under the curve
B(*i*-PrO)$_3$	triisopropyl borate
Bn	benzyl
BSA	bovine serum albumin
Bu	butyl
CHO	Chinese hamster ovary
CL	clearance
C_{max}	maximum drug concentration
Compd	compound
DAG	diacylglycerol
dba	dibenzylideneacetone
DEAD	diethyl azodicarboxylate
DHA	docosahexaenoic acid
DMA	*N*,*N*-dimethylacetamide
DME	1,2-dimethoxyethane
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPP-4	dipeptidyl peptidase-4
dppf	(diphenylphosphino)ferrocene
EC_{50}	50% effective concentration

ED	effective dose
ER	endoplasmic reticulum
Et	ethyl
Et_3N	triethylamine
EtOH	ethanol
F	bioavailability
FFA	free fatty acid
FLIPR	fluorometric imaging plate reader
GK	Goto-Kakizaki
GLP-1	glucagon-like peptide-1
GPCR	G protein-coupled receptor
GPR40	G protein-coupled receptor 40
GSIS	glucose-stimulated insulin secretion
HPLC	high-performance liquid chromatography
IP_3	inositol trisphosphate
iv	intravenous
Leu	leucine
Lys	lysine
m-CPBA	*m*-chloroperbenzoic acid
Me	methyl
MeCN	acetonitrile
MeOH	methanol
MsCl	methanesulfonyl chloride
NBS	*N*-bromosuccinimide
n-Bu_4NBr_3	tetrabutylammonium tribromide
n-BuLi	*n*-butyl lithium
NCS	*N*-chlorosuccinimide
NE	not effective
NH_4OAc	ammonium acetate
Ns	2-nitrobenzenesulfonyl

NT	not tested
OGTT	oral glucose tolerance test
$P(n\text{-}Bu)_3$	tributylphosphine
Pd/C	palladium on carbon
Phe	phenylalanine
PIP_2	phosphatidylinositol 4,5-bisphosphate
PLC	phospholipase C
po	per os
PPh_3	triphenylphosphine
p-TsCl	*p*-toluenesulfonyl chloride
rt	room temperature
SD	standard deviation
siRNA	small interfering ribonucleic acid
SPhos	2-dicyclohexylphosphino-2′,2′-dimethoxybiphenyl
SU	sulfonyl urea
TBAF	tetrabutylammonium chloride
TBSCl	*tert*-butyldimethylsilyl chloride
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TM	transmembrane
T_{max}	time to maximum value
Trp	tryptophan
Tyr	tyrosine

緒論

糖尿病

国際糖尿病連合 (International Diabetes Federation) の最新の発表よると、世界の糖尿病有病数は 3 億 7100 万人で、その数は途上国を中心に急速に増加しており、2030 年時点で 5 億 5200 万人にも達すると予想されている [1)]。全糖尿病の約 90%を占める 2 型糖尿病は、インスリン感受性の低下を特徴とする病態で、しばしば膵 β 細胞からのインスリン分泌能の低下を伴う。長期にわたり高血糖状態が続くと、動脈硬化症、冠動脈性心疾患、腎症、神経障害、網膜症などの大血管障害や小血管障害のリスクが高まる。従って、血糖値を適切にコントロールすることが、糖尿病の管理と治療に重要である。

糖尿病治療薬の現状と期待される新薬候補

これまでスルホニルウレア (sulfonyl urea: SU) 系やグリニド系に分類されるインスリン分泌促進薬が、糖尿病治療の第一選択薬の 1 つとして使用されてきた (Figure 1)[2)]。これらの薬剤は、膵 β 細胞の ATP 依存性カリウムチャネルに結合して膜の脱分極を引き起こし、電位依存性カルシウムチャネルを開口させてインスリン分泌を促進する。しかしながら、その作用は細胞外のグルコース濃度に依存しないため、低血糖を引き起こす懸念がある [3)] とともに、長期間の投与により膵 β 細胞の機能低下やアポトーシスを引き起こす懸念がある (二次無効)[4)]。さらに、英国の一般診療研究データベース (General Practice Research Database) における 9 万人の症例を解析した結果、SU 系薬剤は、同じく糖尿病治療薬として汎用されているビグアナイド系薬剤のメトホルミン (Figure 1) と比較して、循環器系副作用の発生リスクが高いことが明らかとなった [5)]。これら薬剤の安全面における懸念を払拭するためには、薬剤の血中濃度を厳格にコントロールする必要がある。一方、グリニド系薬剤は、短時間作用型のインスリン分泌促進薬であり、SU 系薬剤と比較して低血糖の懸念は低いものの、食事直前の投薬が必須なため患者の利便性に問題があるとともに、その作用は食後

過血糖の改善に限定される。このような背景から、近年、グルコース濃度依存的なインスリン分泌 (glucose-stimulated insulin secretion: GSIS) を促進する薬剤である、ジペプチジルペプチダーゼ-4 (dipeptidyl peptidase-4: DPP-4) 阻害薬 [6] やグルカゴン様ペプチド-1 (glucagon-like peptide-1: GLP-1) アナログ [7] が、次世代の糖尿病治療薬として注目を集めている (Figure 1)。

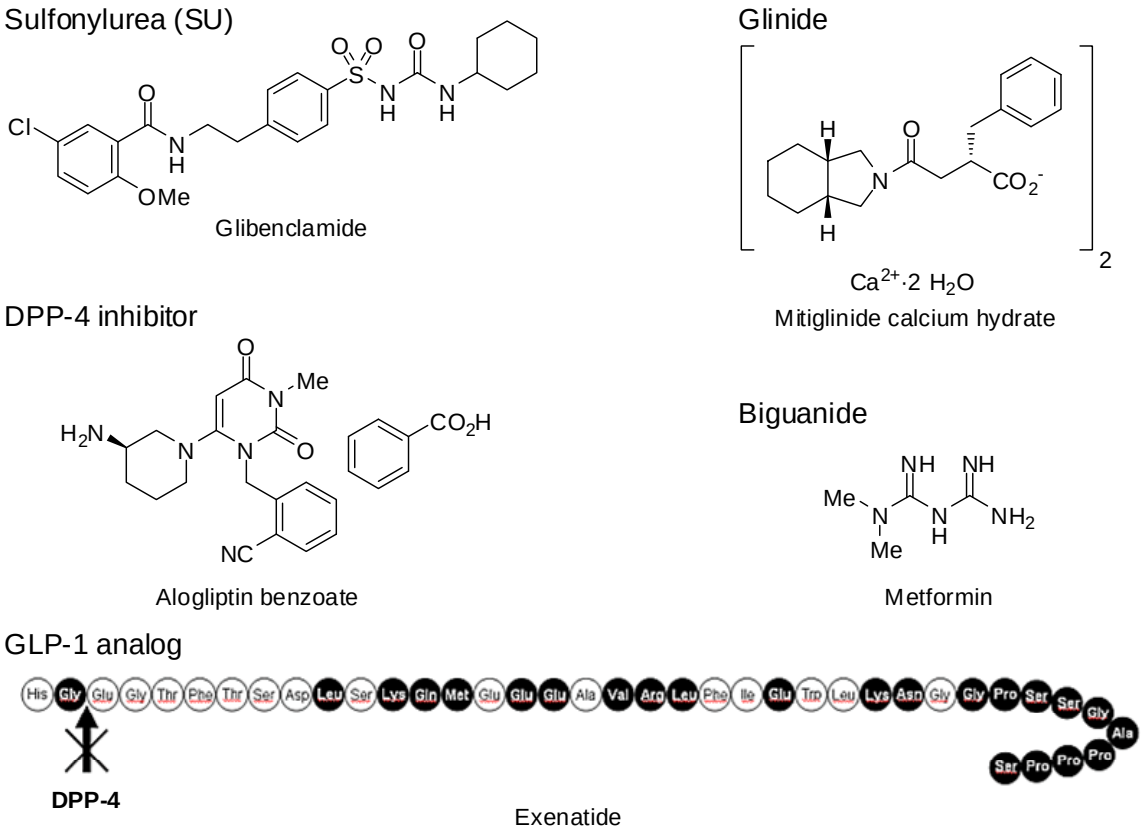


Figure 1. Representative antidiabetic drugs.

遊離脂肪酸と GPR40

遊離脂肪酸 (free fatty acid: FFA) は、エネルギー源として有用であるだけでなく、シグナル伝達分子としても重要な役割を果たしている。FFA は膵 β 細胞に対して二面的な作用を持つことが知られている。膵 β 細胞を使った in vitro の実験から、短時間の FFA 暴露は GSIS を促進することが明らかとなっており [8–11]、また絶食ラットおよびヒトでの in vivo の試験から、FFA が糖依存的なインスリン分泌に重要な役割を果たすことが示されている [9,10]。すなわち、空腹時に上昇する FFA は単なる栄養素となるだけではなく、インスリン分泌を促進する作用

も担っており、食事により吸収された糖質を速やかにエネルギーとして貯蔵するのに役立っていると考えられる。一方、持続的な高濃度の暴露によって膵 β 細胞の機能低下やインスリン分泌能の低下 (脂肪毒性) を促すことが報告されている [11,12]。さらに FFA はインスリン抵抗性惹起の重要な役割を担い、ひいては 2 型糖尿病、肥満、高脂血症などを含むメタボリックシンドロームを引き起こすことも知られている [13]。FFA が GSIS を引き起こすメカニズムは長年にわたり不明であったが、2003 年になって G protein-coupled receptor 40 (GPR40)[14] が FFA による GSIS に関与することが報告された [15]。

GPR40 は、1997 年に内因性リガンドの不明なオーファン G タンパク共役型受容体 (G protein-coupled receptor: GPCR)[16] としてクローニングされた [17]。その後、武田薬品工業を含む 3 つの研究グループからほぼ同時期に、GPR40 が中長鎖遊離脂肪酸を内因性リガンドとし、膵 β 細胞に高発現することが報告された [15,18,19]。GPR40 は G_q ファミリーに属しており、リガンドである FFA が結合すると、GPCR 複合体から $G\alpha_q$ サブユニットが解離してホスホリパーゼ C (phospholipase C: PLC) を活性化し、ホスファチジルイノシトール 4,5-ビスリン酸 (phosphatidylinositol 4,5-bisphosphate: PIP_2) のジアシルグリセロール (diacylglycerol: DAG) とイノシトール-3-リン酸 (inositol trisphosphate: IP_3) への加水分解を促進する。生じた DAG はプロテインキナーゼを活性化し、IP_3 は小胞体 (endoplasmic reticulum: ER) からの細胞内 Ca^{2+}放出を促す (Figure 2)。このように、G_q パスウェイを通じて細胞内 Ca^{2+}濃度を上昇させ、インスリン分泌を促進すると考えられる [15,20,21]。一方、膵 β 細胞株 MIN6 や INS-1 を GPR40 特異的な siRNA (small interfering ribonucleic acid)[22] で処理すると、FFA による GSIS が抑制されることから、その作用の少なくとも一部は GPR40 を介していることが示唆された [15,20]。本結果は、GPR40 が新規インスリン分泌促進薬の標的分子として高い可能性を有していることを支持している。すなわち、強力かつ選択的な GPR40 作動薬は、グルコース濃度依存的なインスリン分泌促進作用を有する、画期的な糖尿病治療薬になり得ることが期待される。

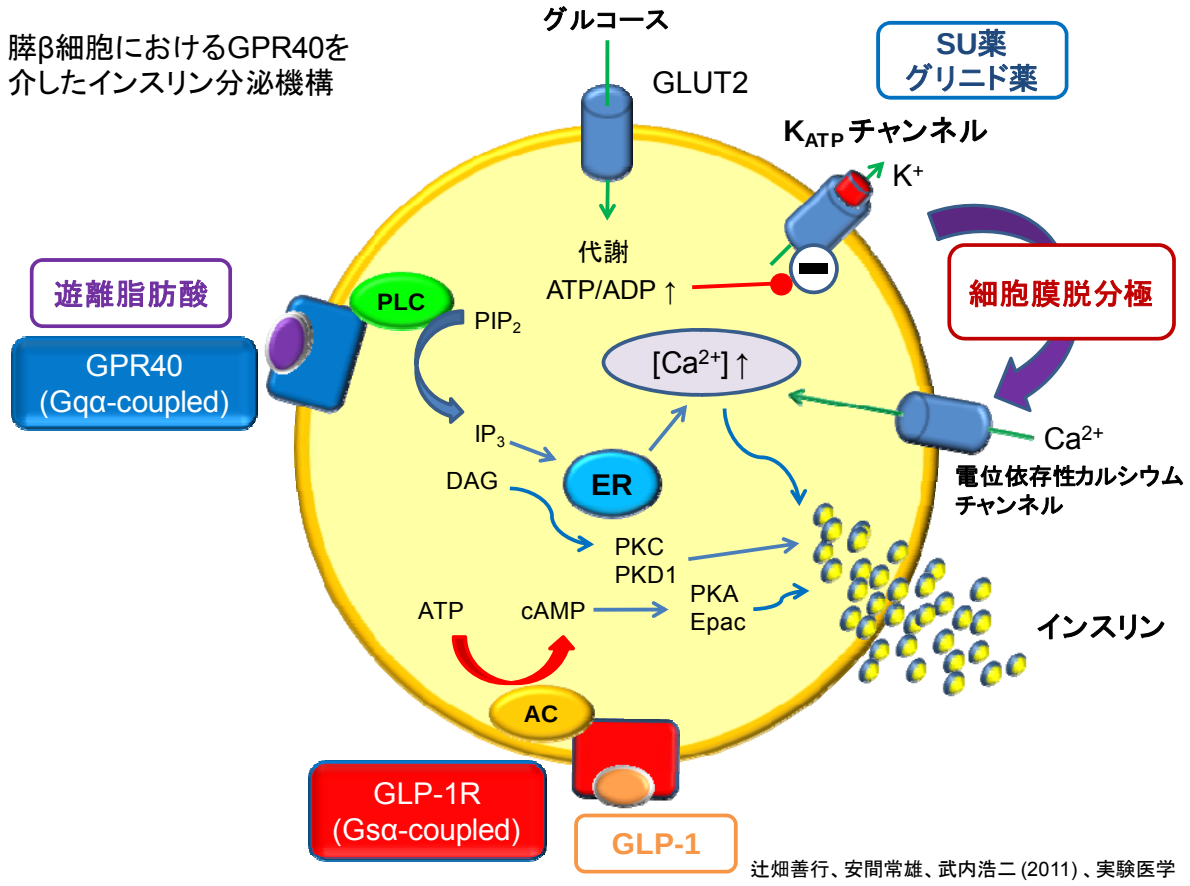


Figure 2. Schematic representation of islet receptors and their main secretory signaling pathways in β cells.

前述のように、武田薬品工業においてオーファン GPCR であった GPR40 の内因性リガンド探索研究が行われ、中長鎖脂肪酸、中でも分子内に不飽和結合を複数含有するドコサヘキサエン酸 (docosahexaenoic acid: DHA) などの多価不飽和長鎖脂肪酸が、強い GPR40 受容体作動活性を示すことが明らかとなった (Figure 3)[15]。この結果から、疎水性相互作用および π 電子相互作用が、受容体との結合に大きく寄与していることが示唆された。一方、リノレイン酸のメチルエステルには GPR40 受容体作動活性が認められなかったことから、カルボン酸が活性の発現に重要な役割を果たしていると推察された。

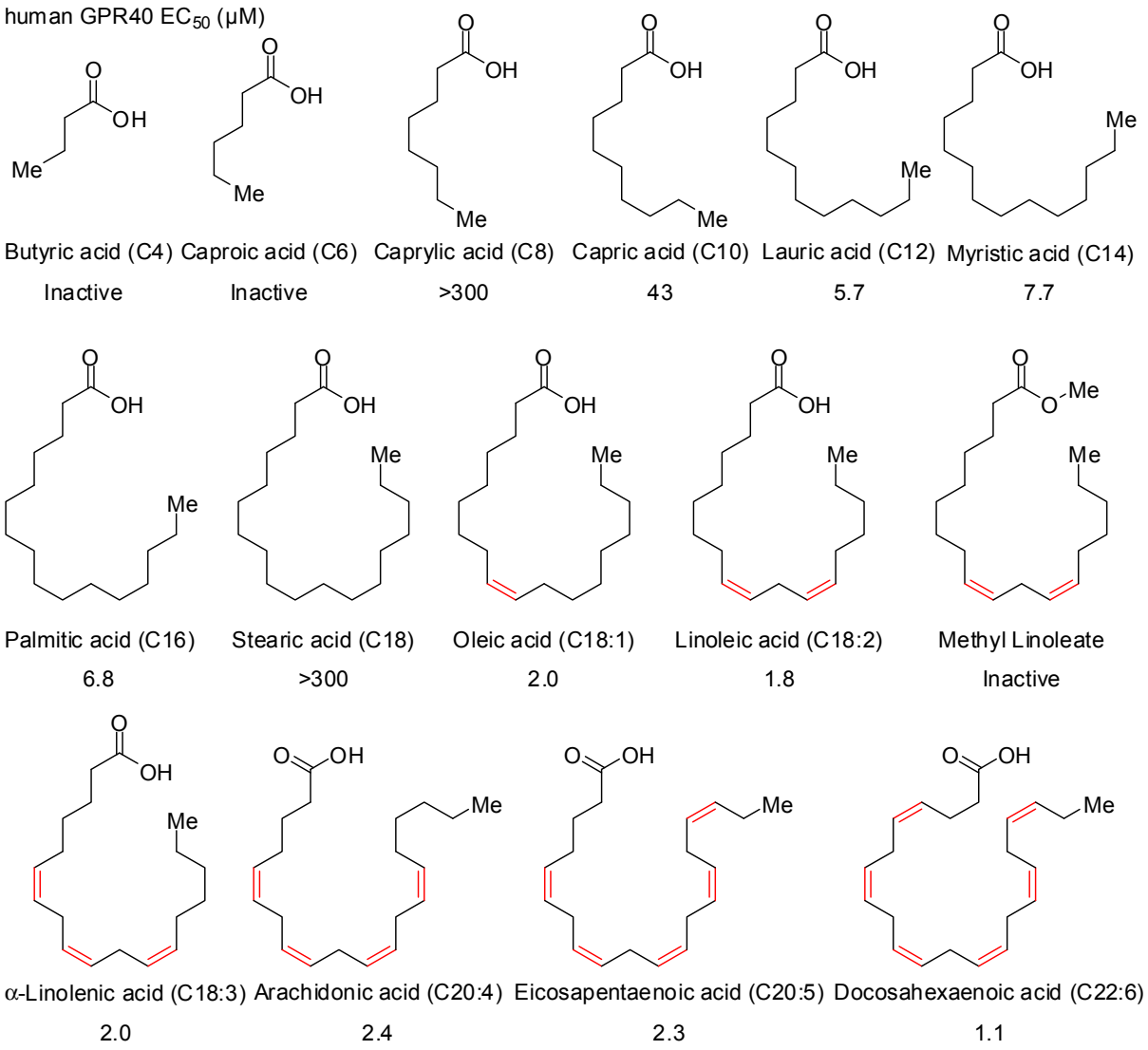
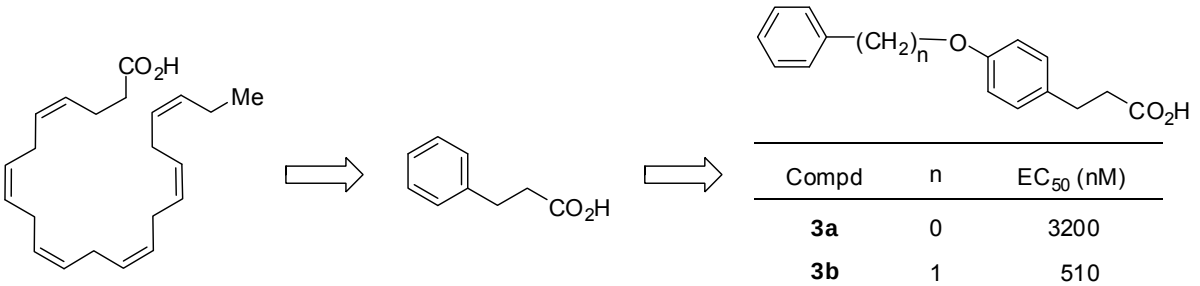


Figure 3. In vitro GPR40 activities of various free fatty acids.

GPR40 作動薬のリード創出とコンセプト検証

上記の知見を基に、低分子 GPR40 作動薬の探索研究が開始された。蛍光イメージングプレートリーダー (fluorometric imaging plate reader: FLIPR) 装置[23] を用い、Ca^{2+}濃度変化を指標として、市販および社内化合物ライブラリーのアリールアルカン酸誘導体を評価したところ、3-フェニルプロパン酸 **2** が 100 μM の濃度においてヒト GPR40 受容体作動活性を示すことが判明した (Figure 4)[24]。そこで、更なる相互作用の獲得を期待して、本化合物のベンゼン環 4 位に炭素鎖長の異なるフェニルアルキルオキシ基の導入が検討され、いずれの誘導体 **3a–e** においても活性の増強が認められた。それらの中で最も活性が強く合成展開が

容易な 4-ベンジルオキシフェニルプロパン酸 **3b** (EC_{50} = 510 nM) が初期リード化合物に選択され、最適化研究が行われた。



1 (DHA)
FLIPR EC_{50}: 1100 nM

2
active at 100 μM

Compd	n	EC_{50} (nM)
3a	0	3200
3b	1	510
3c	2	3500
3d	3	3500
3e	4	1700

Figure 4. Identification of the initial lead compound.

フェニルプロパン酸誘導体の構造活性相関を Figure 5 にまとめた[24]。フェニルプロパン酸部に関しては、カルボン酸が最適であり (A)、フェニル基上の置換基導入位置はオルト位が好ましく、かさ高い置換基の導入は活性の減弱を招いたが、電子的な効果は認められなかった (B)。中央のリンカー部はエーテルもしくは無置換アミンが好ましく、メチレンあるいはチオエーテルでは活性が減弱した (C)。ベンジル基上の置換基はメタ位もしくはパラ位がよく (D)、メタ位直結フェニル体が高活性を示したが、ヘテロ環への置換は活性の低下を招いた (E)。このことから、末端ベンゼン環は π–π 相互作用のみならず疎水性相互作用にも寄与していることが示唆された。次に末端ベンゼン環上の置換基効果を調べたところ、オルト位>パラ位>メタ位の順で高活性を示し、オルト位の置換基としては疎水性のメチル基やクロロ基が好ましく、立体的に小さなフルオロ基や親水性のメトキシ基では活性がやや減弱した (F)。

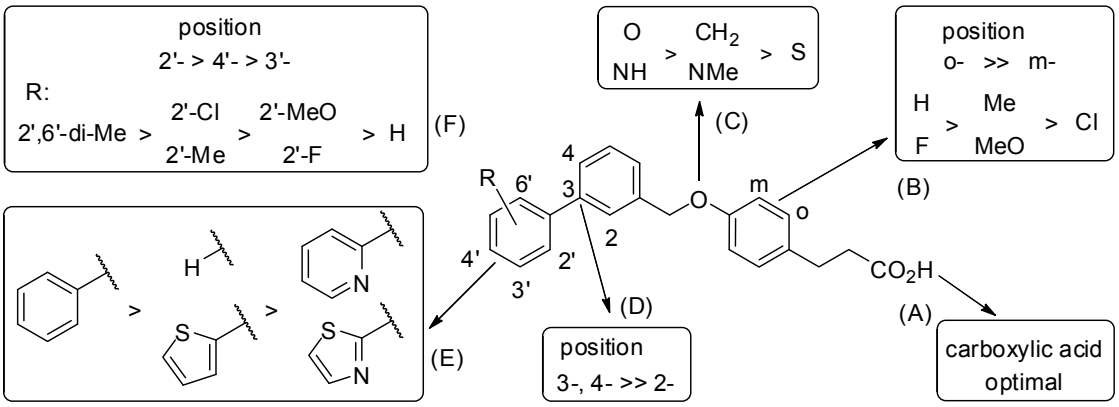
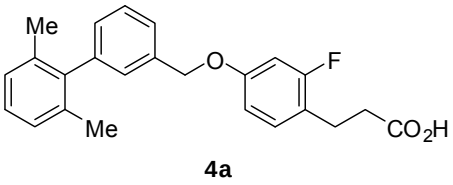


Figure 5. Structure activity relationships of phenylpropanoic acid derivatives.

本検討で見出された 2',6'-ジメチルビフェニリル基を有する *o*-フルオロフェニルプロパン酸誘導体 **4a** は、強力なヒト GPR40 受容体作動活性を有し (EC_{50} = 7.7 nM)、糖尿病モデルラット (雌性 Wistar fatty ラット) を用いた経口グルコース負荷試験 (oral glucose tolerance test: OGTT) で有意なインスリン分泌促進作用とともに血糖上昇抑制作用 (最小有効用量: 3 mg/kg, po) を示したことから、GPR40 作動薬の糖尿病治療薬としてのコンセプト検証が完了した (Figure 6)[24]。



4a

- Potent agonist activity: FLIPR, EC_{50} = 7.7 nM
- In vivo efficacy: 3 mg/kg, po (OGTT, female Wistar fatty rats)
- High clearance: 1032 mL/h/kg (3 mg/kg, SD rats)
- High lipophilicity: Log*D* = 4.19 (at pH = 7.4)

Figure 6. In vitro and in vivo profiles of phenylpropanoic acid **4a**.

臨床開発を指向した GPR40 作動薬の創製

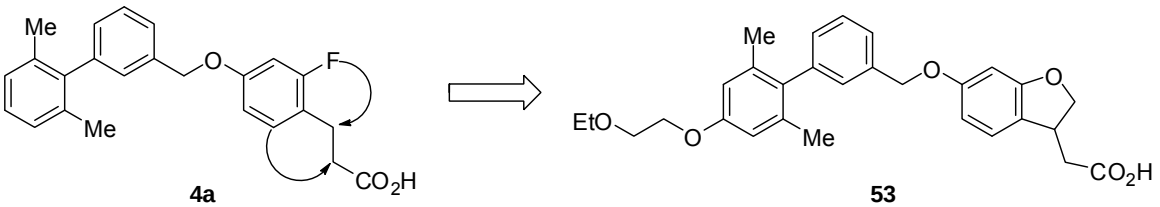
以上の基礎的研究を基に、今回筆者は、臨床開発を指向した GPR40 作動薬の創製を目指して研究を実施した。GPR40 の薬物ターゲットとしての魅力は、グルコース濃度依存的にインスリン分泌促進作用を示すために低血糖の懸念が少なく、受容体が膵 β 細胞に選択的に発現するためターゲット由来の毒性に関する懸念も少ないこと、またリガンドが FFA のような低分子であるため低分子経口薬としての開発が可能であること、などが挙げられる。従って、SU 薬やグリニド薬と異なり薬物の血中濃度を厳密に制御することなく、強力な GPR40 受容体作動作用を持続的に暴露することが可能と考えられる。一方、GPR40 の内因

性リガンドである FFA は、多彩な生理作用を有するとともに、生体内の重要な構成要素でもある。すなわち、FFA に類似した構造をもつ化合物には、FFA に由来するオフターゲット作用[25]を示す可能性が想定される。この懸念を回避するための方策として、リガンドの GPR40 に対する結合親和性を向上させるとともに、脂肪酸に特徴的な性質である、高い脂溶性からの脱却が必要であると考えた。

先の研究で見出したフェニルプロパン酸誘導体 **4a** は、強力な in vitro GPR40 受容体作動活性と in vivo での顕著な薬効を示すものの、ラットに投薬した際の血中からの消失が速い。従って、持続的な抗糖尿病作用を発現させるためには、毎食前の投薬が必要であると推察された。本性質は、臨床における利便性の観点からは好ましくない。また、FFA と同様に脂溶性が高く、非特異的な相互作用を示す懸念があった (Figure 6)。そこで、薬物動態プロファイルの改善と、強力かつ受容体選択性に優れた安全性の高い GPR40 作動薬の創出を目的として、研究を実施した。

第 1 章では、良好な薬物動態プロファイルを有する、縮合環アルカン酸誘導体の創出研究について述べる (Figure 7)[26,27]。リード化合物 **4a** は、そのプロパン酸部位が β 酸化に脆弱であったことから、フェニルプロパン酸のオルト位とプロパン酸の α 位あるいは β 位で環化させ縮環構造を形成することで β 酸化を抑制し、薬物動態プロファイルを改善する戦略を立案した。種々の縮合環アルカン酸誘導体を合成、評価した結果、5～7 員の非芳香環がベンゼン環に縮環した誘導体が、フェニルプロパン酸誘導体に匹敵する受容体作動活性を有することを見出した。一方、これらの誘導体にはヒトーラット間で受容体作動活性に種差が認められたことから、その原因をロドプシンの結晶構造から作成した GPR40 ホモロジーモデルを用いて解析した。ここで見出した縮合環アルカン酸誘導体は、フェニルプロパン酸誘導体と比較して、期待通り、血中からの消失速度が遅く、血中での暴露量も増加した。次に、ビフェニル部位と縮合環部の構造変換を行い、良好な活性と薬物動態プロファイルを有する化合物 **53** を見出した。本化合物は、糖尿病モデルラットを用いた OGTT で、投薬 1 時間後および 4 時間後に実施した糖負荷による血糖上昇を抑制するとともにインスリン分泌を有

意に促進したことから、本結果をもって、血中持続性の高い GPR40 作動薬の創出を達成した。



4a

FLIPR, EC_{50}:	7.7 nM
C_{max}:	86.0 ng/mL
$AUC_{po,\ 0–8h}$:	249.0 ng·h/mL
F:	21.5%

53

FLIPR, EC_{50}:	28 nM
C_{max}:	465.4 ng/mL
$AUC_{po,\ 0–8h}$:	2837.4 ng·h/mL
F:	59.5%

(Rat cassete dosing at 0.1 mg/kg, iv and 1 mg/kg, po)

Figure 7. Design of fused-ring alkanoic acids.

第 2 章では、脂溶性低減を指向して分子末端に極性官能基を導入した、ジヒドロベンゾフラン酢酸誘導体の最適化研究について述べる (Figure 8)[26,28]。上記で見出した化合物 **53** をリード化合物として、脂溶性低減と薬効増強を目的とした構造変換を実施した。その際、化合物の脂溶性の指標である Log*D* 値と、細胞傷害性の指標であるヒト HepG2 細胞におけるカスパーゼ-3/7 活性に着目して化合物を選択した。その結果、ジヒドロベンゾフラン 3 位の立体化学が活性に重要であること、また分子末端ビフェニル部の 4'位にスルホニル基を有する化合物が、活性を保持しつつ低い Log*D* 値を有し、かつ非常に良好な薬物動態プロファイルを示すことを見出した。中でも化合物 **85** は、GPR40 と同様に脂肪酸をリガンドとする GPCR に対する優れた選択性を示し、各種薬効モデル動物において、強力なインスリン分泌促進作用とそれに基づく血糖上昇抑制作用を示した。また、**85** の詳細な薬物動態および代謝物解析から、β 酸化に対する抵抗性を示し、他の動物種においても良好な薬物動態プロファイルを示すことを明らかにした。

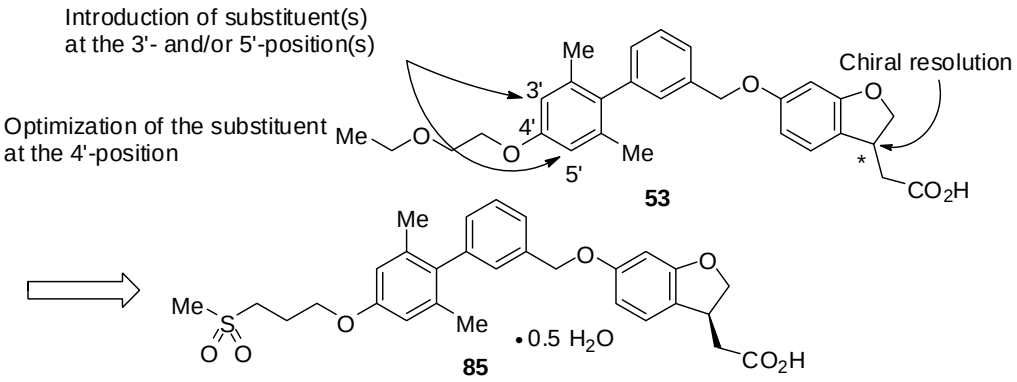


Figure 8. Design of (2,3-dihydro-1-benzofuran-3-yl)acetic acids.

詳細について、以下に論述する。

本論

第 1 章　良好な薬物動態プロファイルを有する GPR40 作動薬の創出：縮合環アルカン酸誘導体の合成と生物活性 [26,27)]

第 1 節　序論

緒論で述べたように、これまでの武田薬品工業における内因性リガンドを基にしたリード創出研究により、フェニルプロパン酸誘導体 **4a** が強力な in vitro GPR40 受容体作動活性および糖尿病モデル動物における血糖上昇抑制作用を示すことが判明している [24)]。本誘導体は、長鎖脂肪酸の炭素鎖をベンゼン環に置換することで、コンフォメーションを固定化および π 電子相互作用を付与した結果、活性が向上したと考えられる。特に、分子末端の 2′,6′-ジメチルフェニル基と中央のベンゼン環が直交するビフェニル構造が、活性向上に寄与していると考えられる。一方、分子右側フェニルプロパン酸部位は受容体作動活性の発現に必須であることが判明しているが、**4a** の代謝物を解析した結果、β 酸化を受けて桂皮酸 **4b**、更には安息香酸 **4c** まで代謝され、未変化体が血中から速やかに消失することも判明した (Figure 9)。

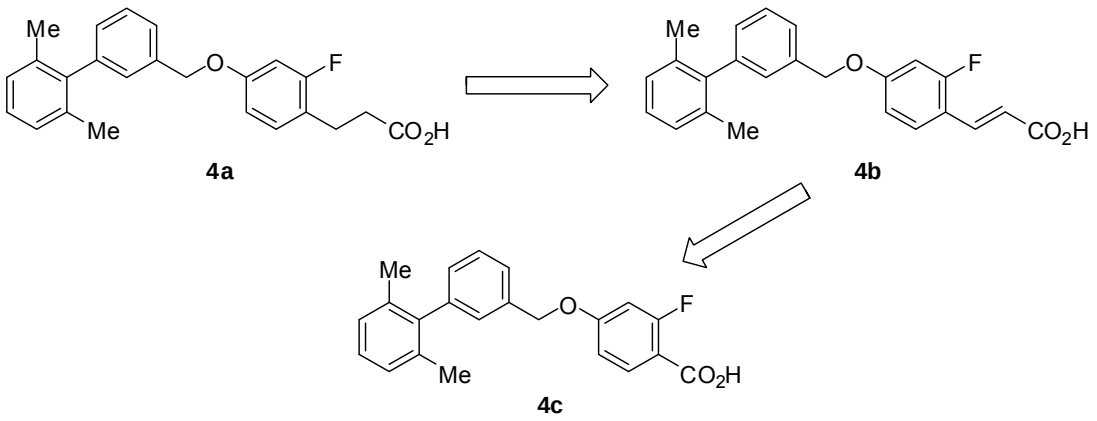


Figure 9.　Plausible metabolic pathway of **4a**.

GPR40 作動薬の特長は、厳格なグルコース濃度依存性にあり、長時間暴露して

も低血糖を引き起こさないと期待されることから、本特長を最大限に活かすためには、長時間作用型の性質を有することが望ましい。そこで、代謝に脆弱なフェニルプロパン酸部位の構造変換による薬物動態プロファイルの改善を試みた。Figure 10 に示すように、β 酸化に関与する酵素との反応点であるプロパン酸の α 位もしくは β 位とベンゼン環のオルト位で縮環構造を形成させることにより、β 酸化に対する耐性を獲得できると考えた。また同時に、コンフォメーションの固定化による活性の向上も期待した。まず、GPR40 受容体に対する高い結合親和性を有する部分構造である 2′,6′-ジメチルビフェニリルメチル基を固定して、縮合環アルカン酸部位の検討を実施した (一般式 A)。さらに、活性と薬物動態面で好ましい縮合環アルカン酸構造を見出した後、再度その骨格に適した脂溶性部分構造を検討する計画を立案した (一般式 B)。一般式 A を代表例として、その逆合成経路を示す。A のエーテル結合は、ビフェニリルメタノールあるいはそのメシレート C とフェノール D との光延反応もしくは置換反応により形成可能であると考えた。ビフェニリルメタノール C は、ボロン酸 E とアリールハライド F から、鈴木反応と続く還元反応により誘導可能であると考えた。D は、それぞれの縮合環に適した合成法を利用あるいは開発して構築した。

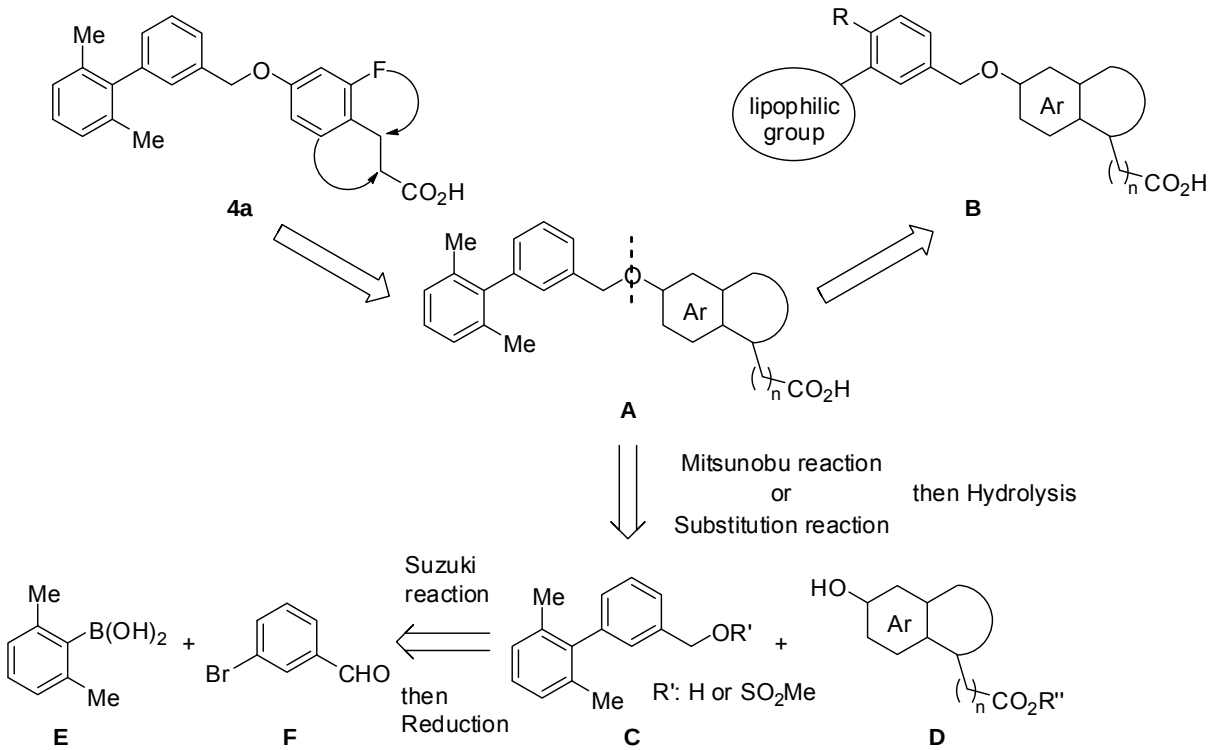


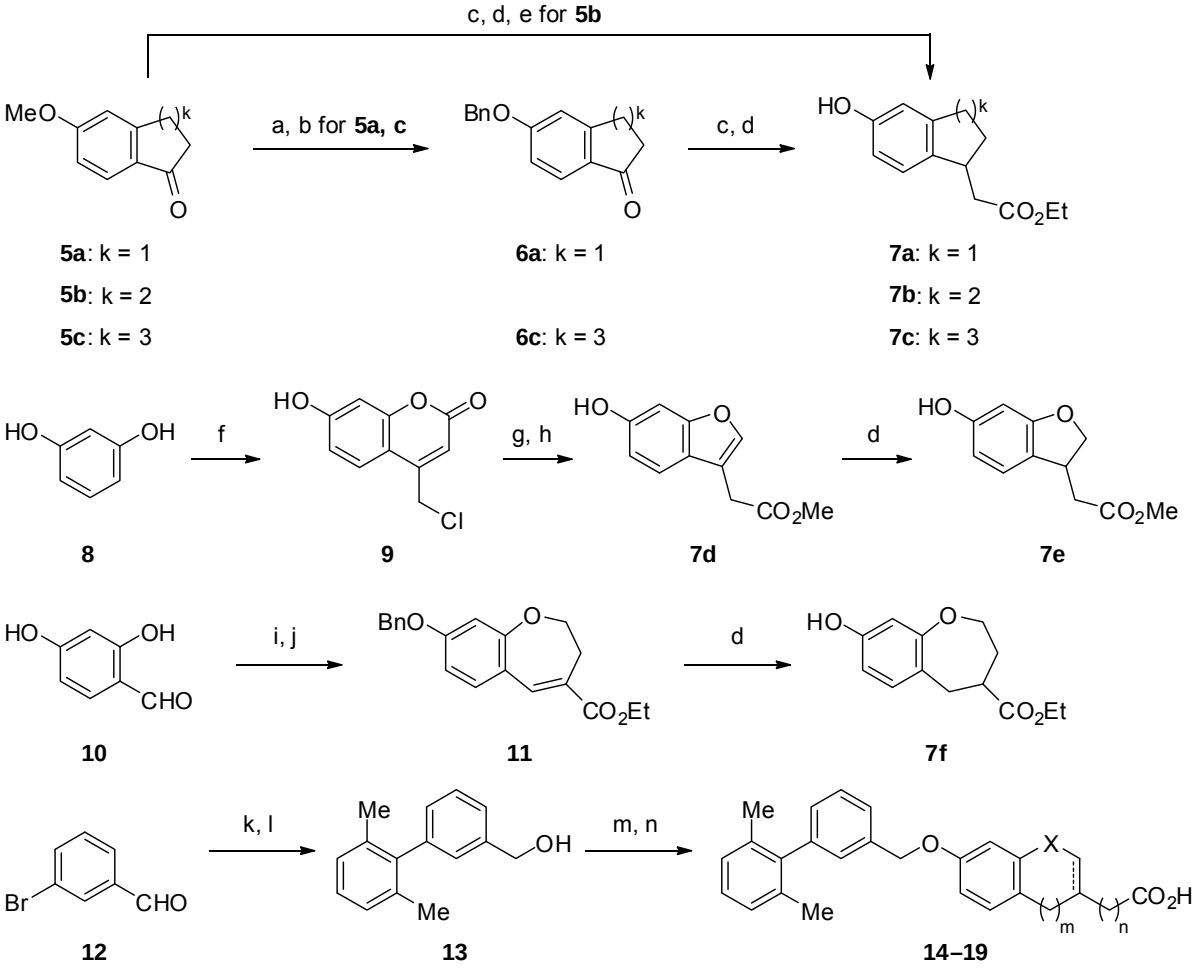
Figure 10. Design and retrosynthesis of fused-ring alkanoic acids.

第 2 節　縮合環アルカン酸誘導体の合成

第 1 項　各種縮合環アルカン酸誘導体の合成

各種縮合環アルカン酸 **14–19** は、Scheme 1 に示す方法で合成した。5-ヒドロキシインダン中間体 **7a** は、市販の 5-メトキシ-1-インダノン (**5a**) を一旦 5-ベンジルオキシ-1-インダノン (**6a**) に変換し、Horner-Wadsworth-Emmons 反応で酢酸ユニットを導入後、接触水素化反応によりオレフィンの還元とベンジル基の脱保護を一挙に行うことで効率良く得た。中間体 **7a** の 6 員環アナログである **7b** は、**7a** の合成法を基に、6-メトキシ-1-テトラロン (**5b**) から保護基を付け替えることなく合成した。すなわち、酢酸ユニットの導入後、野出らの塩化アルミニウム／無臭チオールを用いるメトキシ基の脱メチル化法[29] により **7b** とした。7 員環アナログである **7c** は、3-メトキシベンズアルデヒドから 4 工程で容易に調製可能な 2-メトキシ-6,7,8,9-テトラヒドロ-5*H*-ベンゾ[7]アンヌレン-5-オン (**5c**) から、**7a** の合成と同様にして調製した。ベンゾフラン中間体 **7d** の合成は、レゾルシノール (**8**) を出発原料として、Pechmann 反応[30] により 7-ヒドロキシ-4-クロロメチルクマリン (**9**) を得た後、塩基処理にてクマリンの開環とベンゾフラン環の再構築を誘導し[31]、続いてエステル化することで目的とする **7d** を得た。さらに、**7d** を接触水素化に付し、ジヒドロベンゾフラン中間体 **7e** を得た。テトラヒドロベンゾオキセピン中間体 **7f** の合成は、2,4-ジヒドロキシベンズアルデヒド (**10**) のモノベンジル化によりパラ位のヒドロキシ基を保護し、4-ブロモ酪酸エチルを用いたアルキル化、それに伴う閉環により環化体 **11** を得、続いて接触水素化に付すことで、二重結合の還元とベンジル基の脱保護を一気に行うことにより達成した。分子左側のビフェニリルメタノール **13** は、3-ブロモベンズアルデヒド (**12**) と 2,6-ジメチルフェニルボロン酸との鈴木カップリング、続く水素化ホウ素ナトリウムを用いた還元により合成した。上記のようにして合成したアルコール **13** とフェノール **7a–f** を光延反応により縮合させ、最後に加水分解に付すことにより、目的とするカルボン酸 **14–19** へと誘導した。

Scheme 1[a]

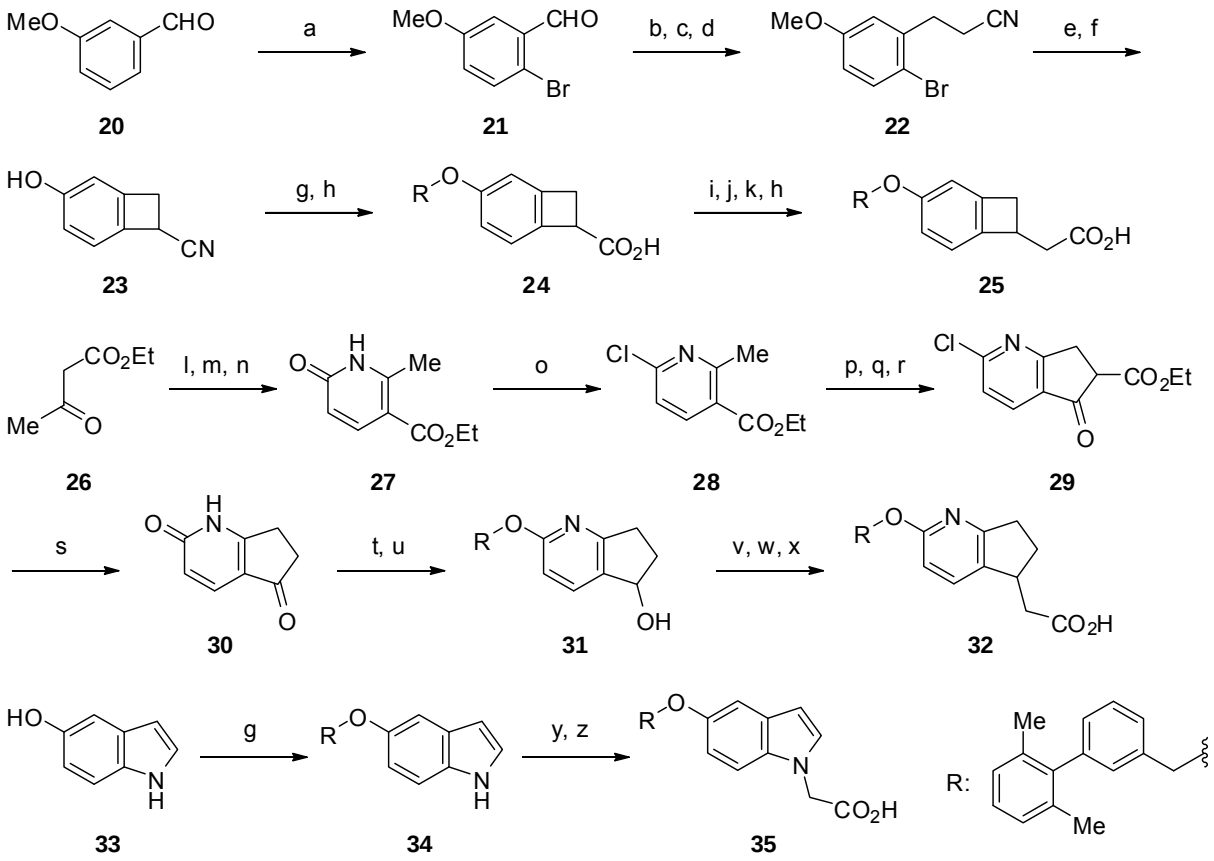


	14	15	16	17	18	19
X	CH_2	-$(CH_2)_2$-	-$(CH_2)_3$-	O	O	-OCH_2-
m	0	0	0	0	0	1
n	1	1	1	1	1	0
bond type (⋯⋯)	single	single	single	double	single	single

[a] Reagents and conditions: (a) $AlCl_3$, toluene, reflux; (b) benzyl bromide, K_2CO_3, acetone, reflux; 91–94% (2 steps); (c) triethyl phosphonoacetate, NaH, toluene, reflux; (d) H_2 (balloon pressure), 10% Pd/C, EtOH or MeOH, rt, 54–89% (2 steps), 76–100% for **7e**, **f**; (e) $AlCl_3$, 1-dodecanethiol, toluene, rt, 98%; (f) ethyl 4-chloroacetoacetate, H_2SO_4, rt, 84%; (g) 1 M NaOH aq., reflux, 83%; (h) H_2SO_4, MeOH, reflux, 70%; (i) benzyl chloride, KF, MeCN, reflux; 43%; (j) ethyl 4-bromobutyrate, Cs_2CO_3, DMF, 80 °C, 41%; (k) 2,6-dimethylphenylboronic acid, $Pd(PPh_3)_4$, 1 M Na_2CO_3 aq., EtOH, toluene, reflux, 97%; (l) $NaBH_4$, DME, THF, 0 °C, 83%; (m) **7a**–**f**, ADDP, P(*n*-Bu)$_3$, toluene, rt, 23–96%; (n) 2 M NaOH aq., MeOH or EtOH, THF, rt, 53–80%.

その他の縮合環アナログ (**25**, **32** および **35**) の合成は、Scheme 2 に示す方法で実施した。

Scheme 2[a]



[a] Reagents and conditions: (a) Br_2, AcOH, rt, 88%; (b) cyanoacetic acid, NH_4OAc, pyridine, toluene, reflux, 80%; (c) $NaBH_4$, MeOH, sat. $NaHCO_3$ aq., rt, 98%; (d) DMA, 180 °C, 88%; (e) $NaNH_2$, NH_3 aq., −33 °C, 48%; (f) $AlCl_3$, 1-dodecyl methyl sulfide, toluene, 0 °C, 79%; (g) **13**, ADDP, P(*n*-Bu)$_3$, toluene or THF, rt, 62−90%; (h) KOH, EtOH, H_2O, rt to reflux, 82−99%; (i) $LiAlH_4$, THF, rt, 88%; (j) *p*-TsCl, pyridine, rt, 89%; (k) NaCN, DMSO, rt, 90%; (l) silica gel, NH_3 aq., rt, 67%; (m) methyl propiolate, toluene, reflux; (n) heat, DMF, reflux, 34% (2 steps); (o) $POCl_3$, 120 °C, 89%; (p) NBS, AIBN, CCl_4, reflux, 63%; (q) diethyl malonate, NaH, toluene, rt, 74%; (r) NaH, toluene, reflux, 99%; (s) 85% H_3PO_4, 185 °C, 85%; (t) **13**, MsCl, Et_3N, THF then **30**, K_2CO_3, DMF, 70 °C; (u) $NaBH_4$, MeOH, THF, 0 °C, 6% (2 steps); (v) $SOCl_2$, pyridine, toluene, rt, (w) diethyl malonate, NaH, THF, rt, 53% (2 steps); (x) 2 M NaOH aq., EtOH, THF, 0 °C then toluene, reflux, 38%; (y) ethyl bromoacetate, NaH, THF, DMF, 4 °C to rt, 83%; (z) KOH aq., EtOH, THF, rt, 76%.

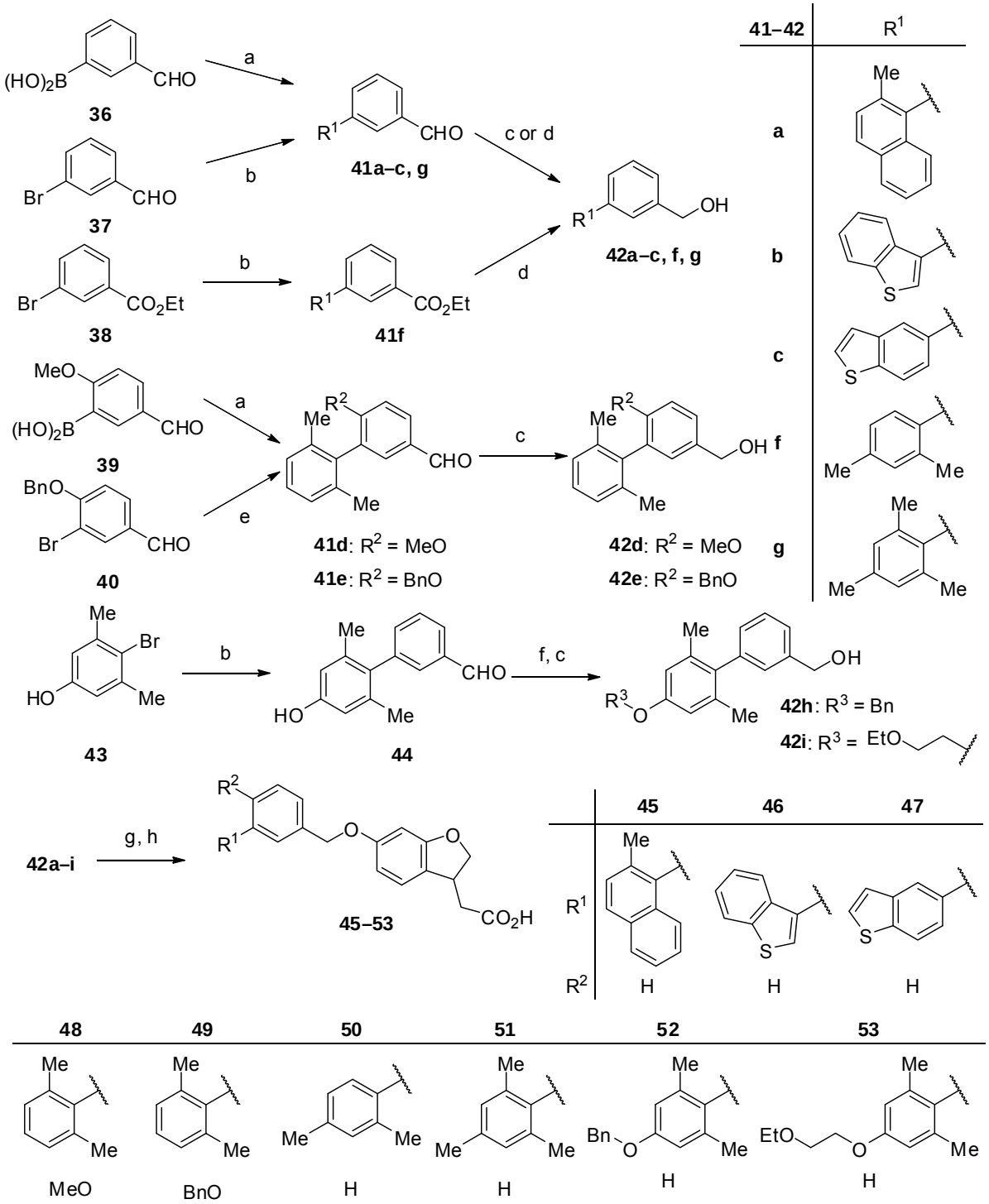
ベンゾシクロブテン骨格の構築は、亀谷らの方法[32]に準じて行った。すなわち、3-メトキシベンズアルデヒド (**20**) から 4 工程で合成したプロパンニトリル **22** を液体アンモニア中ナトリウムアミドで処理してベンザイン中間体経由で閉環させ、塩化アルミニウム／ドデシルメチルスルフィドを用いる方法[33]でメトキシ基の脱メチル化を行い所望の **23** を得た。この際、スルフィドではなく汎

用されるチオールを用いると、構造未同定の副生成物が生じ、目的物を得ることはできなかった。得られた **23** にビフェニル側鎖を導入後、4 工程を経て増炭し、最後にニトリルの加水分解を行って目的とするベンゾシクロブテンカルボン酸 **25** へと誘導した。ジヒドロシクロペンタ[*b*]ピリジン酢酸 **32** に関しては、まずピリドン **27** を公知の方法[34,35]に従って 3 工程で合成し、続いてオキシ塩化リンで処理してクロロピリジン **28** とした。得られた **28** のメチル基を *N*-ブロモスクシンイミドで臭素化後、マロン酸ユニットを導入し、続いて水素化ナトリウムで処理したところ、炭酸ジエチルの脱離を伴って環化体 **29** が得られた[36]。さらにリン酸で処理し、脱炭酸とクロロピリジン部の加水分解を同時に行って、ピリドン **30** とした。次に、ビフェニル側鎖を導入後、カルボニル基を還元してアルコール **31** を得た。ここで生じたヒドロキシ基を塩化チオニルで塩素化し、再度マロン酸ユニットを導入後、エステルを加水分解し、得られたジカルボン酸を熱的に脱炭酸させることにより目的とする **32** へと誘導した。インドール酢酸 **35** は、5-ヒドロキシインドール **33** にビフェニル側鎖と酢酸ユニットを順次導入し、最後にエステルを加水分解することで合成した。

第 2 項　種々の脂溶性側鎖を有するジヒドロベンゾフラン酢酸誘導体の合成

続いて、縮合環アルカン酸部位を (2,3-ジヒドロ-1-ベンゾフラン-3-イル)酢酸に固定して、脂溶性側鎖を変換した誘導体を合成した (Scheme 3)。各種アルコール **42a**–**g** は、対応する臭素体とボロン酸との鈴木カップリングを行った後、それぞれのエステルあるいはホルミル基を還元することにより合成した。4′位にアルコキシ基を有するアルコール **42h**, **i** は、フェノール **43** から合成したアルデヒド **44** のアルキル化、続くホルミル基の還元により合成した。得られた **42a**–**i** とジヒドロベンゾフラン中間体 **7e** との光延反応を行い、最後にエステルを加水分解することで、目的とするカルボン酸 **45**–**53** へと誘導した。

Scheme 3[a]



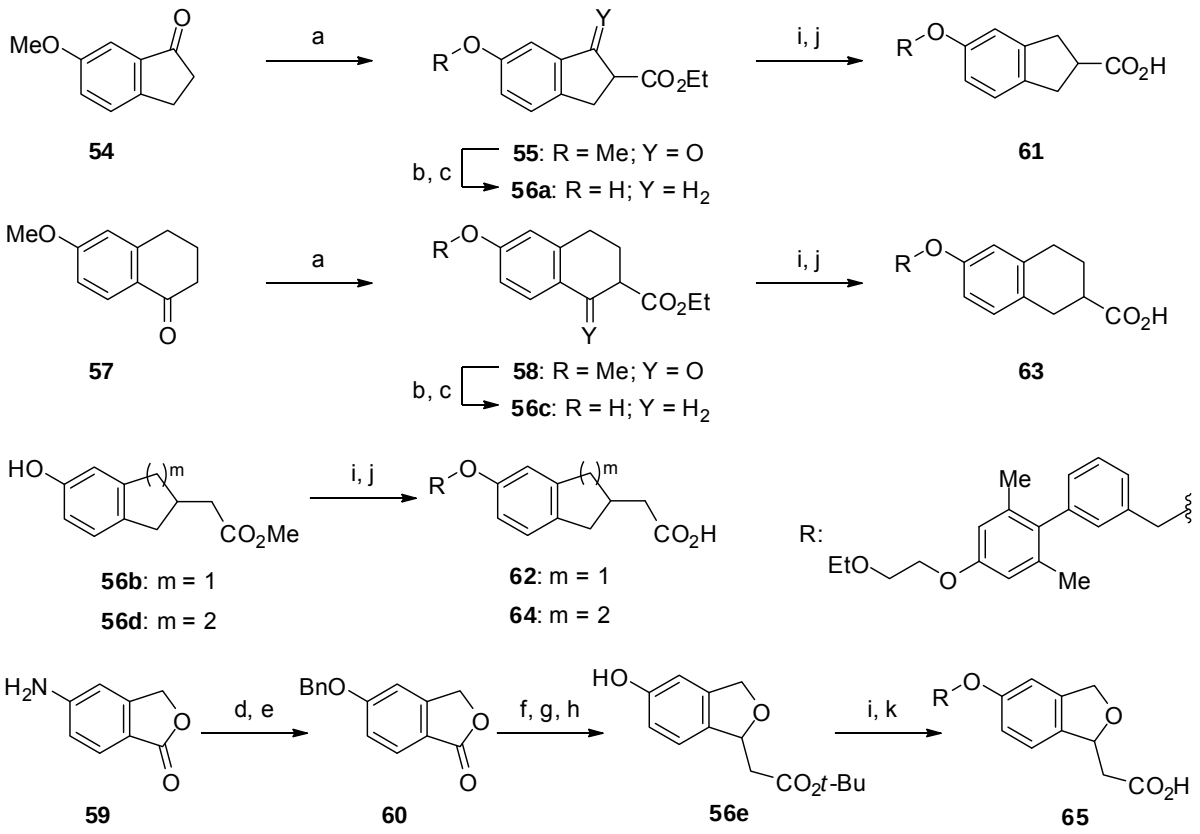
[a] Reagents and conditions: (a) ArBr, $Pd(PPh_3)_4$, Cs_2CO_3 or Na_2CO_3, EtOH, toluene, H_2O, 70 °C, 65−94%; (b) $ArB(OH)_2$, $Pd(PPh_3)_4$, Cs_2CO_3 or Na_2CO_3, EtOH, toluene, H_2O, 70−80 °C, 76%−quant.; (c) $NaBH_4$, EtOH or DME-THF or MeOH-THF, 0 °C, 70−99%; (d) $LiAlH_4$, THF, 0 °C to rt, 95−96%; (e) 2,6-dimethylphenylboronic acid, $Pd_2(dba)_3$, SPhos, K_3PO_4, toluene, H_2O, 100 °C, quant.; (f) R^3-X, K_2CO_3, (KI), DMF, 70 °C, 89−92%; (g) **7e**, ADDP, P(*n*-Bu)$_3$, toluene, rt, 50−93%; (h) 2 M NaOH aq., MeOH, THF, rt, 55−92%.

第 3 項　[4′-(2-エトキシエトキシ)-2′,6′-ジメチルビフェニル-3-イル]メチル基を有する各種縮合環アルカン酸誘導体の合成

脂溶性側鎖を[4′-(2-エトキシエトキシ)-2′,6′-ジメチルビフェニル-3-イル]メチル基に固定して、縮合環アルカン酸部位を変換した誘導体は、Scheme 4 および 5 に示す方法で合成した。まず Scheme 4 に、インダン、テトラヒドロナフタレン、およびジヒドロイソベンゾフラン誘導体 **61**−**65** の合成法を示す。

インダン-2-カルボン酸エステル中間体 **56a** あるいはテトラヒドロナフタレン-2-カルボン酸エステル中間体 **56c** の合成は、6-メトキシ-1-インダノン (**54**) あるいは 6-メトキシ-1-テトラロン (**57**) を出発物質とし、炭酸ジエチルによるエトキシカルボニル基の挿入、トリフルオロ酢酸およびトリエチルシランを用いたカルボニルのメチレンへの還元、続くメトキシ基の脱メチル化により達成した。ジヒドロイソベンゾフラン中間体 **56e** は 5 工程を経て合成した。すなわち、5-アミノフタリド (**59**) のアミノ基を Sandmeyer 反応でヒドロキシ基に変換し、続いてそのヒドロキシ基をベンジル基で保護して **60** を得た。次に、酢酸 *tert*-ブチルから発生させた有機リチウム試薬を反応させ、生じたヒドロキシ基をトリフルオロ酢酸とトリエチルシランを用いて還元的に除去後、脱ベンジル化を行って **56e** を得た。なお、**56b** および **56d** は社内の化合物ライブラリーに保管されているものを用いた。これらの中間体 **56a**−**e** から、第 1 項の手法と同様にして目的とするカルボン酸 **61**−**65** を得た。

Scheme 4[a]

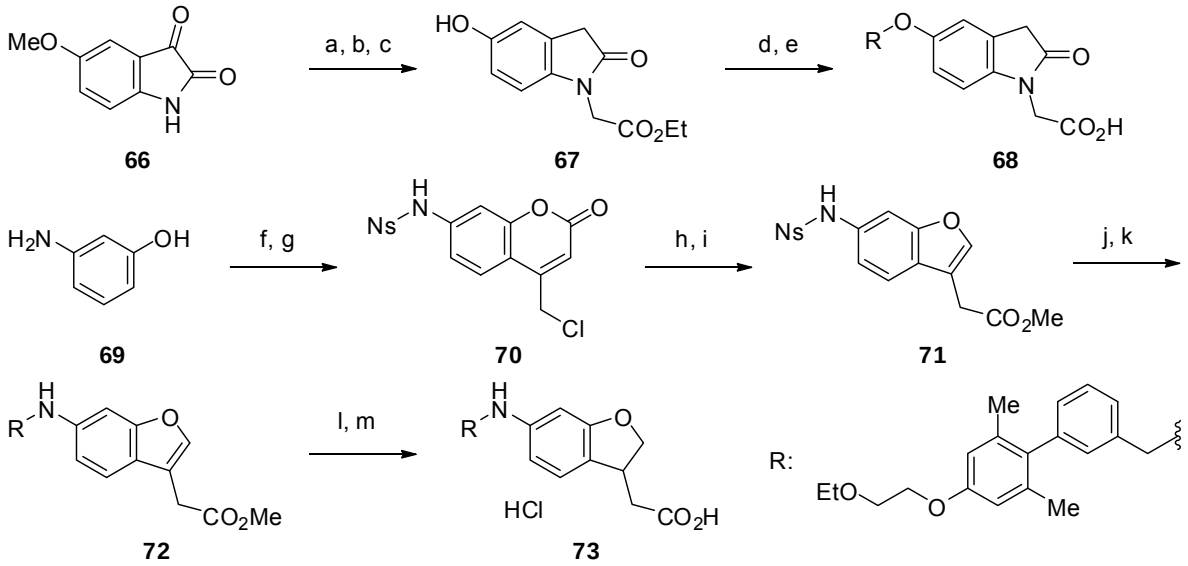


[a] Reagents and conditions: (a) diethyl carbonate, NaH, toluene, rt to 120 °C, 51–70%; (b) triethylsilane, TFA, rt; (c) $AlCl_3$, 1-octanethiol, CH_2Cl_2, 0 °C, 32–67% (2 steps); (d) H_2SO_4, sodium nitrite, H_2O, 0 °C to reflux; (e) benzyl bromide, K_2CO_3, DMF, 60 °C, 21% (2 steps); (f) *tert*-butyl acetate, lithium diisopropylamide, THF, −78 °C, (g) triethylsilane, TFA, CH_2Cl_2, rt, 16% (2 steps); (h) H_2, Pd/C, EtOH, rt, 86%; (i) **42i**, ADDP, P(*n*-Bu)$_3$, toluene, rt, 41–85%; (j) 1 M or 2 M NaOH aq., MeOH or EtOH, THF, rt, 57–91%; (k) TFA, toluene, rt, 72%.

続いて Scheme 5 に、オキシインドール酢酸 **68** およびジヒドロベンゾフラン酢酸 **53** の 6 位エーテルリンカーをアミンリンカーに変換した 6-アミノジヒドロベンゾフラン酢酸 **73** の合成法を示す。オキシインドール酢酸 **68** は、5-メトキシイサチン (**66**) を出発物質として合成した。すなわち、酢酸ユニット導入の後、酸性条件下での水素化反応によりカルボニル基の還元を行ったが、同時にエステルが加水分解されたことから再エステル化し、続いて 5 位メトキシ基の脱メチル化を行ってフェノール **67** を得た。次に、光延反応により脂溶性側鎖を導入し、最後にエステルの加水分解を酸性条件下で行い、目的とする **68** を得た。この際、常法の塩基性条件下での反応では、ラクタム環の開環が起こり

基質の分解を招いた。最後に、6-アミノジヒドロベンゾフラン酢酸 **73** は、3-アミノフェノール (**69**) を出発原料とし、福山らが開発した 2-ニトロベンゼンスルホニル (Ns) 基[37] を利用して合成した。すなわち、**69** への Ns の導入後、ベンゾフラン中間体 **7d** の合成と同様のスキームにて **71** を合成した。この際、**69** に対して無保護の状態で Pechmann 反応を行った場合、反応が複雑化し目的物を得ることはできなかった。続いて光延反応により脂溶性側鎖を導入し、メルカプト酢酸を用いて脱 Ns 化[37] 後、得られた **72** の接触還元、さらにエステル加水分解を行って、目的とするカルボン酸 **73** を塩酸塩として得た。本化合物の合成において、Ns 基は Pechmann 反応の際の保護基としての役割とともに、光延反応の際の NH プロトンの活性化基としての役割も果たしており、非常に有用である。

Scheme 5[a]



[a] Reagents and conditions: (a) ethyl bromoacetate, NaH, DMF, 0 °C to rt, 78%; (b) H_2 (balloon pressure), 10% Pd/C, 70% perchloric acid, AcOH, 50 °C, then $SOCl_2$, EtOH, rt, 36%; (c) $AlCl_3$, 1-octanethiol, CH_2Cl_2, 0 °C, 81%; (d) **42i**, ADDP, P(*n*-Bu)$_3$, toluene, rt, 22%; (e) 60% $HClO_4$, AcOH, 50 °C, 18%; (f) 2-nitrobenzenesulfonyl chloride, pyridine, rt, 77%; (g) ethyl 4-chloroacetoacetate, H_2SO_4, rt, 51%; (h) 1 M NaOH aq., rt, quant.; (i) $SOCl_2$, MeOH, rt, 62%; (j) **42i**, DEAD, PPh_3, toluene, rt; (k) mercaptoacetic acid, LiOH·H_2O, DMF, rt 76% (2 steps); (l) H_2 (balloon pressure), 10% Pd/C, EtOH, rt, 66%; (m) 2 M NaOH aq., EtOH, THF, rt, then 4 M HCl/AcOEt, Et_2O, rt, 85%.

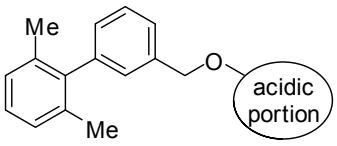
第 3 節　縮合環アルカン酸誘導体の in vitro 活性

合成した化合物について、ヒト GPR40 受容体を強制発現させたチャイニーズハムスター卵巣 (Chinese hamster ovary: CHO) 由来細胞を用いて、0.1% ウシ血清アルブミン (bovine serum albumin: BSA) 存在下 [38]、FLIPR を用いて Ca^{2+}濃度の変化を測定し、GPR40 受容体作動活性を評価した。また、ヒトおよびラット GPR40 受容体を強制発現させた CHO 細胞の膜画分を用いて、0.2% BSA の存在下、受容体結合親和性 (binding affinity) を評価した。化合物の脂溶性の指標である Log*D* 値は、pH 7.4 における HPLC 分析により、標準化合物の保持時間との相対的な比較により算出した [39]。

第 1 項　縮合環アルカン酸の変換

最初に、β酸化抑制を目的としてデザインした縮合環アルカン酸誘導体が GPR40 受容体作動活性を保持するかどうかを確認した (Table 1)。

Table 1.　In Vitro Activities of Fused-Ring Alkanoic Acids



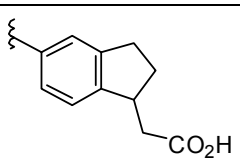
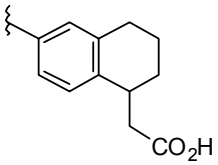
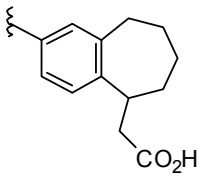
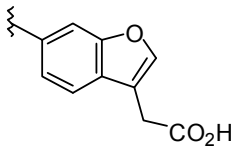
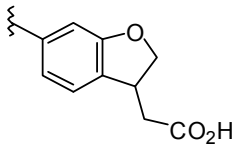
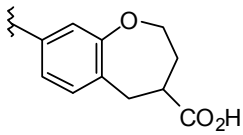
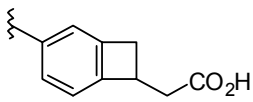
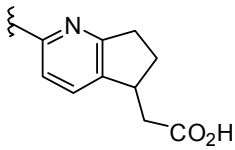
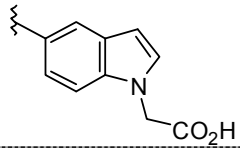
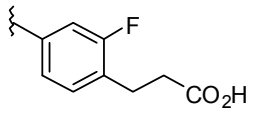
compd	acidic portion	FLIPR human EC_{50} (μM)[a]	binding affinity human K_i (μM)[b]	binding affinity rat K_i (μM)[b]	Log*D*[c]
14		0.033 (0.019−0.057)	0.21	0.52	4.40

Table 1. (Continued)

compd	acidic portion	FLIPR human EC_{50} (μM)[a]	binding affinity human K_i (μM)[b]	binding affinity rat K_i (μM)[b]	Log*D*[c]
15		0.027 (0.016−0.045)	0.059	3.7	4.70
16		0.94 (0.64−1.4)	2.4	>10	5.02
17		4.9 (2.4−10)	>10	>10	4.04
18		0.027 (0.019−0.038)	0.17	1.1	3.88
19		0.070 (0.049−0.10)	0.27	>10	4.05
25		0.069 (0.042−0.11)	1.2	>10	4.12
32		0.57 (0.38−0.86)	>10	>10	3.88
35		ND[d]	>10	>10	3.93
4a		0.0077 (0.0051−0.012)	0.032	0.054	4.21

[a] EC_{50} values are averages of $n = 3$ in the presence of 0.1% BSA. EC_{50} values and 95% confidence intervals of each compound were obtained with Prism 5 software (GraphPad). [b] All values are averages of $n = 2$ in the presence of 0.2% BSA. [c] Log*D* values were determined at pH 7.4 according to a reported method.[39] [d] Not determined (101% increase of control at 10 μM, 2% increase of control at 1 μM).

非芳香4–6員環が縮環した縮合環アルカン酸誘導体 **14**, **15** および **25** は、フェニルプロパン酸誘導体 **4a** に匹敵する受容体作動活性を示した。インダン誘導体 **14** における 3 位のメチレンリンカーをエーテルリンカーに置換したジヒドロベンゾフラン誘導体 **18** が受容体作動活性を保持したことから、酸素原子は活性にほとんど影響を与えず、化合物の脂溶性の指標である Log*D* 値を約 0.5 低下させることが判明した。7 員環が縮環したテトラヒドロベンゾアンヌレン-5-イル酢酸誘導体 **16** では活性が減弱したが、興味深いことに、**16** におけるプロパン酸の結合部位を β 位から α 位に移動させたテトラヒドロ-1-ベンゾオキセピン-4-カルボン酸誘導体 **19** では、受容体作動活性が回復した。この結果から、縮環の大きさやプロパン酸のどの位置で縮環構造を形成しているかに因らず、カルボン酸とベンゼン環が適切な配置を取ることが、強力な GPR40 活性を示すために重要であることがわかった。ベンゾフラン体 **17** およびインドール体 **35** のような平面性の高い芳香族縮合環誘導体では活性が減弱した。本結果から、プロパン酸部位の β 位に sp^3 炭素を有する非芳香環が縮環したベンゼン誘導体の方が、sp^2 炭素を有する誘導体よりも適切にカルボン酸を配置させていると考えられる。最後に、ベンゼン環上の炭素原子を窒素原子に置換した縮合ピリジン誘導体 **32** は、対応する誘導体 **14** と比較して受容体作動活性が減弱した。我々はリガンド－受容体間の π–π 相互作用が、活性発現に重要な役割を果たすことを既に報告している [24]。一般的にピリジン環は π 電子不足ヘテロ環として知られていることから、**32** における活性の減弱は、化合物と GPR40 受容体との π 電子相互作用能が低下したことに起因すると考えられる。

次に、化合物のヒトおよびラット GPR40 受容体に対する結合親和性を評価し、ヒト－ラット間の種差を検討した。我々は化合物のインスリン分泌能および血糖上昇抑制作用を、軽度肥満型糖尿病モデルである雌性 Wistar fatty ラットを用いて評価することから、本データは in vitro と in vivo の相関およびヒトとラットのデータの外挿性を確認する上で重要な情報である。Table 1 に示すように、ヒト受容体結合親和性についてはヒト受容体作動活性との良い相関が認められた。一方、ラット受容体結合親和性については、縮環部が小さい誘導体の方が強い傾向を示し (5 員環 **14**, **18** > 6 員環 **15** > 7 員環 **19**)、ヒト受容体結合親和性とは異なる傾向を示した。ただし、4 員環誘導体 **25** はその傾向を示さなかった。

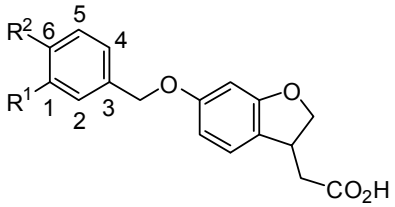
このように、ヒト受容体作動活性およびヒト／ラット受容体結合親和性を示す、非芳香環が縮環したベンゼン誘導体を見出すことに成功した。中でも 5 員環が縮環した誘導体 **14** および **18** は強力な活性を有し、ヒト－ラット間の種差に関してもラットを用いた薬効試験が可能な活性プロファイルを示した。ところで、脂溶性の高い化合物は ADME-Tox (Absorption: 吸収; Distribution: 分布: Metabolism: 代謝; Excretion: 排泄; Toxicology: 毒性) プロファイルの観点から好ましくないことが多数の研究から明らかとなっている [40]。また、後述する薬物動態試験の結果も踏まえ、脂溶性の低いジヒドロベンゾフラン誘導体 **18** をテンプレートとして選択し、さらなる検討を実施した。

第 2 項　ジヒドロベンゾフラン酢酸誘導体における脂溶性側鎖の最適化

第 1 項において、β 酸化に対して安定で優れた血中持続性が期待されるジヒドロベンゾフラン骨格を見出すことに成功したことから、骨格をジヒドロベンゾフランに固定して、リガンドの脂溶性側鎖を検討した (Table 2)。初期構造活性相関研究において、ビフェニル部位の 2 つのベンゼン環が直交した配置が強力な受容体作動活性の発現と血清アルブミンに対する結合に重要であることが判明している [24]。この知見を基に、まず R^1 の置換基としてねじれ構造を取りやすいと推定される二環性芳香環の導入を検討した。最も立体的にかさ高い 2-メチル-1-ナフチル基を有する **45** は、対照化合物 **18** と同等の受容体作動活性および強力なヒト／ラット受容体結合親和性を示した。一般的にチオフェン環はベンゼン環の生物学的等価体として知られていることから、ナフタレン環をベンゾチオフェン環に変換した 3-ベンゾチエニル体 **46** を評価したところ、ほぼ同程度の受容体作動活性を示したものの、特にラット受容体に対する結合親和性が低下した。ベンゼン環のオルト位に相当する 2 位にメチル基を持たないために、中央のベンゼン環との立体配置が固定されにくくなり、結合親和性が低下したと推察される。また、中央ベンゼン環との立体障害がより小さい 5-ベンゾチエニル体 **47** では受容体作動活性が低下した。続いて、分子中央ベンゼン環 6 位への置換基 R^2 の導入を検討したところ、メトキシ体 **48** は対照化合物 **18**

と同等の活性を示し、立体的によりかさ高いベンジルオキシ体 **49** は本系統誘導体の中で最強の受容体作動活性とヒト受容体結合親和性を示した。これらの結果から、ジヒドロベンゾフラン誘導体においても、ビアリール部分の立体的なかさ高さが受容体作動活性発現に重要な役割を果たしていることが明らかとなった。次に、ビフェニル 4′位への置換基導入を検討した。2′,6′-ジメチル体 **18** の片方のメチル基を 4′位に移動させた 2′,4′-ジメチル体 **50** は、作動活性／結合親和性ともに維持し、さらに 2′,4′,6′-トリメチル体 **51** は、より強力なヒト／ラット受容体結合親和性を示した。さらによりかさ高い 4′-ベンジルオキシ基を導入した **52** やある程度の極性を有する 4′-(2-エトキシエトキシ)体 **53** も強力な受容体作動活性と受容体結合親和性を示した。本結果は、ビフェニル 4′位への置換基導入がヒト／ラット受容体結合親和性に好ましい影響を与えるだけでなく、ドラッグライクネス[41] の調節が可能となるさまざまな官能基が許容される可能性を示した重要な知見である。

Table 2. In Vitro Activities of (2,3-Dihydro-1-benzofuran-3-yl)acetic Acids



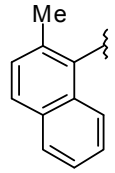
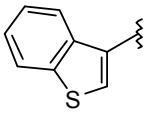
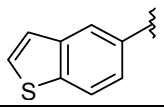
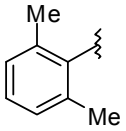
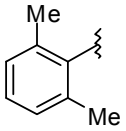
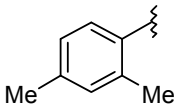
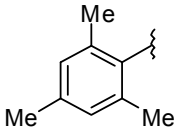
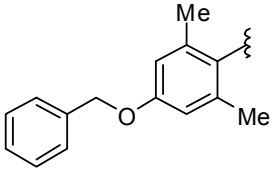
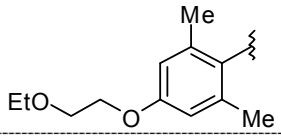
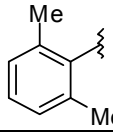
compd	R^1	R^2	FLIPR human EC_{50} (μM)[a]	binding human K_i (μM)[b]	binding rat K_i (μM)[b]	Log*D*[c]
45		H	0.021 (0.014−0.032)	0.041	0.47	4.33
46		H	0.039 (0.024−0.062)	0.29	>10	3.99
47		H	0.18 (0.11−0.28)	0.57	>10	3.97

Table 2. (Continued)

compd	R^1	R^2	FLIPR human EC_{50} (μM)[a]	binding human K_i (μM)[b]	binding rat K_i (μM)[b]	Log*D*[c]
48		OMe	0.024 (0.015−0.040)	0.13	1.6	3.31
49		OBn	0.010 (0.0075−0.014)	0.0069	0.17	4.53
50		H	0.029 (0.018−0.045)	0.057	1.6	4.15
51		H	0.033 (0.018−0.061)	0.036	0.13	4.47
52		H	0.028 (0.017−0.047)	0.018	0.10	5.03
53		H	0.028 (0.018−0.043)	0.032	0.20	3.83
18		H	0.027 (0.019−0.038)	0.17	1.1	3.88

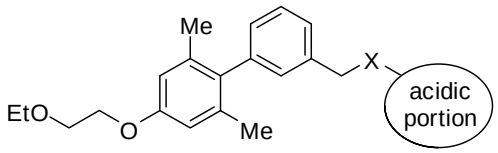
[a] EC_{50} values are averages of $n = 3$ in the presence of 0.1% BSA. EC_{50} values and 95% confidence intervals of each compound were obtained with Prism 5 software (GraphPad). [b] All values are averages of $n = 2$ in the presence of 0.2% BSA. [c] The Log*D* values were determined at pH 7.4 according to a reported method.[39]

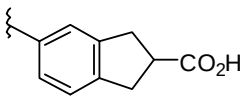
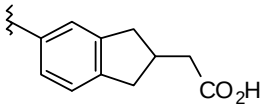
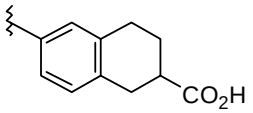
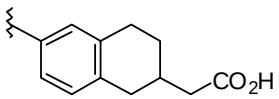
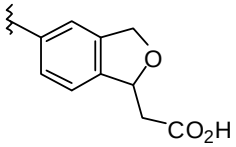
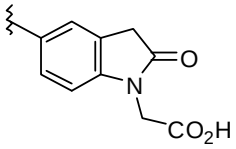
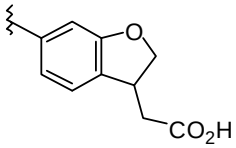
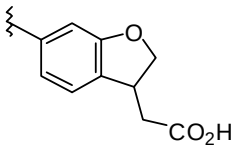
第 3 項　ジヒドロベンゾフラン骨格の再変換

上述のように、ジヒドロベンゾフラン誘導体はヒト GPR40 受容体に対して高い結合親和性を示すものの、薬効評価動物であるラット GPR40 受容体に対しての結合親和性は低下する。そこで、縮環部の結合位置や、環とカルボキシル基

間の距離が活性に与える影響を確認する目的で、再度カルボン酸部分の変換を実施した (Table 3)。その際、分子左側の脂溶性側鎖は、前項の検討で良好なプロファイルを示しかつ比較的脂溶性が低かった 4′-(2-エトキシエトキシ)-2′,6′-ジメチルビフェニリル基に固定した。まず、インダン骨格とテトラヒドロナフタレン骨格を用いて、ベンゼン環とカルボン酸間の炭素鎖長を 2 に固定した状態で閉環位置を α 位に移動させた **61** および **63** や、良好な活性プロファイルを示した **14** と **15** の酢酸ユニット結合位置を隣接位に移動させた **62** および **64** を評価したが、いずれも活性は低下した。特にラット受容体結合親和性が大幅に減弱した。一方、ジヒドロイソベンゾフラン誘導体 **65** は、ヒト受容体に対する活性は若干低下したものの、ラット受容体に対して高い結合親和性を示し、本系統の誘導体の中で唯一ヒトとラットの受容体結合親和性が逆転した。縮合環部の極性向上を指向したオキシインドール体 **68** は活性が大幅に減弱した。極性官能基であるラクタム部位が、受容体との結合を妨げていると考えられる。最後に、対照化合物である **53** のジヒドロベンゾフラン環とビフェニル骨格間のリンカーを、酸素原子から窒素原子に置換した誘導体 **73** は、活性を保持しつつ Log*D* 値は 0.7 程度低下した。結論として、縮環部としては(2,3-ジヒドロ-1-ベンゾフラン-3-イル)酢酸が最適で、6 位リンカーとしては酸素原子だけでなく窒素原子も好ましいことがわかった。

Table 3. In Vitro Activities of Fused-Ring Alkanoic Acids



compd	X	acidic portion	FLIPR human EC_{50} (μM)[a]	binding human K_i (μM)[b]	binding rat K_i (μM)[b]	Log*D*[c]
61	O		0.96 (0.64−1.5)	>10	>10	4.11
62	O		0.084 (0.055−0.13)	0.27	>10	4.39
63	O		0.14 (0.091−0.23)	0.28	>10	4.33
64	O		0.51 (0.32−0.80)	2.1	>10	4.67
65	O		0.091 (0.055−0.15)	0.39	0.067	3.61
68	O		ND[d]	>10	>10	3.41
73	NH		0.048 (0.031−0.076)	0.043	0.11	3.13
53	O		0.028 (0.018−0.043)	0.032	0.20	3.83

[a] EC_{50} values are averages of $n = 3$ in the presence of 0.1% BSA. EC_{50} values and 95% confidence intervals of each compound were obtained with Prism 5 software (GraphPad). [b] All values are averages of $n = 2$ or 3 in the presence of 0.2% BSA. [c] The Log*D* values were determined at pH 7.4 according to the reported method.[39] [d] Not determined (59% increase of control at 10 μM, 2% increase of control at 1 μM).

第 4 節　縮合環アルカン酸誘導体の受容体結合モデル

次に、ウシロドプシンの結晶構造 [42] を基に作成した GPR40 ホモロジーモデルを用いて、縮合環アルカン酸誘導体の結合モードを検討した。ロドプシンは GPR40 と同様にクラス A GPCR ファミリーに属し、かつ唯一結晶が得られていた GPCR であったことから、本検討に用いた。まず、ヒト－ラット間の種差が生じた原因を、5 員環が縮環したジヒドロベンゾフラン誘導体 **18**、6 員環が縮環したテトラヒドロナフタレン誘導体 **15**、および 7 員環が縮環したベンゾオキセピン誘導体 **19** を用いて解析した。GPR40 受容体のアミノ酸配列はヒト－ラット間でよく保存されており、DNA レベルで 75%、タンパクレベルで 82%の相同性がある [18]。リガンド結合ポケット付近のアミノ酸残基で相違が認められるのは、ヒト受容体における Leu186 (TM5, TM: transmembrane) のみと考えられ、この残基がラット受容体では Phe に置換されている。まず、ヒト GPR40 受容体とジヒドロベンゾフラン誘導体 **18** とのドッキングを行い、テトラヒドロナフタレン誘導体 **15** およびベンゾオキセピン誘導体 **19** との重ね合わせを行った (Figure 11A)。その結果、これら 3 つの誘導体は、ビフェニル部位とカルボン酸部位が非常に良い重なりを示し、予想通り共通のファーマコフォアをとっていることが示唆された。ここでヒト受容体の Leu186 を Phe に置換したモデルを重ね合わせたところ、ちょうどこれら誘導体の縮環部が Phe 近傍に位置する結合モードが得られた。従って、縮環部が大きくなるほど Phe との立体反発が大きくなると推定され、このことは縮環部が大きくなるにつれてラット受容体に対する結合親和性が低下した実験結果と合致する。一方、ヒト受容体では十分な空間があり、3 つの化合物間で結合親和性に差がほとんど認められない結果と一致する。このように、ヒト－ラット間の結合親和性に関する種差は、アミノ酸残基の置換により生じたリガンド結合ポケットの大きさの違いにより生じていることが、GPR40 受容体ホモロジーモデルから示唆された。

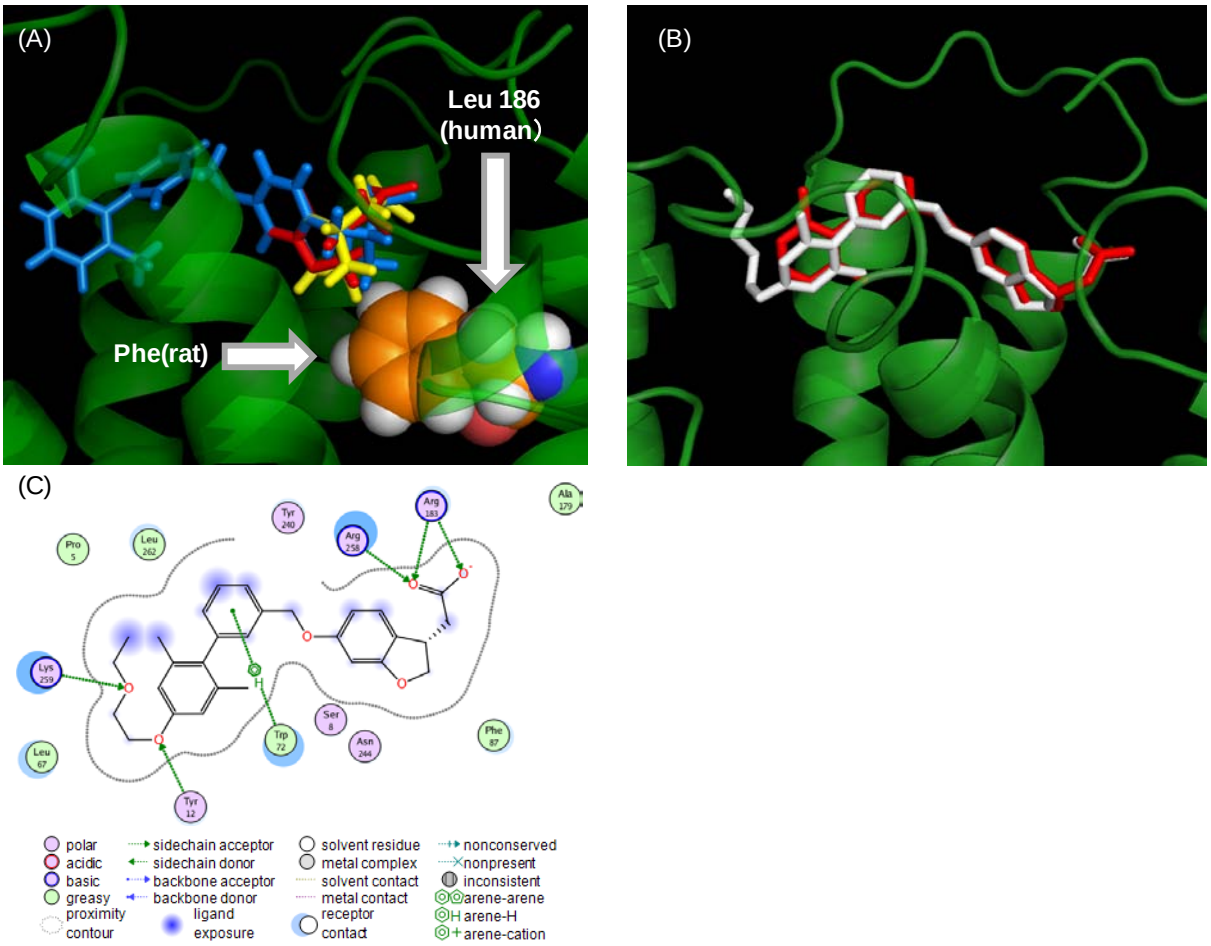


Figure 11. (A) Docking model of GPR40 in complex with **15** (yellow), **18** (red), and **19** (blue). (B) Overlay of **18** (red) and **53** (white) in complex with human GPR40. (C) Two-dimensional diagram showing the interaction of **53** with human GPR40 constructed by MOE.

次に、ビフェニル 4′位に 2-エトキシエトキシ基を有する代表化合物 **53** と、無置換の鋳型化合物 **18** との結合モードの比較を行った (Figure 11B)。その結果、これら誘導体は非常に良い重なりを示した。化合物 **53** は、カルボン酸部位で Arg183 (TM5) および Arg258 (TM7) と静電的相互作用をし、ビフェニル部位で Trp72 (E-I loop) との π–π 相互作用や、Leu67 (TM2)、Phe82 (TM3)、Leu262 (TM7) などの疎水性アミノ酸残基との疎水性相互作用をしていることが示唆された (Figure 11C)。また、化合物 **53** の特徴的な置換基である 2-エトキシエトキシ基は、TM1 と TM7 の間の空間に延び、2 つの酸素原子が近傍の Tyr12 (TM1) や Lys259 (TM7) と相互作用している可能性が示唆された。この知見は、ビフェニル 4′位の置換基効果を検討する価値があることを示している。

第 5 節　縮合環アルカン酸誘導体の薬物動態

強力な GPR40 受容体作動活性および受容体結合親和性を示した縮合環アルカン酸誘導体について、ラット薬物動態試験を実施した (Table 4)。まず、縮合環部の構造と薬物動態プロファイルとの関係を、インダン誘導体 **14**、テトラヒドロナフタレン誘導体 **15**、ジヒドロベンゾフラン誘導体 **18**、およびベンゾオキセピン誘導体 **19** を用いて検討した。その結果、リード化合物であるフェニルプロパン酸誘導体 **4a** と比較して、いずれの誘導体も期待通り良好な薬物動態プロファイルを示し、特に化合物の消失速度を示すクリアランス値 (CL_{total})[43] の低下と、血中での暴露を示す曲線下総面積値 ($AUC_{po,0-8h}$)[44] の向上が認められた。これら誘導体間の比較では、環状エーテル誘導体 **18** および **19** がシクロアルケン誘導体 **14** および **15** よりも良好なプロファイルを示した。環内のメチレンを酸素原子に置換することで、代謝的に脆弱なベンジル位 (Figure 12, site A) がマスクされ、薬物動態プロファイルの改善に繋がったと推察される。最も強力な活性を示した 6-ベンジルオキシビフェニル体 **49** は、良好な経口吸収性 (F = 52.4%) を示すものの、クリアランス値が大きく $AUC_{po,0-8h}$ も低かった。化合物 **49** が血中から速く消失してしまう要因として、脂溶性が高い (Log$D$ = 4.53) ことに起因する組織移行性の高さと、中央ベンゼン環上に電子供与性のベンジルオキシ基を有することでビフェニル環とジヒドロベンゾフラン環をつなぐメチレン部 (Figure 12, site B) の電子密度が高くなり、代謝酵素による酸化を受けやすくなったためと推察される。ビフェニル 4′位にメチル基を導入すると (**51**)、血漿中での暴露量は減少したが ($AUC_{po,0-8h}$ = 698.7 ng·h/mL)、そのベンジル位 (Figure 12, site C) を酸素原子に置き換えた 4′-アルコキシアナログ (**52** および **53**) は、良好な薬物動態プロファイルを示した。特に、4′-(2-エトキシエトキシ)体 **53** は、本系統の化合物の中で最もクリアランス値が低く (211 mL/h/kg)、最も高い血漿中への暴露を示した ($AUC_{po,0-8h}$ = 2837.4 ng·h/mL)。また、窒素リンカーを有する誘導体 (**73**) も酸素リンカーを有する誘導体 (**53**) に匹敵する高い AUC 値とバイオアベイラビリティー値を示した。

Table 4. Rat PK Profiles for Fused-Ring Alkanoic Acids[a]

compd	CL_{total} (mL/h/kg)	C_{max} (ng/mL)	T_{max} (h)	$AUC_{po,0-8h}$ (ng·h/mL)	F (%)
14	464	285.1	1.67	1701.3	47.8
15	782	220.7	1.33	925.7	56.9
18	296	449.7	2.67	2357.3	69.4
19	293	606.6	1.33	2801.4	68.6
49	1708	84.9	1.00	308.9	52.4
51	625	162.8	2.67	698.7	43.3
52	414	316.9	2.00	1687.8	69.4
53	211	465.4	2.67	2837.4	59.5
65	573	193.1	1.33	871.2	49.2
73	348	439.0	1.67	2230.6	70.2
4a	900	86.0	1.50	249.0	21.5

[a] Rat cassette dosing at 0.1 mg/kg, iv and 1 mg/kg, po. All values are averages of 3 rats. *F* indicates bioavailability.

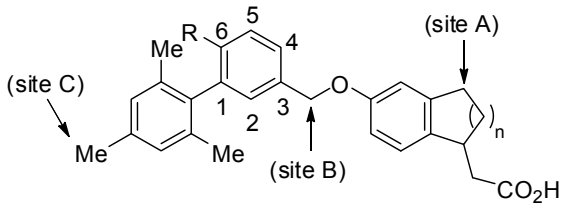


Figure 12. Presumed metabolic sites of fused-ring alkanoic acids.

このように、縮合環アルカン酸誘導体は総じて低いクリアランス値と高い $AUC_{po,0-8h}$ 値を示したが、その効果はフェニルプロパン酸部のβ酸化が抑制されたことに起因すると考えられる。このように、縮合環を形成させることは、血中持続性の高い化合物を見出すための効果的な戦略になると考える。

Table 4 に記載の化合物のうち、**18**、**19**、**53** および **73** が特に優れた薬物動態プロファイルを示したが、in vitro 活性と C_{max}、$AUC_{po,0-8h}$ について総合的に最も優れていた **53** を in vivo 試験に供する化合物として選択した。

第 6 節　化合物 **53** の in vivo 薬理作用

第 5 節の結果に基づき、化合物 **53** の薬効試験を実施した。薬効は、高インスリン血症や高脂血症などの肥満に関連した症状を呈する軽度肥満型糖尿病モデルである雌性 Wistar fatty ラット [45] を用いて OGTT を実施し、化合物 **53** のインスリン分泌促進能と血糖上昇抑制作用を評価した。

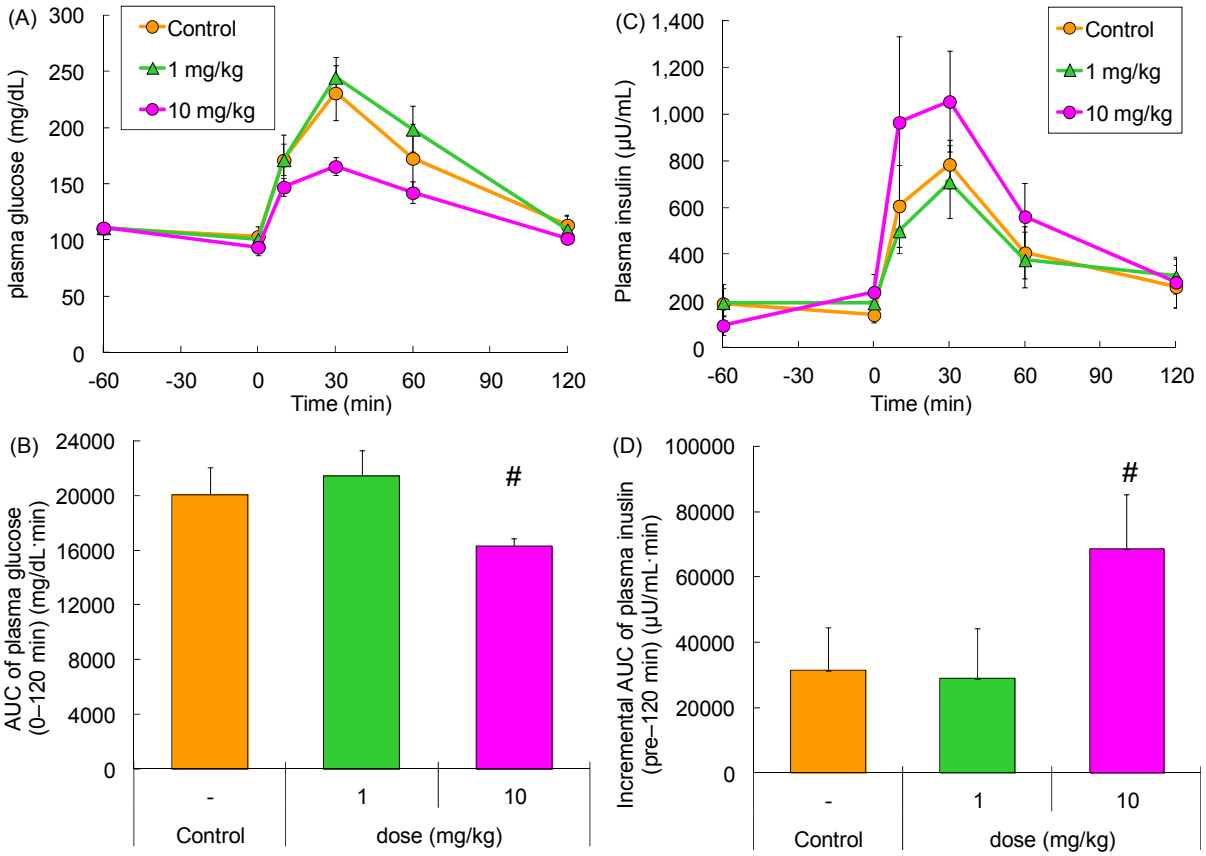


Figure 13. Effects of **53** during a 1H-OGTT in female Wistar fatty rats. (A) and (C) show time-dependent changes of plasma glucose (PG) and plasma insulin levels 1 hour after single oral doses of **53** (1, 10 mg/kg) followed by 1 g/kg oral glucose challenge, respectively. Data in (B) and (D) represent $AUC_{0–120\ min}$ of PG levels and incremental $AUC_{0–120\ min}$ of plasma insulin levels shown in (A) and (C), respectively. Values are mean ± SD (n = 6). #: $P \leq 0.025$ compared with control by one-tailed Williams' test.

また、**53** の最大薬効と薬効持続性を評価する目的で、グルコース負荷 (1 g/kg) の 1 時間前と 4 時間前に薬物を投与した (以降、それぞれの試験を 1H-OGTT、4H-OGTT と記す)。1H-OGTT の血漿中グルコース濃度推移を Figure 13A、その

時間曲線下面積値を Figure 13B に、同様に血漿中インスリン濃度推移を Figure 13C、その時間曲線下面積値を Figure 13D に示す。化合物 **53** を 1 あるいは 10 mg/kg 投薬した結果、10 mg/kg 投薬した際に顕著なインスリン分泌促進作用と血糖上昇抑制作用が認められた。さらに、**53** は 4H-OGTT においても同様に、10 mg/kg の用量で顕著なインスリン分泌促進作用 (Figure 14C, D) と血糖上昇抑制作用 (Figure 14A, B) を示した。

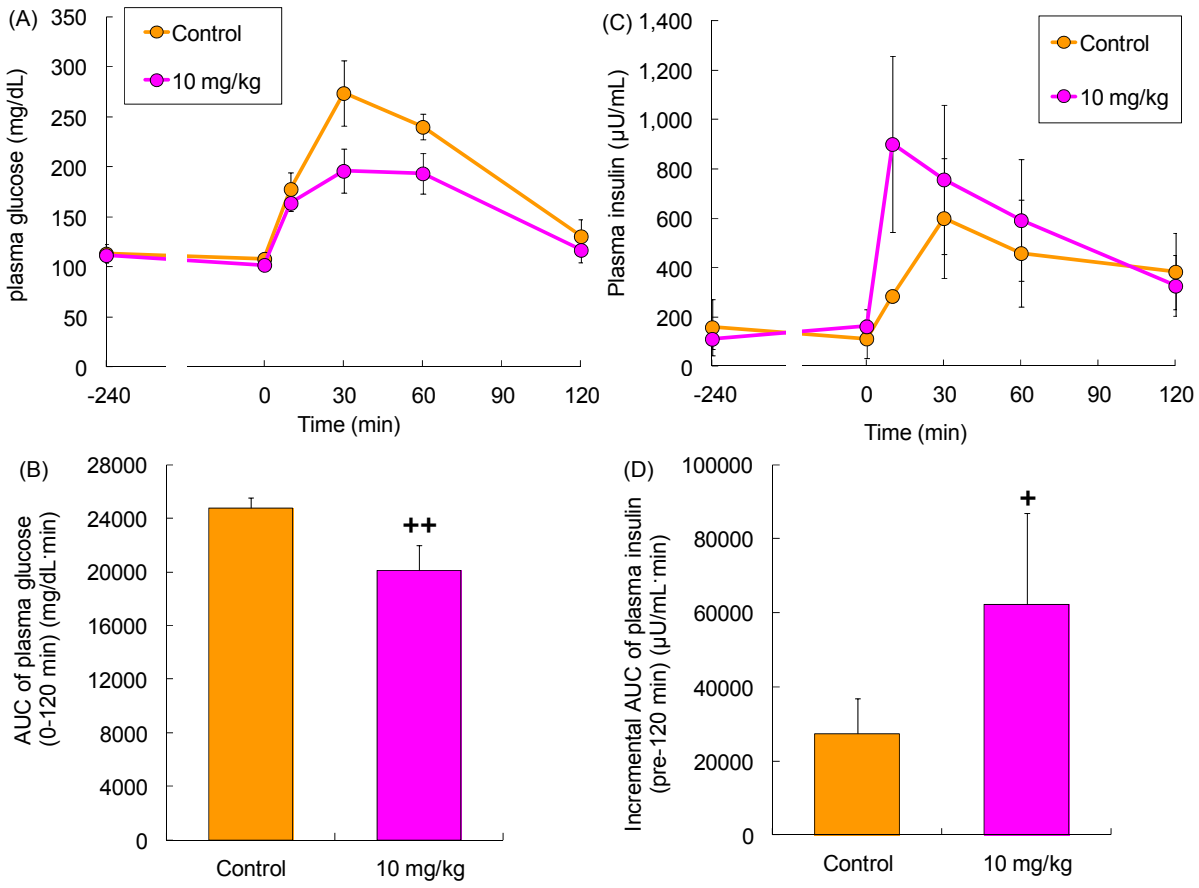


Figure 14. Effects of **53** during a 4H-OGTT in female Wistar fatty rats. (A) and (C) show time-dependent changes of plasma glucose (PG) and plasma insulin levels 4 hour after single oral doses of **53** (10 mg/kg) followed by 1 g/kg oral glucose challenge, respectively. Data in (B) and (D) represent $AUC_{0–120\ min}$ of PG levels and incremental $AUC_{0–120\ min}$ of plasma insulin levels shown in (A) and (C), respectively. Values are mean ± SD (n = 5−6). +: $P \leq 0.05$; ++: $P \leq 0.01$ compared with control by Aspin-Welch test.

このように、ジヒドロベンゾフラン誘導体が良好な薬物動態プロファイルに基づく持続的な薬効を示したことから、縮合環を有する本化合物群は、高いクリアランス値を示すフェニルプロパン酸系誘導体よりも優れた薬物特性を有する

と考えられる。さらに、ジヒドロベンゾフラン誘導体はラット受容体よりもヒト受容体に対する親和性が高いことから、ヒトにおいてより強力な薬効が期待できる。本結果は、ジヒドロベンゾフラン誘導体が強力な薬効、持続性、および高い安全性を有する臨床化合物を見出すための良好なリード化合物であることを支持するものである。

第 7 節　結論

以上述べてきたように、薬物動態プロファイルの優れた GPR40 作動薬創出を目的として合成研究を行った。リード化合物 **4a** の β 酸化に脆弱なフェニルプロパン酸部位に環状構造を導入することにより、強力な活性と良好な薬物動態プロファイルを併有する複数の誘導体を見出した。それらの中で、ヒト受容体作動活性とラット受容体結合親和性に関して優れていた (2,3-ジヒドロ-1-ベンゾフラン-3-イル)酢酸骨格に固定してビアリール部位の探索を実施したところ、4′-(2-エトキシエトキシ)-2′,6′-ジメチルビフェニリル基を有する化合物 **53** が強力な受容体作動活性を有し、低いクリアランス値と高い血中暴露量を示すことを見出した。化合物 **53** は雌性 Wistar fatty ラットを用いた OGTT において、糖負荷 1 時間前および 4 時間前の投与で有意なインスリン分泌促進作用と血糖上昇抑制作用を示した。本知見は、化合物 **53** が長時間作用型の GPR40 作動薬として有効に機能したことを示すものである。本章で示したフェニルプロパン酸部位を縮合環アルカン酸に置換し β 酸化を抑制する方法論は、筆者の知る限り報告例が無く、ターゲット分子に対する活性を保持しつつ薬物動態プロファイルを改善するための有用な戦略となりうると考えられる。

第 2 章　創薬を指向した GPR40 作動薬の最適化研究：極性官能基を有するジヒドロベンゾフラン酢酸誘導体の合成と生物活性 [26,28)]

第 1 節　序論

これまでに数多くの GPR40 の合成リガンドが報告されている (Figure 15) [46−52)]。これらの多くは、内因性リガンドである FFA を基にデザインされていることから、多彩な生理作用を有する FFA に由来するオフターゲット作用を併有している可能性がある。臨床開発可能な安全性の高い GPR40 作動薬を創出するためには、FFA 様のプロファイルからの脱却、すなわち脂溶性の低減が必要であると考えた。

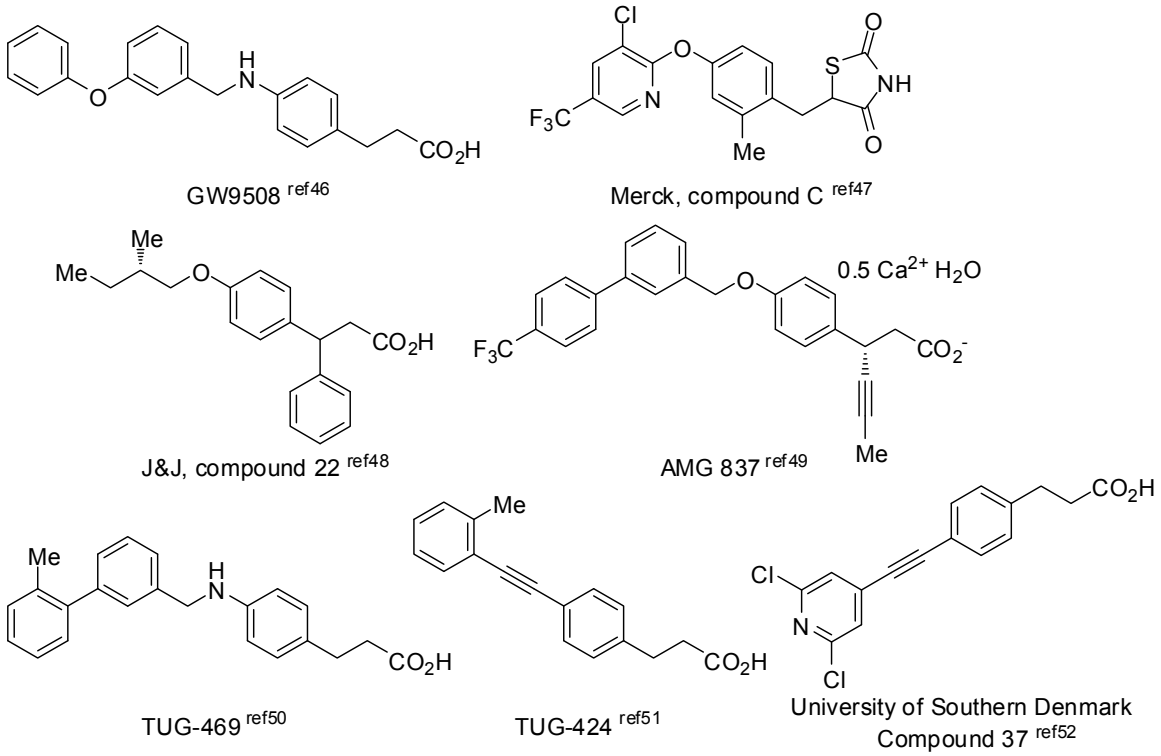


Figure 15. Representative GPR40 agonists.

第 1 章において、縮合環アルカン酸誘導体がフェニルプロパン酸誘導体の強力な GPR40 受容体作動活性を保持しつつ、薬物動態プロファイルを大幅に改善すること、さらに、4′-(2-エトキシエトキシ)-2′,6′-ジメチルビフェニリル基を有するジヒドロベンゾフラン誘導体 **53** が、ラット GPR40 受容体に対してやや結合親和性が低い (human/rat: 0.032/0.20 μM, Table 2) にもかかわらず、化合物投与 4 時間後の OGTT においても有意な薬効を示すことがわかった。一方、化合物 **53** は長時間作用型 GPR40 作動薬のコンセプトを立証したものの、in vivo 試験における有効薬効量はまだ高く (10 mg/kg)、加えて脂溶性も依然として高かった (Log*D*: 3.83)。そこで、脂溶性を低下させるべく、極性置換基の導入が可能かどうかを検討した。その際、FFA が毒性を示すことが報告されている、ヒト肝細胞由来細胞株 HepG2 細胞を用いて、その毒性を評価することとした [53]。第 1 章において、ビフェニル 4′位にメチル基、エトキシエトキシ基やベンジルオキシ基が許容されることを報告した。また、別の化合物群における検討結果から、ビフェニル 4′位へのさまざまな置換基導入が可能であることも判明している [54]。これらの知見を踏まえ、Figure 16 に示す合成戦略を立案した。第一に、脂溶性を低減させるべく、ビフェニル 4′位への極性置換基の導入を検討した。第二に、ジヒドロベンゾフラン 3 位の立体化学の重要性を確認するために、エナンチオマーの分離を行った。最後に、in vitro 活性と薬物動態プロファイルを最適化する目的で、ビフェニル骨格への置換基導入を検討した。一般式 A に示す化合物の逆合成経路は 2 通り考えられる。ルート I は第 1 章で述べた方法と同様であり、ベンジルエーテル部 **a** で分割してビフェニリルメタノールあるいはそのメシレート B と 6-ヒドロキシジヒドロベンゾフラン C から光延反応もしくは置換反応で形成させることを計画した。中間体 B は、各種置換基を有する 4′-ヒドロキシビフェニル D と、アルコールとの光延反応もしくはハライド、メシレートあるいはエポキシドとの置換反応により調製可能と考えた。一方、ルート II は、ビフェニル 4′位のアルコキシ部 **b** で分割して、鍵中間体 E から各種アルコールやハライドとの光延反応もしくは置換反応を利用することとした。本方法は、ビフェニル骨格 4′位の変換に特化した方法であり、最終工程の 1 工程前での置換基変換が可能である。中間体 E は、D から容易に調製可能なビフェニル 4′位

ヒドロキシ基を保護したアルコール F と 6-ヒドロキシジヒドロベンゾフラン C から誘導できると考えた。

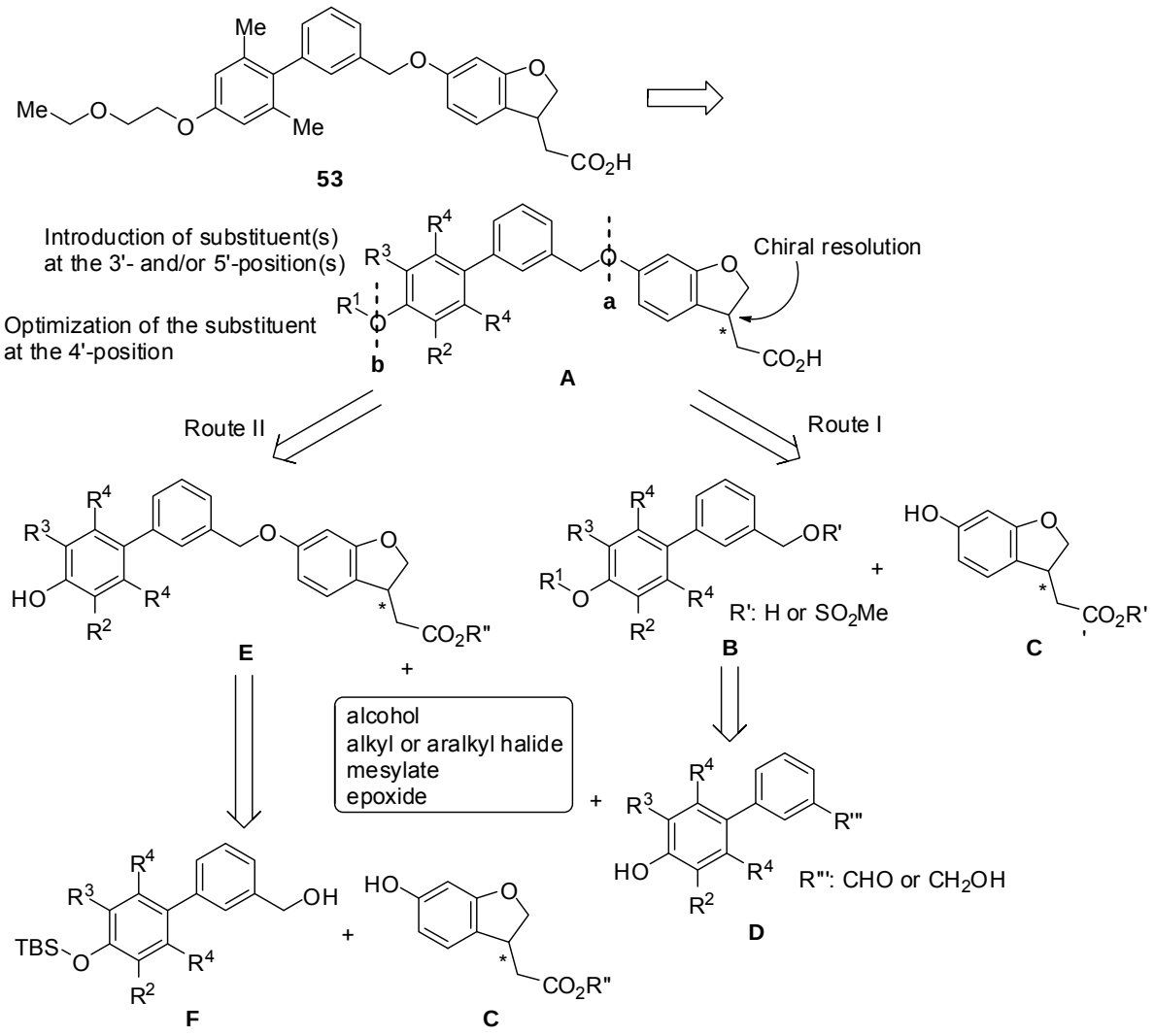


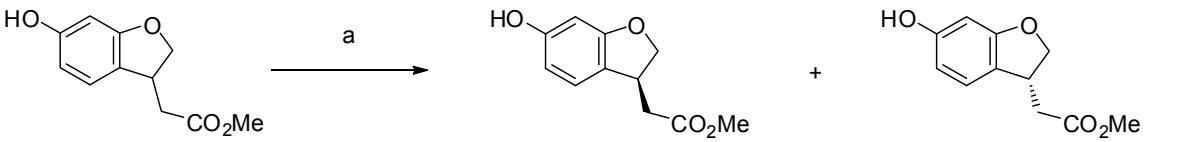
Figure 16. Design of (2,3-dihydro-1-benzofuran-3-yl)acetic acids.

第 2 節　ジヒドロベンゾフラン酢酸誘導体の合成

第 1 項　ジヒドロベンゾフラン中間体の光学分割

ラセミ体のジヒドロベンゾフラン中間体 **7e** を第 1 章第 2 節第 1 項の方法にて合成し、CHIRALPAK AD カラムを用いたキラル HPLC 分割により、エナンチオマー **7g** および **7h** を得た (Scheme 6)。ジヒドロベンゾフラン 3 位の絶対配置は、後述するように、**7g** から誘導したユートマー[55] **85** の X 線結晶構造解析により決定した。

Scheme 6[a]



7e (racemate)　　**7g** (short retention time, *S*-isomer)　**7h** (long retention time, *R*-isomer)

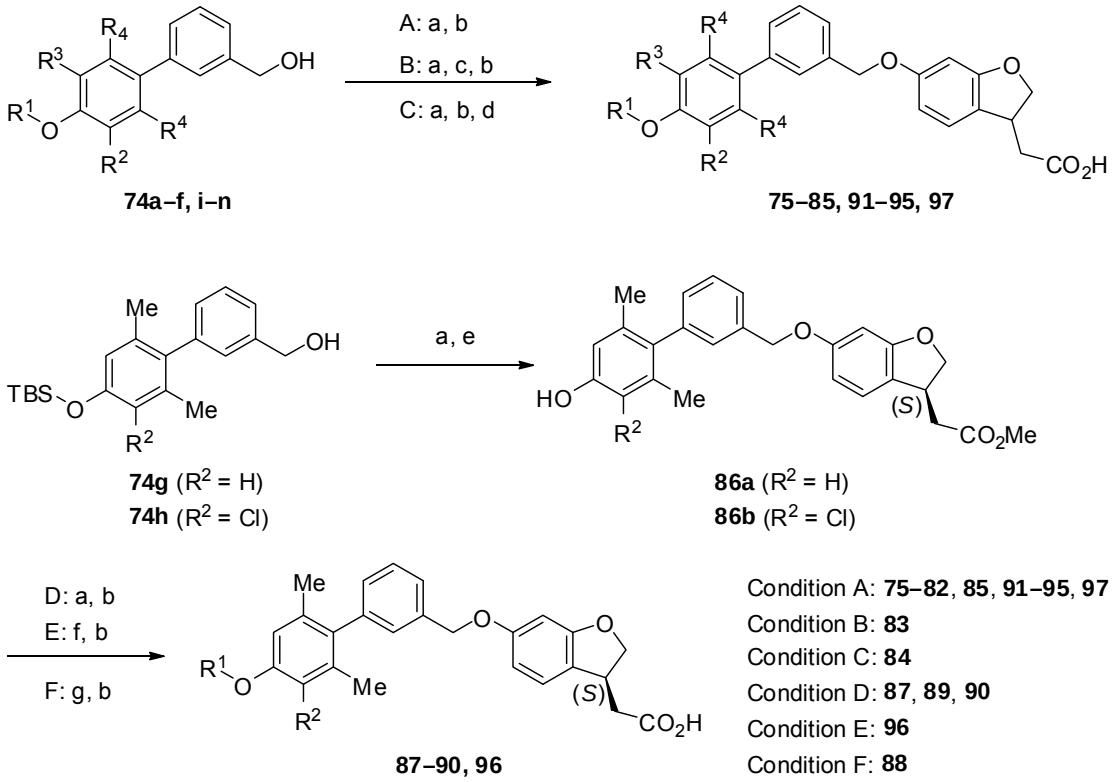
[a] Reagents and conditions: (a) CHIRALPAK AD HPLC separation.

第 2 項　ジヒドロベンゾフラン誘導体の合成

目的とする GPR40 作動薬は、Scheme 7 に示す方法で合成した。フェノール中間体 **7e**, **7g** および **7h** をビフェニリルメタノール **74a–f** または **74i–n** (後述) との光延反応、あるいは対応するメシル体とのアルキル化により縮合させ、続くエステル加水分解によりカルボン酸 **75–85**, **91–95** および **97** を得た。なお、分子内にスルホニル基を有する **83** と **84** については、スルフィドの酸化工程を経て合成した。一方、ビフェニル骨格 4′位アルコキシ基の変換については、別法にて実施した。すなわち、アルコール体 **74g** および **74h** から誘導される鍵中間体 **86a** および **86b** とアルコール体あるいはトシル体との反応により置換

基を導入後、エステルの加水分解を行い、目的とする化合物 **87–90** および **96** を合成した。

Scheme 7[a]



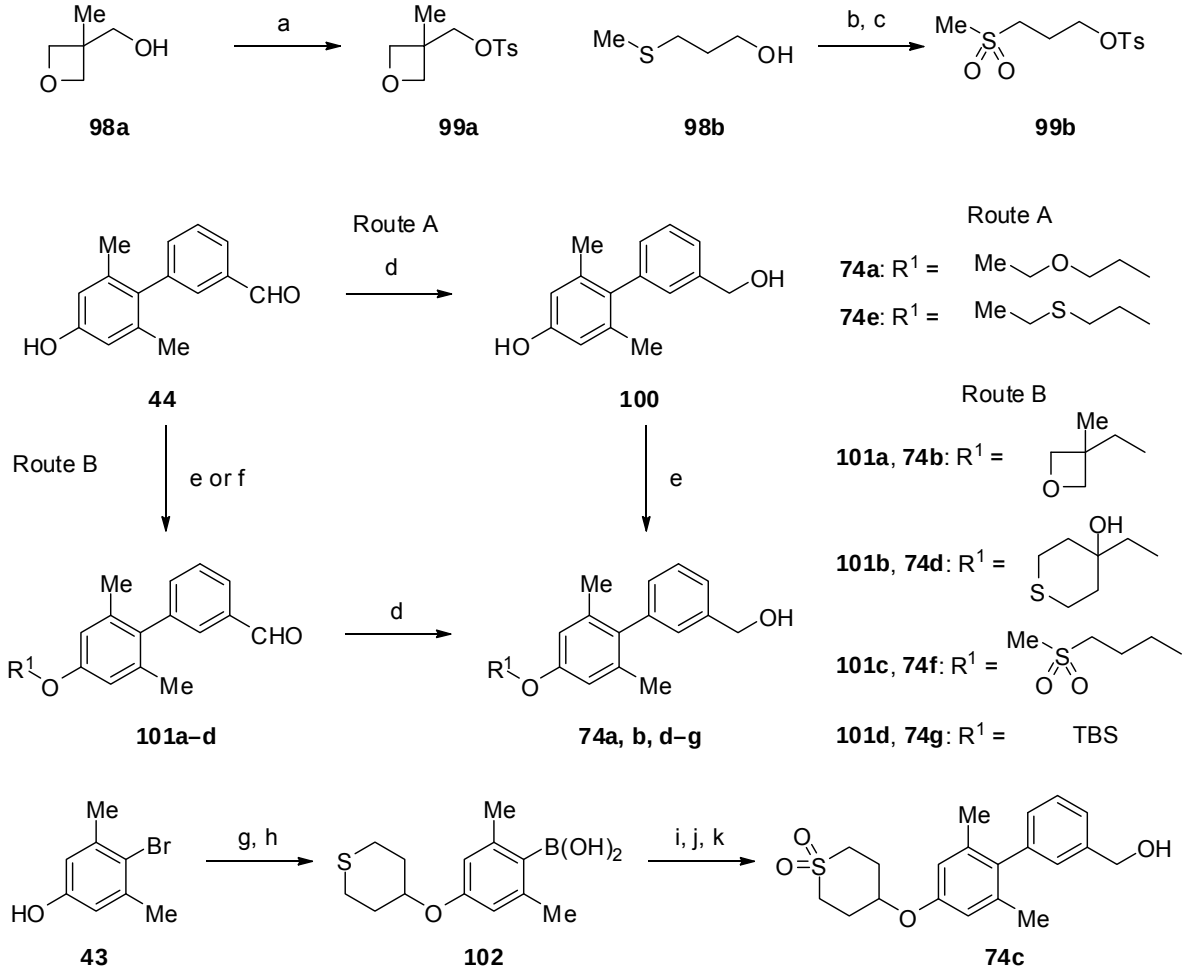
[a] Reagents and conditions: (a) **7e**, **g**, **h** (condition A–C) or R^1OH (condition D), ADDP, P(*n*-Bu)$_3$, toluene, rt, 60–95%; (b) 2 M NaOH aq., MeOH, THF, 50 °C, 32–94% except for **87**, **89**, **90**, 16–42% (from **86a** in 2 steps); **94**, 15% (from **111c** in 3 steps); **88** as a HCl-salt; (c) *m*-CPBA, AcOEt, 0 °C, 79%; (d) Oxone®, MeOH, H_2O, 0 °C to rt, 73%; (e) TBAF, THF, rt, 88–94%; (f) **99b**, K_3PO_4, DMF, 90 °C, 88%; (g) 1-methylpiperidin-4-ol, DEAD, PPh_3, toluene, rt, 63%.

第 3 項　ビフェニリルメタノール中間体の合成

ビフェニリルメタノール中間体 **74a–g** の合成を Scheme 8 に示す。トシレート **99a** は通常の条件で合成したが、(3-メチルチオ)-1-プロパノール (**98b**) を同様の条件に付すとピリジン塩酸塩の弱い求核性によりトシル基が塩素原子に置換された塩素体を副生した。そこで、吉田、田辺らの報告[56] に従い、触媒量の

ジアミン存在下、塩化トシルと反応させたところ、収率良くトシレートを得ることができた。続いて、オキソン®を用いてスルフィドの酸化を行い、目的とするスルホン **99b** へと誘導した。

Scheme 8[a]



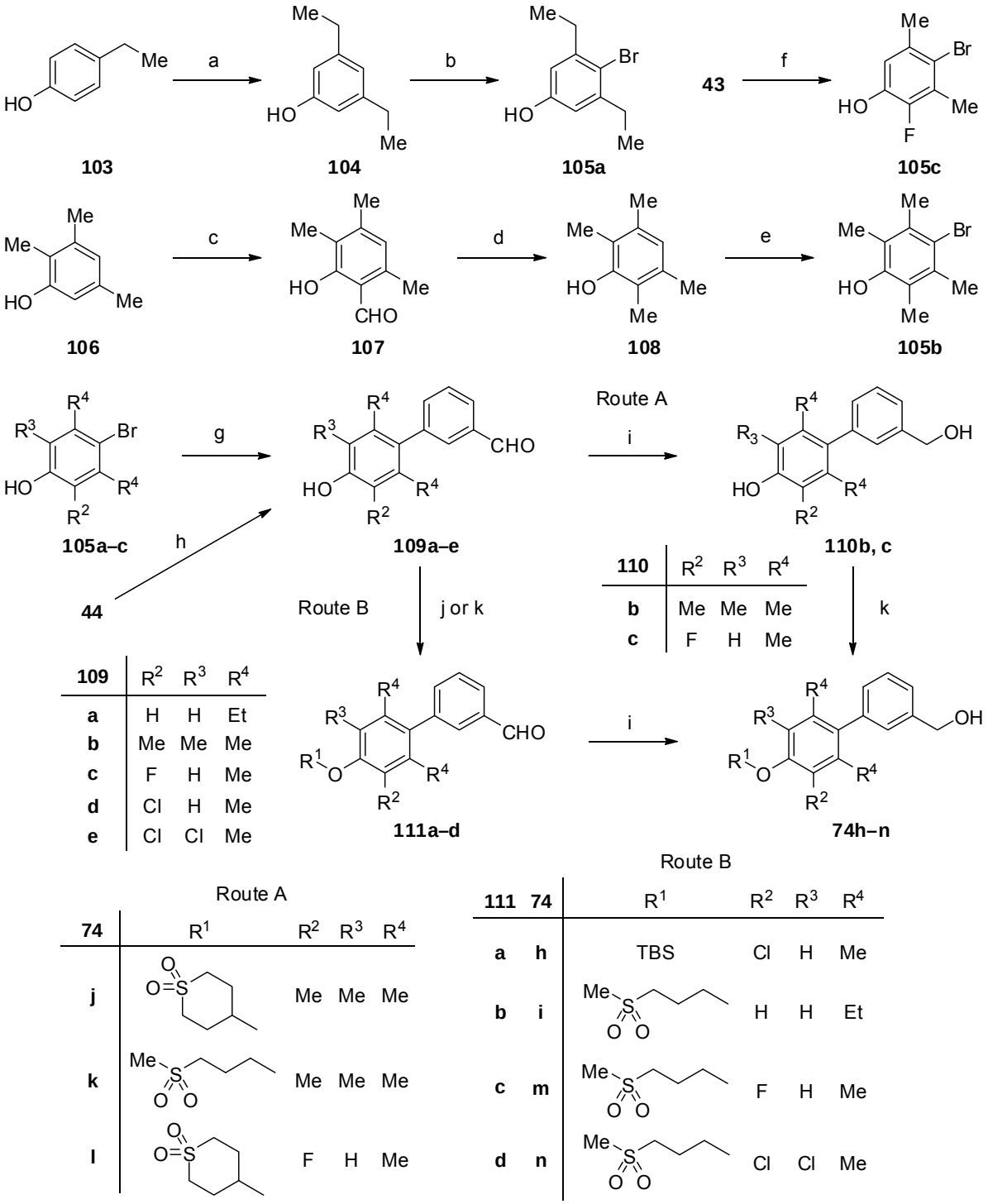
[a] Reagents and conditions: (a) *p*-TsCl, pyridine, 0 °C, 70%; (b) *p*-TsCl, *N*,*N*,*N*′,*N*′-tetramethyl-1,6-hexanediamine, Et_3N, toluene, 0 °C, 94%; (c) Oxone®, MeOH, H_2O, 0 °C to rt, 96%; (d) $NaBH_4$, MeOH, THF, 0 °C to rt, 92–97%; (e) R^1Cl (for **74a** and **74e**), R^1OTs (for **74b** and **74f**) or 1-oxa-6-thiaspiro[2.5]octane (for **74d**), K_2CO_3, (KI), DMF, 70–100 °C, 47–98%; (f) TBSCl, imidazole, DMF, rt, 77%; (g) tetrahydro-4*H*-thiopyran-4-ol, DEAD, PPh_3, THF, rt, 86%; (h) i) 1.6 M *n*-BuLi in hexanes, THF, −78 °C; ii) B(*i*-PrO)$_3$, −78 °C to rt; iii) 2 M HCl aq., rt, 71% (from **43** in 2 steps); (i) methyl 3-bromobenzoate, $Pd(PPh_3)_4$, 2 M Cs_2CO_3 aq., DME, reflux, 86%; (j) *m*-CPBA, AcOEt, 0 °C, 85%; (k) $LiAlH_4$, THF, 10 °C to rt, 93%.

前章で報告した中間体 **44** を用い、2 通りの合成ルートで **74a**, **74b** および **74d–g** を合成した。ルート A では、まず **44** を水素化ホウ素ナトリウムで還元してアルコール **100** とし、続いてフェノール性ヒドロキシ基を選択的にアルキル化し **74a** および **74e** を得た。一方、ルート B では **44** をトシレート **99a**、1-オキサ-6-チアスピロ[2.5]オクタン、あるいは **99b** でアルキル化、もしくは *tert*-ブチルジメチルクロロシランでシリル化し、続いて還元に付すことで **74b**, **74d**, **74f** および **74g** へと誘導した。また、1,1-ジオキシドテトラヒドロチオピラニル基を有するアルコール **74c** は、別法により合成した。すなわち、フェノール **43** を光延反応により 4-ヒドロキシチオピランと縮合させ、得られたエーテル体をボロン酸に変換して **102** とした。続いて、3-ブロモ安息香酸メチルとの鈴木カップリング反応に付し、チオエーテル部位を酸化してスルホンに変換後、水素化アルミニウムリチウムを用いてエステルの還元を行い、所望のアルコール **74c** を得た。

第 4 項　3′位あるいは 5′位に置換基を有するビフェニリルメタノール中間体の合成

3′位あるいは 5′位に置換基を有するビフェニリルメタノール中間体 **74h–n** の合成を Scheme 9 に示す。ジエチル基を有する **105a** は、Baddeley の報告 [57] に従い 4-エチルフェノール (**103**) を塩化アルミニウムで処理して 3,5-ジエチルフェノール (**104**) を得、三臭化 *n*-テトラブチルアンモニウムでパラ位選択的に臭素化して合成した。テトラメチル体 **105b** は、2,3,5-トリメチルフェノール (**106**) を出発物質として、四塩化チタン存在下、ジクロロメチルメチルエーテルと反応させることで生じた所望のオルトホルミルフェノール **107** と副生成物のパラホルミルフェノールをシリカゲルカラムで分離して約 2:1 の比率で得、続いて接触水素化を行うことによりテトラメチルフェノール **108** へと変換後、臭素化することで合成した。モノフルオロ体 **105c** は、**43** にトリフルオロメタンスルホン酸 1-フルオロピリジニウムを作用させてフッ素化することにより、中程度の収率で得ることに成功した。

Scheme 9[a]



109	R^2	R^3	R^4
a	H	H	Et
b	Me	Me	Me
c	F	H	Me
d	Cl	H	Me
e	Cl	Cl	Me

110	R^2	R^3	R^4
b	Me	Me	Me
c	F	H	Me

Route A

74	R^1	R^2	R^3	R^4
j		Me	Me	Me
k		Me	Me	Me
l		F	H	Me

Route B

111	74	R^1	R^2	R^3	R^4
a	h	TBS	Cl	H	Me
b	i		H	H	Et
c	m		F	H	Me
d	n		Cl	Cl	Me

[a] Reagents and conditions: (a) $AlCl_3$, 115 °C, 78%; (b) *n*-Bu_4NBr_3, MeOH, rt, 72%; (c) dichloromethyl methyl ether, $TiCl_4$, CH_2Cl_2, 0 °C, 40%; (d) H_2, Pd/C, MeOH, toluene, rt, 97%; (e) Br_2, AcOH, rt, 83%; (f) 1-fluoropyridinium triflate, 1,2-dichloroethane, reflux, 36%; (g) 3-formylphenylboronic acid, $PdCl_2(dppf)\cdot CH_2Cl_2$, K_3PO_4, THF, 80 °C, 49–79%; (h) NCS, DMF, rt, 60–65%; (i) $NaBH_4$, MeOH, THF, 0 °C, 65–98%; (j) TBSCl, imidazole, DMF, rt, 88%; (k) R^1OTs, K_2CO_3, (KI), DMF, 90–95 °C, 53–95%.

このようにして得られたブロモフェノール **105a–c** を 3-ホルミルフェニルボロン酸との鈴木カップリング反応に付し、ビフェニル **109a–c** とした。モノクロロ体およびジクロロ体 **109d** および **109e** は、ビフェニル **44** を *N*-クロロスクシンイミドで処理することによりそれぞれ合成した。続いて、ビフェニル **109a–e** を Scheme 8 と同様に 2 通りの合成法を活用して、目的とする **74h–n** へと誘導した。

第 5 項　絶対配置の決定

化合物 **85** の絶対立体配置は、X 線結晶構造解析により *S* 体と決定した (Figure 17)[58]。化合物 **85** は鍵中間体 **7g** から誘導していることから、**7g** の立体配置は *S* 体、逆の立体配置を有する **7h** は *R* 体と決定した。

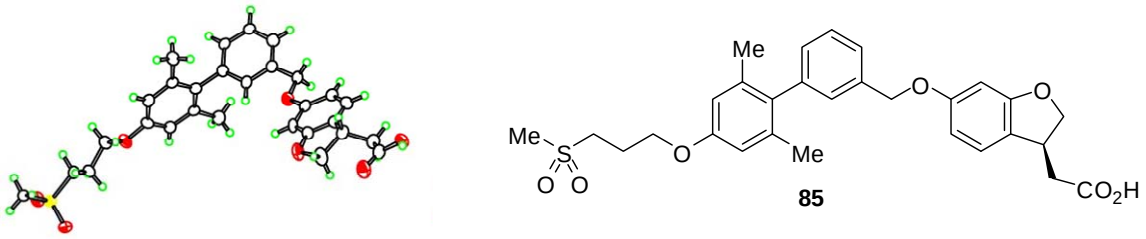


Figure 17.　Stereoscopic molecular view and chemical structure of compound **85**.

第 3 節　ジヒドロベンゾフラン誘導体の in vitro 活性

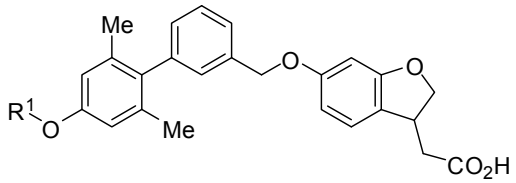
合成した化合物のヒト受容体作動活性およびヒト／ラット受容体に対する結合親和性を評価した。また、GPR40 の内因性リガンドである FFA は、HepG2 細胞におけるカスパーゼ 3/7 の酵素活性を促進しアポトーシスを誘導することが報告されている [53] ことから、化合物のアポトーシス誘導能をそのマーカーであるカスパーゼ 3/7[59] 活性を測定することで評価した [60]。

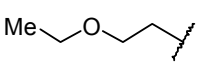
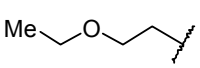
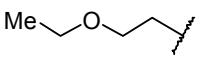
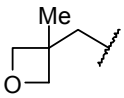
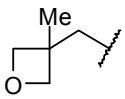
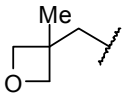
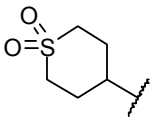
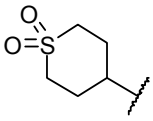
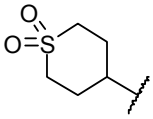
第 1 項　ジヒドロベンゾフラン 3 位光学異性体の検討

最初に、ビフェニル骨格 4′位の置換基に対する許容性を、2-エトキシエトキシ基とは異なる 2 つの官能基を用いて確認した (Table 5)。2-エトキシエトキシ体 **53** のエトキシ部位を環化させたタイプの (3-メチルオキセタン-3-イル)メトキシ体 **77** は強力な活性を示し、さらによりかさ高く極性の高い (1,1-ジオキシドテトラヒドロ-2*H*-チオピラン-4-イル)オキシ体 **80** もまた受容体作動活性およびヒト/ラット結合親和性を保持した。これらの結果は、末端置換基周辺の結合ポケットが極性官能基を含む幅広い置換基を許容することを示唆している。

また、平行してジヒドロベンゾフラン環 3 位立体化学の影響を検証した。GPCR は細胞膜表面に存在するタンパクで、リガンドによる活性化に伴ってそのコンフォメーションをダイナミックに変化させる。GPR40 に関しては、さまざまな中長鎖脂肪酸が内因性リガンドとして機能することが知られている。すなわち GPR40 のリガンド認識に関しては、脂溶性のアルキル鎖が結合する部位はさほど厳密ではないことが示唆される。一方、内因性リガンドに共通する部分構造であるカルボン酸が結合する部位は、リガンド結合後のシグナル伝達に重要であることから、非常に厳密であることが示唆される。そこで、ジヒドロベンゾフラン誘導体におけるカルボン酸近傍に存在するジヒドロベンゾフラン環 3 位の絶対立体配置が、リガンドの結合とその後のシグナル伝達に重要であると想定し、その確認を行った。

Table 5. In Vitro Activities of (2,3-Dihydro-1-benzofuran-3-yl)acetic Acids



compd	R^1	stereo	FLIPR human EC_{50} (μM)[a]	binding human K_i (μM)[b]	binding rat K_i (μM)[b]	Caspase -3/7[c]	Log*D*[d]
53		*rac*	0.030 (0.019−0.047)	0.032	0.30	3.2	3.83
75		*S*	0.016 (0.012−0.023)	0.023	0.24	1.5	3.86
76		*R*	0.29 (0.19−0.44)	0.28	7.4	10.5	3.86
77		*rac*	0.024 (0.016−0.035)	0.021	0.083	23.6	3.86
78		*S*	0.018 (0.013−0.024)	0.025	0.11	21.1	3.88
79		*R*	0.27 (0.18−0.42)	0.27	2.2	20.9	3.88
80		*rac*	0.039 (0.024−0.065)	0.083	0.21	0.4	2.77
81		*S*	0.022 (0.016−0.030)	0.036	0.17	−2.5	2.75
82		*R*	0.29 (0.20−0.43)	0.30	0.51	1.3	2.84

[a] All values are average of $n = 3$ in the presence of 0.1% BSA. Efficacies of compounds at 10 μM were 103–113% of γ-linolenic acid at 10 μM. [b] All values are average of $n = 2$ in the presence of 0.2% BSA. [c] Percent of activation at 30 μM was compared to maximal activity of staurosporine as a reference compound. [d] The Log*D* values were determined at pH 7.4 according to the reported method.[39]

その結果、(*S*)-エナンチオマー **75** は対応する(*R*)-エナンチオマーよりも強力なヒト受容体作動活性およびヒト／ラット受容体に対する高い結合親和性を示した。この *R* 配置よりも *S* 配置が好ましい傾向は、他の 4′-アルコキシビフェニ

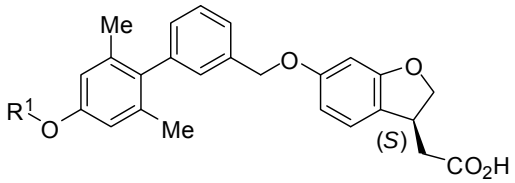
ル誘導体においても同様に認められた (**78** vs. **79** および **81** vs. **82**)。これらの結果から、ジヒドロベンゾフラン環に結合している酢酸部位の立体配置が GPR40 活性の発現に非常に重要な役割を果たしていることが明らかとなった。このように、GPR40 受容体のリガンド結合ポケットは、ジヒドロベンゾフラン環の芳香環とカルボン酸部位の相対的な位置関係を厳密に認識していることが示唆された。以上の検討で、(2,3-ジヒドロ-1-ベンゾフラン-3-イル)酢酸部位の好ましい立体配置を同定したことから、以降の検討は *S* 体を用いて実施した。

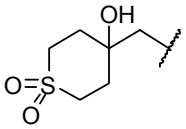
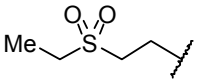
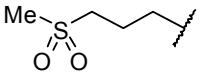
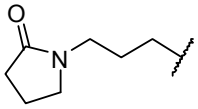
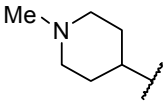
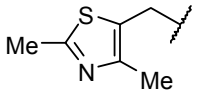
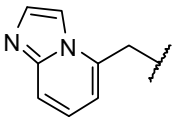
カスパーゼ-3/7 活性に関しては、脂溶性の指標である Log*D* 値の低い (2.8 程度) (1,1-ジオキシドテトラヒドロ-2*H*-チオピラン-4-イル)オキシ体 (**80**–**82**) が、エーテル誘導体 (**53**, **76**–**79**) と比較して低い傾向を示した。これらの結果から、**81** をさらなる評価対象化合物に選定した。

第 2 項　ビフェニル 4′位への極性基の導入

期待通り末端フェニル基の 4′位にエーテルやスルホンのような極性官能基が許容されたことから、化合物の脂溶性を最適化すべくこの部位の置換基変換を行った (Table 6)。環状および直鎖状のスルホンアナログ **83**–**85** は脂溶性が低いにもかかわらず (Log*D*: 2.43–2.73)、強力な受容体作動活性と受容体結合親和性を示した。ラクタムアナログ **87** もまた受容体作動活性および受容体結合親和性を保持した。化合物 **81** における 1,1-ジオキシド-2*H*-チオピラン環のスルホン部位をメチルアミンに置換した 1-メチルピペリジン-4-イル体 **88** は、受容体作動活性を保持し、より強力な受容体結合親和性を示した。また、チアゾール (**89**) やイミダゾピリジン (**90**) のような芳香族複素環アナログもまた、強力な受容体作動活性と受容体結合親和性を保持した。しかしながら、**89** および **90** はカスパーゼ-3/7 活性を示した。この作用は、これら化合物の高い脂溶性 (Log*D*: 4.27 および 3.80) に関連があると考えられる。

Table 6. In Vitro Activities of (2,3-Dihydro-1-benzofuran-3-yl)acetic Acids



compd	R^1	FLIPR human EC_{50} (μM)[a]	binding human K_i (μM)[b]	binding rat K_i (μM)[b]	Caspase -3/7[d]	Log*D*[e]
83		0.013 (0.010−0.017)	0.024	0.17	–1.8	2.43
84		0.014 (0.011−0.019)	0.037	0.17	–1.3	2.73
85		0.016 (0.012−0.021)[c]	0.038	0.14	–2.1[c]	2.58[c]
87		0.017 (0.013−0.023)	0.031	0.17	3.9	3.22
88		0.019 (0.014−0.025)	0.0088	0.066	–1.3	3.10
89		0.018 (0.013−0.025)	0.012	0.36	23.1	4.27
90		0.017 (0.013−0.022)	0.015	0.19	17.7	3.80

[a] All values are average of $n = 3$ in the presence of 0.1% BSA. Efficacies of compounds at 10 μM were 107–113% of γ-linolenic acid at 10 μM. [b] All values are average of $n = 2$ or 3 in the presence of 0.2% BSA. [c] The activity was measured with anhydrous **85**. [d] Percent of activation at 30 μM was compared to maximal activity of staurosporine as a reference compound. [e] The Log*D* values were determined at pH 7.4 according to the reported method.[39]

上記のように、ビフェニル 4′位にスルホン、アミド、アミン、およびヘテロ芳香環などのさまざまなかさ高さと極性を有する官能基が、受容体作動活性および受容体結合親和性の面で許容されることがわかった。これらの結果から、ヒト／ラット GPR40 受容体のリガンド結合ポケットは、ジヒドロベンゾフラン誘導体のビフェニル 4′位が位置する近傍に大きな空間を有することが示唆され

た。従って、この部位は化合物の脂溶性、毒性や薬物動態などの ADME-Tox (absorption, distribution, metabolism, excretion and toxicology) プロファイルの調節に利用可能と考えられる。これらの結果を基に、Table 6 の化合物から **83**–**85**, **87** および **88** を精査化合物として選定した。

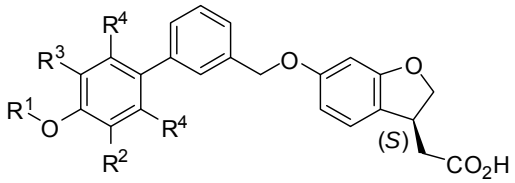
第 3 項　ビフェニル環への置換基導入効果

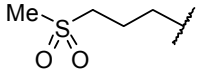
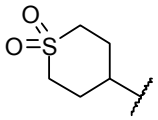
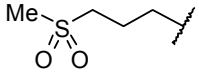
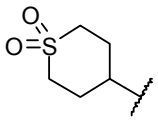
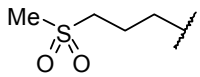
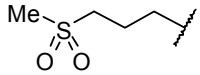
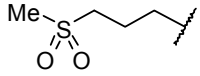
第 1 章第 3 節第 4 項において示したように、リガンド結合ポケットのビフェニル基が位置する近傍には、疎水性アミノ酸残基が多く存在することが示唆されている。そこで、さらなる活性の向上と ADME-Tox プロファイルの調節を意図して、ビフェニル環へのメチル基やハロゲン原子などの疎水性置換基の導入を行った (Table 7)。小さな疎水性置換基を導入した誘導体 **91**–**97** は、おおむね同等の受容体作動活性と同等もしくは若干高い受容体結合親和性を示した。中でも、2′,6′-ジエチルアナログ **91** および 3′,5′-ジクロロアナログ **97** は、ジメチルアナログ **85** と比較して強力な受容体結合親和性を示した。

カスパーゼ-3/7 活性に関しては、(1,1-ジオキシドテトラヒドロ-2*H*-チオピラン-4-イル)オキシ基を有する 2′,3′,5′,6′-テトラメチル体 **92** およびモノフルオロ体 **94** は弱い活性化を示した。一方、3-(メチルスルホニル)プロポキシ基を有する誘導体では、2′,3′,5′,6′-テトラメチル体 **93** およびモノフルオロ体 **95** ともにカスパーゼ-3/7 活性を示さなかったものの、塩素原子を有する誘導体 (**96** および **97**) はカスパーゼ-3/7 活性が残存した。

このように、いずれの化合物も同等のヒト受容体作動活性を示したが、幾つかの誘導体はその脂溶性の向上に伴ってカスパーゼ-3/7 活性が発現する結果となった。以上の結果から、本系統の誘導体においてアポトーシス誘導を惹起することが懸念される Log*D* の境界値は、2.9–3.2 程度であると考えられる。Table 7 に示す結果に基づき、**93** と **95** の 2 化合物を精査化合物として選定した。

Table 7. In Vitro Activities of (2,3-Dihydro-1-benzofuran-3-yl)acetic Acids



compd	R^1	R^2	R^3	R^4	FLIPR human EC_{50} (μM)[a]	binding human K_i (μM)[b]	binding rat K_i (μM)[b]	Caspase -3/7[c]	Log*D*[d]
91		H	H	Et	0.017 (0.013−0.023)	0.011	0.056	12.4	3.17
92		Me	Me	Me	0.029 (0.019−0.044)	0.040	0.40	12.3	3.35
93		Me	Me	Me	0.018 (0.012−0.027)	0.033	0.12	–1.1	3.12
94		F	H	Me	0.019 (0.014−0.026)	0.031	0.27	7.9	2.95
95		F	H	Me	0.016 (0.012−0.020)	0.018	0.20	–1.1	2.68
96		Cl	H	Me	0.016 (0.013−0.020)	0.019	0.11	8.3	3.07
97		Cl	Cl	Me	0.017 (0.013−0.022)	0.012	0.059	16.1	3.53

[a] All values are average of $n = 3$ in the presence of 0.1% BSA. Efficacies of compounds at 10 μM were 104–114% of γ-linolenic acid at 10 μM. [b] All values are average of $n = 2$ in the presence of 0.2% BSA. [c] Percent of activation at 30 μM was compared to maximal activity of staurosporine as a reference compound. [d] The Log*D* values were determined at pH 7.4 according to the reported method.[39]

第 4 節　ジヒドロベンゾフラン酢酸誘導体の薬物動態

強力な GPR40 受容体作動活性を示し、カスパーゼ-3/7 活性化作用をもたない化合物について、絶食ラットを用いたカセットドージング試験[61] により、経口投与での薬物動態プロファイルを評価した。In vivo での薬効評価試験を念頭に置き、化合物の投薬 1 時間後と 4 時間後の血中濃度 (C_{1h} および C_{4h}) とその他の薬物動態パラメータ (C_{max}, T_{max}, $AUC_{0–8h}$) を算出した (Table 8)。(1,1-ジオキシドテトラヒドロ-2*H*-チオピラン-4-イル)オキシ体 **81** は非常に好ましい薬物動態プロファイルを示した。すなわち、速やかな吸収 (T_{max} = 0.7 h)、高い最高血中濃度 (C_{max} = 2667.7 ng/mL) および良好な血中持続性 (C_{4h} = 1621.0 ng/mL) とその結果に基づく高い血中での暴露量 ($AUC_{0–8h}$ = 13007.6 ng·h/mL) を示した。さらに極性の高いヒドロキシ基を有するアナログ **83** は、**81** と比較して C_{max} および $AUC_{0–8h}$ 値は低かった。その要因の 1 つとして、膜透過性が低下したことが考えられる。化合物 **81** の 1,1-ジオキシドテトラヒドロチオピラン部を開環し、フレキシビリティーを向上させた 2-エチルスルホニルエトキシ体 **84** は、良好な薬物動態プロファイルを示したが、**81** と比較するとその血中濃度は低かった。類似誘導体の検討において 2-エチルスルホニルエトキシ基は、塩基性条件下で β 脱離により分解することを確認しており、経口投与においても同様の分解が起こった可能性がある。そこで、2-エチルスルホニルエトキシ基のスルホニル基を分子末端に 1 原子分移動させ β 脱離の可能性を排除した 3-(メチルスルホニル)プロポキシ体 **85** は、期待通り非常に好ましい薬物動態プロファイルを示した。ラクタム体 **87** や環状アミン体 **88** の $AUC_{0–8h}$ 値は、対応するスルホン体と比較して低い値を示したが、これら誘導体の C_{1h} 値および C_{4h} はともに C_{max} 値と同程度であったことから、良好な血中持続性を示すことが示唆された。2′,3′,5′,6′-テトラメチル体 **93** は対応する 3′,5′-無置換体 **85** と比較して $AUC_{0–8h}$ 値が低く、脂溶性の向上に伴って血中からの消失が速くなったと考えられる。一方、3′-フルオロ体 **95** は高い C_{max} 値と早い T_{max} 値から良好な経口吸収性を示したが、相対的に $AUC_{0–8h}$ 値と C_{4h} 値は低く、対応する 3′,5′-無置換体 **85** と比較して血中からの消失が速いことが示唆された。

結論として、スルホニル誘導体は概して好ましい薬物動態プロファイルを示し、ビフェニル 3′-位および 5′位への脂溶性置換基導入は血中濃度の低下を招く傾向が認められた。上記の結果に基づき、良好な薬物動態プロファイル、とりわけ長い血中持続性を示した化合物 **81**, **83**–**85**, **88** および **95** を経口投与での薬理試験に供した。

Table 8. Pharmacokinetic Profiles for (2,3-Dihydro-1-benzofuran-3-yl)acetic Acids[a]

compd	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-8h} (ng·h/mL)	C_{1h} (ng/mL)	C_{4h} (ng/mL)
81	2667.7	0.7	13007.6	2614.2	1621.0
83	1082.4	1.0	5210.4	1082.4	638.8
84	1941.3	1.17	9621.7	1737.7	1240.0
85	1883.5	2.00	11840.4	1855.6	1601.7
87	626.4	1.83	3474.9	566.8	477.3
88	743.6	1.58	4372.3	578.0	574.5
93	1275.2	0.67	3963.9	1152.2	340.5
95	2033.3	0.50	8036.1	1541.1	966.6

[a] Rat cassette dosing at 1 mg/kg, po (fasted). All values are averages of 3 rats.

第 5 節　ジヒドロベンゾフラン酢酸誘導体の in vivo 薬理作用

選択した化合物の経口投与での薬理作用を、雌性 Wistar fatty ラットを用いた 2 通りの OGTT で評価した (Table 9)。一方は、糖負荷の 1 時間前に 1 mg/kg の用量を投薬して化合物の即効性と薬効ポテンシャルを評価する 1H-OGTT、もう一方は、糖負荷の 4 時間前に 3 mg/kg の用量を投薬し、化合物の持続性を評価する 4H-OGTT である。試験に供した化合物の中でとりわけ良好な薬物動態プロファイルを有する **81**, **85** および **95** は、1H-OGTT および 4H-OGTT の双方で有意な血糖上昇抑制作用を示した。それらと比較してやや血中濃度の低かった 3 級アルコール誘導体 **83** は 4H-OGTT においてのみ薬効を示し、1H-OGTT では有意な薬効を示さなかった。鎖状スルホン誘導体 **84** は 4H-OGTT で有意な血糖上昇抑制作用を示したが、その効果は環状スルホン誘導体と比較して低かった。化合物 **81** のメチルアミノアナログである **88** は、4H-OGTT で無効であったが、その要因はおそらく血中濃度が低いためと考えられる。

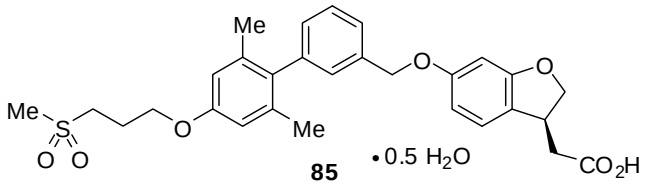
このように、ジヒドロベンゾフラン誘導体はラット受容体に対して結合親和性が低いものの、その優れた薬物動態プロファイルで補完して、薬効の即効性と持続性を示した。評価した化合物のうち 3 化合物 (**81**, **85** および **95**) が顕著な in vivo 作用を示したが、中でも化合物 **85** が最も低い log*D* 値 (2.58) を有していた。

Table 9. Effects of Selected Compounds during an OGTT in Female Wistar Fatty Rats[a]

	ED (mg/kg)[b]	
compd	1H–OGTT	4H–OGTT
81	1	3
83	NE[c]	3
84	NT[d]	3
85	1	3
88	NT[d]	NE[c]
95	1	3

[a] Effects of compounds during an OGTT in female Wistar fatty rats (n = 6). See experimental section for details. [b] Effective dose (ED) was determined by statistical significance on AUC of plasma glucose. [c] NE: not effective. [d] NT: not tested.

上記の各種結果 (in vitro 活性、in vivo 作用、ADME-Tox プロファイルおよびドラッグライクネス等) を基に、化合物 **85** (Figure 18) を臨床試験候補化合物に選択し、さらなる精査を実施した。



85

Figure 18. Chemical structure of **85**.

第 6 節　化合物 **85** の脂肪酸をリガンドとする受容体に対する選択性

GPR40 は、GPR41[62)]、GPR43[62)]、GPR120[63)] を含む、遊離脂肪酸を内因性リガンドとする受容体ファミリーに属している。GPR41 および GPR43 は短鎖遊離脂肪酸により活性化されるが、GPR40 および GPR120 は中長鎖遊離脂肪酸および幾つかのエイコサノイドにより活性化される。GPR41、GPR43 および GPR120 は、いずれもインスリン分泌など糖脂質代謝に関与することが報告されているが、まだそれらの機能は十分に解明されていない。そこで、**85** を始めとする合成リガンドの薬効が真に GPR40 を介したものかどうかを確認する目的、および類似の脂肪酸関連受容体に対する作用に起因する副作用の懸念を払拭する目的で、上記受容体に対する選択性を評価した。Table 10 に示すように、**85** は他の脂肪酸をリガンドとする受容体に対しては作用を示さず、優れた GPR40 選択性を示した。リガンド探索研究開始当初、いずれの受容体のリガンドにもなりうると推察されるフェニルプロパン酸からデザインおよび合成を進めたが、結果的に優れた受容体選択性を獲得するに至った。それは以下の 2 つの合成戦略により達成できたと考えている。すなわち、1) 遊離脂肪酸の特徴であるフレキシブルな構造を固定化することによる GPR40 に対する結合親和性の向上、2) 分子末端に極性官能基を導入することによる高脂溶性構造からの脱却である。

Table 10.　Selectivity Profile for **85** (Anhydrous)

	human GPR40	human GPR41	human GPR43	human GPR120
FLIPR EC_{50} (μM)[a]	0.016	>10	>10	>10

[a] All values are average of $n = 2$ in the presence of 0.5% BSA. [b] The value is average of $n = 4$ in the presence of 0.1% BSA.

第 7 節　化合物 **85** の受容体結合モデル

ヒト GPR40 受容体と精査化合物 **85** とのドッキングモデリングを利用して、結合モードを考察した (Figure 19)。本モデルにおいて、3 つの相互作用ポイントが確認できる。1 点目は極性相互作用である。すなわち、**85** のカルボキシ基が Arg183 (TM5) と 2 点で、および Arg258 (TM7) と 1 点で水素結合を形成している。さらに、Arg258 (TM7) はジヒドロベンゾフラン環のフェニル基とカチオン–π 相互作用 [64] をする位置にあり、Asn244 (TM6) のカルバモイル基が Arg258 (TM7) のアルギニン残基の位置を水素結合で固定化している。2 つ目は、2′,6′-ジメチルビフェニル骨格周辺の Tyr12 (TM1)、Leu67 (TM2)、Trp72 (E-I loop)、Phe82 (TM3) および Leu262 (TM7) によって形成される、ビフェニル骨格と疎水性アミノ酸残基による疎水性相互作用である。3 つ目は Ser8 (TM1) および Lys259 (TM7) と末端スルホニル側鎖との極性相互作用である。このドッキングモデルの結果から、化合物 **85** は多くの異なる相互作用を効果的に利用して、GPR40 受容体と強く結合すると推察される。

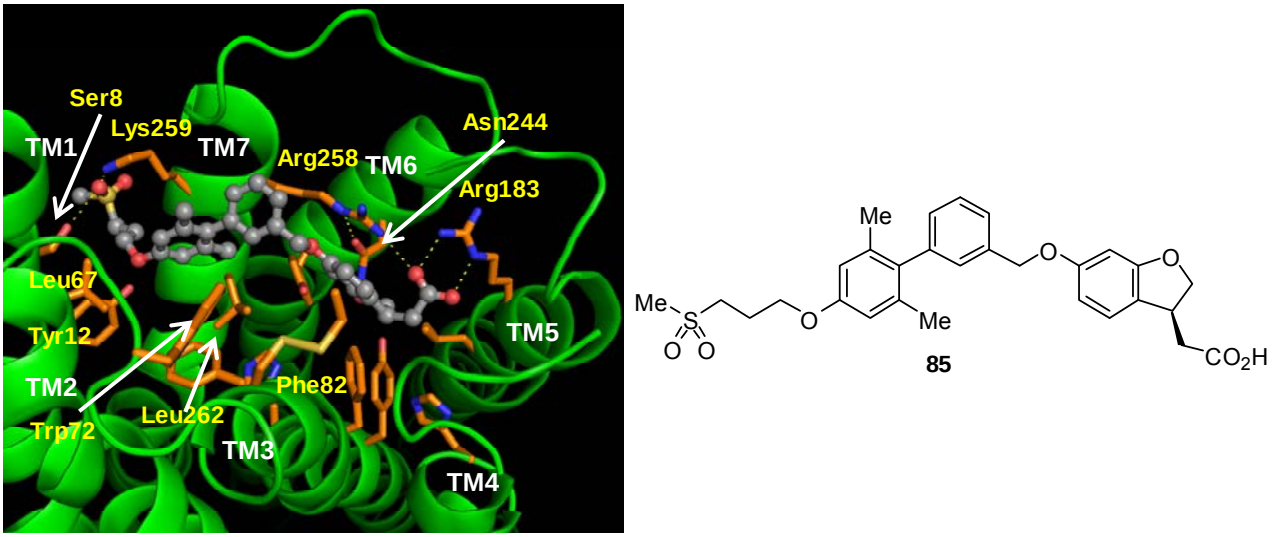


Figure 19.　Docking model of GPR40 in complex with **85** (gray).

第 8 節　化合物 **85** の薬物動態プロファイルと代謝物解析

化合物 **85** の薬物動態プロファイルを、ラットおよびイヌを用いて評価した (Table 11)。化合物 **85** は血中からの消失 (CL) が比較的遅く、薬物の組織移行性の指標である分布容積 ($V_{d(ss)}$)[65] は比較的低い値を示し、これらの性質が両動物種における血中持続性 (iv $t_{1/2}\lambda$: rat, 4.7 h; dog, 5.9 h) に繋がったと考えられる。さらに、経口投与においても **85** は速やかな吸収 (T_{max})、高い最高血中濃度 (C_{max}) および十分な血中での暴露量 ($AUC_{po,\ 0\text{-}24h}$) と生物学的利用能 (F) (rat: 76.0%; dog: 92.4%) を示した。

Table 11. Pharmacokinetic Parameters for **85** (Hemihydrate) in Fasted Rats and Dogs[a]

	iv			po				
species	CL (mL/h/kg)	$V_{d(ss)}$ (mL/kg)	$t_{1/2}\lambda$ (h)	$t_{1/2}\lambda$ (h)	C_{max} (μg/mL)	T_{max} (h)	$AUC_{po,\ 0\text{-}24h}$ (μg·h/mL)	F (%)
rat	34.16	208.49	4.7	4.1	5.77	1.0	65.00	76.0
dog	29.79	224.67	5.9	7.5	3.29	2.0	29.45	92.4

[a] Administered at a dose of 1 mg/kg, iv; 3 mg/kg, po in rats. Administered at a dose of 0.5 mg/kg, iv; 1 mg/kg, po in dogs. The values for C_{max} and AUC were expressed as equivalent of anhydrous **85**. Data are expressed as mean value (rats, n = 3; dogs, n = 4).

次に、化合物 **85** が優れた薬物動態プロファイルを示した要因を探るべく、[^{14}C] でラベル化した **85** を用いて代謝物解析を行った (Figure 20)。血漿中の主要構成成分はラット、イヌの両方において **85** の未変化体であり、ラットにおいては少量のビフェニリルカルボン酸 **113** が認められた。また、ラット胆汁中においては、タウリン抱合体 **114** とグルクロン酸抱合体 **115** が認められ、これら 3 種の代謝物は、両種の糞中においても同定された。このように、**85** の β 酸化代謝物はラット、イヌの双方において確認されず、フェニルプロパン酸に縮環構造を導入することで β 酸化を抑制するという合成デザインの妥当性が証明された。化合物 **85** の代謝プロファイルが、ヒトにおいて 1 日 1 回投与可能な優れ

た薬物動態プロファイルの実現に寄与していると考えられる [66,67]。これらの結果を基に、さらなる薬効精査を実施した。

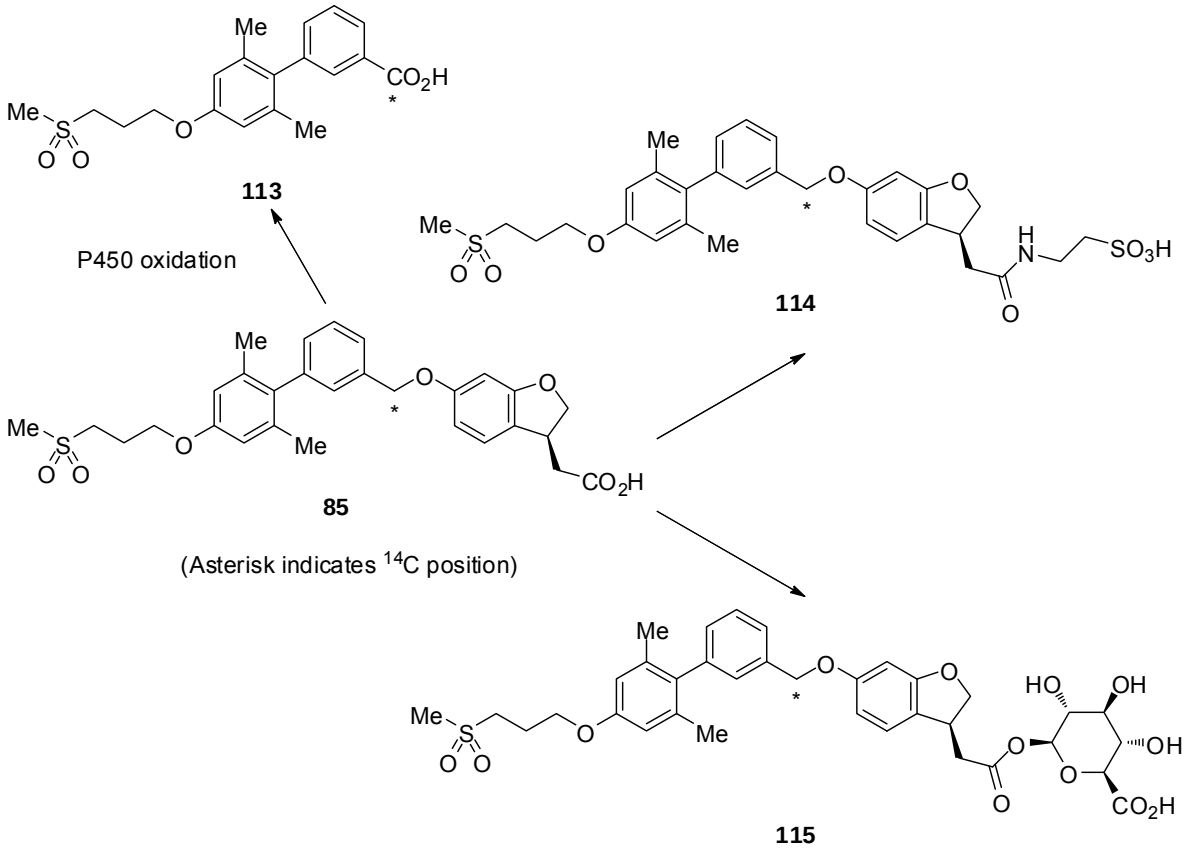


Figure 20. Structures of metabolites of **85**.

第 9 節　化合物 **85** の in vivo 薬理作用

化合物 **85** のインスリン分泌促進作用および血糖上昇抑制作用に関して、雌性 Wistar fatty ラットを用いた用量依存性試験を実施した (Figure 21)。化合物 **85** を 0.3–3 mg/kg の用量で糖負荷の 1 時間前に単回経口投与したところ、用量依存的に血糖上昇を抑制し (Figure 21A)、インスリン分泌を促進した (Figure 21C)。血漿中グルコース濃度の時間曲線下面積値 ($AUC_{0–120\ min}$) および血漿中インスリン濃度の時間曲線下面積値 ($AUC_{pre–30\ min}$) の最小有効量は、それぞれ 1 mg/kg であった (Figure 21B および 21D)。

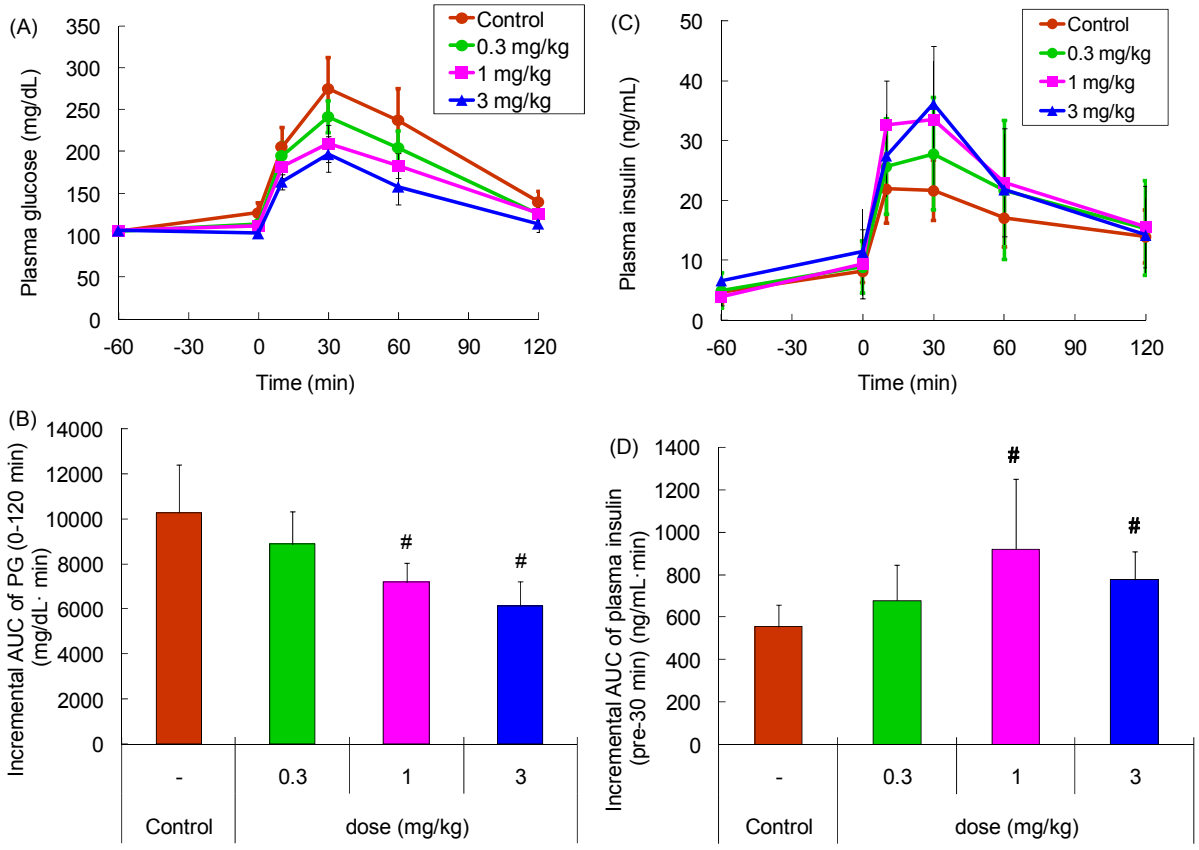


Figure 21. Effects of **85** (hemihydrate) during a 1H-OGTT in female Wistar fatty rats. (A) and (C) show time-dependent changes of plasma glucose (PG) and plasma insulin 1 hour after oral administration of **85** followed by 1 g/kg oral glucose challenge, respectively. Data in (B) and (D) represent incremental $AUC_{0–120\ min}$ of PG levels and incremental $AUC_{pre–30\ min}$ of plasma insulin levels shown in (A) and (C), respectively. Values are mean ± SD (n = 6). #, $p \leq 0.025$ compared with control by one-tailed Williams' test.

このように、**85** は軽度肥満型糖尿病モデル動物で顕著な薬効を示したことから、欧米型の肥満糖尿病患者において有効であることが期待される。

次に、グルコース応答性のインスリン分泌に障害をきたす自然発症糖尿病モデルである雄性 Goto-Kakizaki (GK) ラットを用いた OGTT を実施した (Figure 22)。

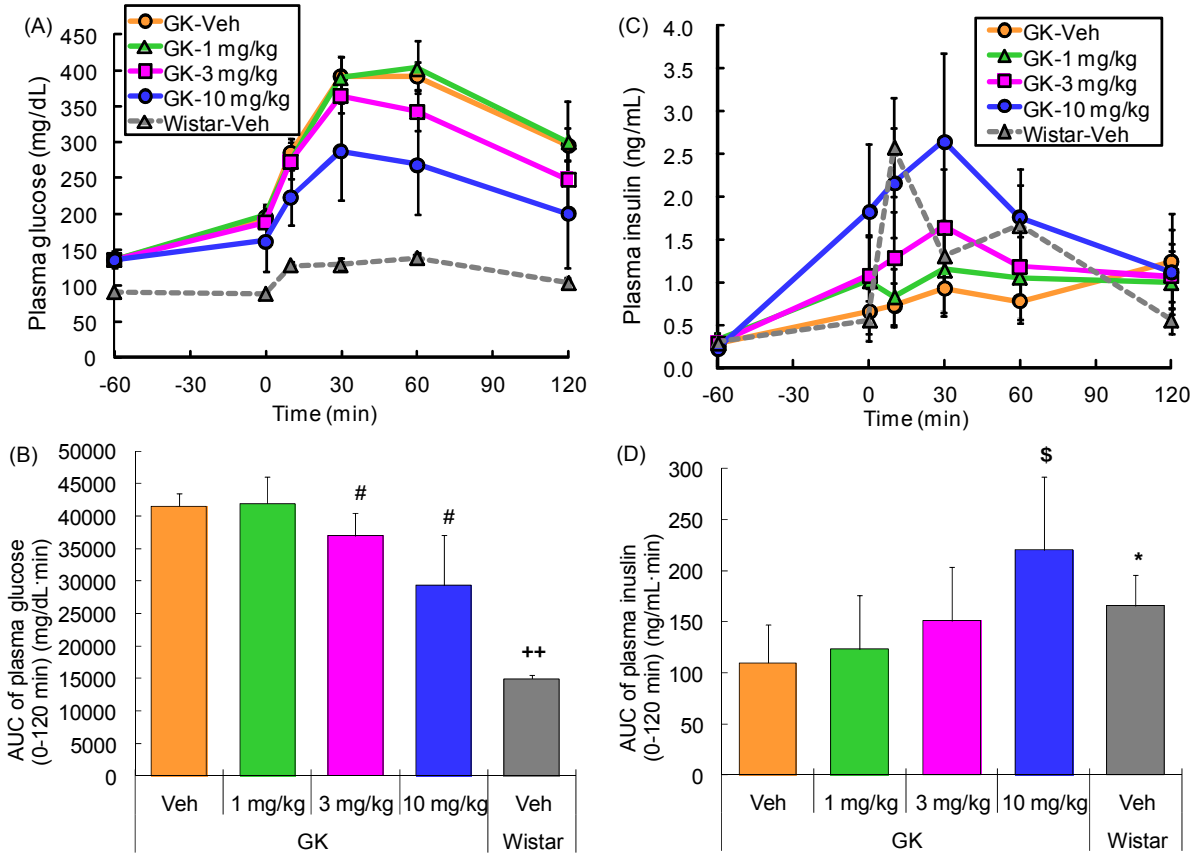


Figure 22. Effects of **85** (hemihydrate) during a 1H-OGTT in male GK rats. (A) and (C) show time-dependent changes of plasma glucose and plasma insulin 1 hour after oral administration of **85** followed by 1 g/kg oral glucose challenge, respectively. Data in (B) and (D) represent $AUC_{0–120\ min}$ of plasma glucose levels and $AUC_{0–120\ min}$ of plasma insulin levels shown in (A) and (C), respectively. Values are mean ± SD (n = 6). #, $P \leq 0.025$ compared to vehicle-treated GK rats by one-tailed Shirley–Williams test. $, $P \leq 0.025$ compared to vehicle-treated GK rats by one-tailed Williams' test. ++, $P \leq 0.01$ compared to vehicle-treated GK rats by Aspin–Welch test. *, $P \leq 0.05$ compared to vehicle-treated GK rats by Student's t-test.

対照動物である正常な Wistar Kyoto ラットではグルコース負荷後速やかにインスリン分泌が認められるのに対し、GK ラットでは初期のインスリン分泌が障害されている (Figure 22C)。またインスリン分泌障害を反映して、血糖上昇が顕著である (Figure 22A)。GK ラットに対して化合物 **85** (1–10 mg/kg) をグルコー

ス負荷 1 時間前に経口投与したところ、用量依存的にインスリン分泌を促進し (Figure 21C)、血漿中グルコース濃度の上昇を抑制した (Figure 22A)。血漿中グルコース濃度の時間曲線下面積値 ($AUC_{0–120\ min}$) (Figure 22B) および血漿中インスリン濃度の時間曲線下面積値 ($AUC_{0–120\ min}$) (Figure 22D) から算出した最小有効用量はそれぞれ 3 mg/kg および 10 mg/kg であった。興味深いことに、**85** を投薬した群では、GK ラットの絶食時血糖値の高さを反映して、グルコース負荷時点 (0 min) において既にインスリン分泌が認められていた。われわれは、正常な糖代謝恒常性を保つ Sprague-Dawlay (SD) ラットに対して高用量の化合物 **85** (30 mg/kg) を経口投与しても絶食血糖値やインスリン分泌に影響がないことを確認している [68,69]。これらの結果は、**85** のインスリン分泌能がグルコース濃度に厳格に依存していることを示唆している。本特長から、**85** は膵 β 細胞の機能が低下している病態においても顕著なインスリン分泌を促進する一方、低血糖を引き起こすリスクは低いと考えられる。このように、**85** はインスリン分泌不全型モデルである GK ラットにおいても顕著な薬効を示したことから、日本人に多いインスリン分泌不全型糖尿病患者にも有効であると期待される。また in vitro 評価において、薬効評価動物であるラット GPR40 受容体に対する結合親和性よりもヒト GPR40 受容体に対する結合親和性がより強力であることから、**85** は 2 型糖尿病患者において強力かつ安全なインスリン分泌促進薬になる可能性を秘めている。

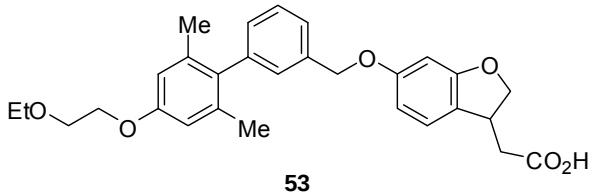
第 10 節　結論

以上述べてきたように、内因性リガンドである FFA が内在する高脂溶性および毒性プロファイルの低減した新規GPR40作動薬の創出を目的に合成研究を行った。第 1 章で見出した (2,3-ジヒドロ-1-ベンゾフラン-3-イル)酢酸誘導体 **53** をリード化合物として、ビフェニル4′位にさまざまな極性官能基を導入した結果、受容体作動活性を保持しつつ、Log*D* 値の低減に伴って毒性プロファイル (カスパーゼ-3/7 活性) が低減し、さらに薬物動態プロファイルが向上した **85** を創製することに成功した。化合物 **85** は、脂肪酸をリガンドとする GPCR に対する優れた選択性を示す、GPR40 特異的リガンドであった。また **85** はラットおよびイヌにおいて良好な経口吸収性および血中持続性を示し、各種薬効モデル動物において、強力なインスリン分泌促進作用とそれに基づく血糖上昇抑制作用を示した。さらに、**85** の代謝物研究の結果、期待通り、カルボン酸のβ酸化に対して高い抵抗性を示すことがわかった。ラットおよびイヌでの安全性試験の結果を受けて、**85** (TAK-875: Fasiglifam) を臨床試験の候補化合物として選定した。

結語

本研究で著者は、臨床投与可能な糖尿病治療薬の創製を目的として、縮合環アルカン酸系 GPR40 作動薬の合成研究を行い、下記の知見を得た。

1. フェニルプロパン酸誘導体の薬物動態プロファイル改善を目的として、β 酸化に脆弱なフェニルプロパン酸部位を環化させた縮合環アルカン酸をデザインし、それぞれの縮合環に適した合成法を適用することにより、効率良く誘導体合成を達成した。その構造活性相関研究の結果、5〜7 員の非芳香環がベンゼン環に縮環した誘導体が、フェニルプロパン酸誘導体に匹敵するヒト受容体作動活性および受容体結合親和性を有することを見出した。また、薬物動態試験の結果、良好な経口吸収性および血中持続性を示すことを見出した。中でも、分子末端にエトキシエトキシ基を有するジヒドロベンゾフラン酢酸誘導体 **53** は、強力なヒト受容体作動活性 (EC_{50} = 28 nM) およびラットにおける良好な薬物動態プロファイル (C_{max} = 465.4 ng/mL; $AUC_{po,0-8h}$ = 2837.4 ng·h/mL, 1 mg/kg, po) を示した。



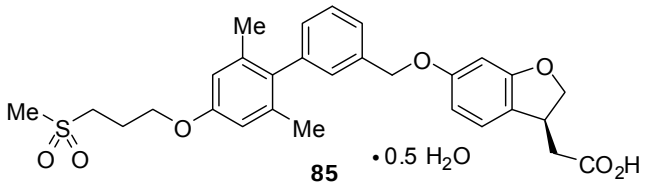
53

2. ウシロドプシンの結晶構造を基に作成した GPR40 ホモロジーモデルを用いて、縮合環アルカン酸誘導体においてヒト－ラット間で受容体結合親和性に種差が認められた要因を考察した。リガンド結合部位付近のヒトとラットのアミノ酸残基の違いを調べた結果、ヒト受容体の Leu86 がラット受容体では Phe に置換され、リガンド結合ポケットの大きさに違いが生じたためであることを明らかにした。

3. 化合物 **53** は、糖尿病モデルラットを用いた OGTT において、投薬 1 時間後

および4時間後に実施した糖負荷によるインスリン分泌を有意に促進するとともに、血糖上昇を抑制した。本結果により、血中持続性の高い GPR40 作動薬の創出を達成した。

4. 第1章で見出したジヒドロベンゾフラン酢酸誘導体 **53** をリード化合物として、脂溶性低減と薬効増強を目的とした構造変換を実施した。その結果、ジヒドロベンゾフラン3位の立体化学は活性に重要であり*S*体がユートマーであること、また分子末端ビフェニル部の 4'位にスルホニル基を有する化合物が、活性を保持しつつ低い Log*D* 値を有し、かつ非常に良好な薬物動態プロファイルを示すことを見出した。中でも化合物 **85** は、低脂溶性 (Log*D* = 2.58) でかつ強力なヒト GPR40 受容体作動活性 (EC_{50} = 16 nM) を示し、脂肪酸をリガンドとする受容体 (GPR41、GPR43、GPR120) に対する優れた選択性を示した。



85

5. 化合物 **85** の詳細な薬物動態および代謝物解析から、β酸化に由来する代謝物は確認されず、ラットおよびイヌにおいて良好な薬物動態プロファイルを示した。本結果は、第 1 章で示したフェニルプロパン酸誘導体のフェニルプロパン酸部位を環化させ、β酸化を抑制することで薬物動態プロファイルを改善するアプローチの妥当性を示唆する結果である。

6. 化合物 **85** は、軽度肥満型糖尿病モデルである雌性 Wistar fatty ラットおよびインスリン分泌不全型糖尿病モデルである雄性 GK ラットを用いた OGTT において、経口投与で有意なインスリン分泌促進作用および血糖上昇抑制作用を示した。これらの結果より、**85** はさまざまなタイプの糖尿病患者に適用可能であると考えられる。化合物 **85** (TAK-875: Fasiglifam) は、世界初の GPR40 作動薬としての上市を目指し、現在臨床第3相試験を実施中である[70–73]。

謝辞

本論文の執筆にあたり、御懇篤な御指導、御高配を賜りました　神戸薬科大学　薬品化学研究室　宮田興子教授に心より感謝いたします。

本研究論文審査にあたり、有益な御助言とご指導を賜りました　神戸薬科大学　守安正恭教授、吉野 伸教授、和田昭盛教授に深謝いたします。

本研究の機会を与えて下さいました　武田薬品工業株式会社　元取締役　大川滋紀博士、元化学研究所長　三宅昭夫博士、元化学研究所長（医薬研究本部主席部員）石原雄二博士に深く感謝いたします。

本研究は、終始、武田薬品工業株式会社　元化学研究所リサーチマネジャー（現医薬研究本部本部長室主席部員）百瀬　祐博士、ならびに元化学研究所主席研究員（現製薬研究所主席研究員）安間常雄氏のもと遂行されたものであり、ここに厚く御礼申し上げます。

本論文作成にあたり、有益な御助言と御指導を賜りました　武田薬品工業株式会社　化学研究所所長　内川　治博士、ならびに循環代謝創薬ユニットリサーチマネジャー　前川毅志博士に深謝いたします。

本研究に際し、共同研究者として種々の御協力いただきました武田薬品工業株式会社　医薬研究本部本部長室主席部員　松永伸一郎博士、循環代謝創薬ユニット主席部員　北村周治博士、化学研究所主任研究員　佐々木忍氏、中枢疾患創薬ユニット主任研究員　味上　達氏、化学研究所主任研究員　伊東昌宏氏、循環代謝創薬ユニットリサーチマネジャー　武内浩二氏、同主任研究員　辻畑善行博士、伊藤　亮氏、鈴木正美博士、同所員　原田絢子氏、本部長室主席部員　鈴木伸宏博士、生物分子研究所リサーチマネジャー　神崎直之氏、同主任研究員　山東　尚氏ならびに小谷知之氏、EHS 推進部チームリーダー　宮崎純一氏、化学研究所リサーチマネジャー　田中稔祐博士、製薬企画部課長代理　船見みゆき氏、薬物動態研究所主任研究員　小亀暁史氏、諸橋昭雄博士、ならびに野中雅美氏に深く感謝いたします。

最後に、本研究に御協力いただいた全ての方に感謝いたします。

実験の部

General. Reagents and solvents were obtained from commercial sources and used without further purification. Reaction progress was determined by thin layer chromatography (TLC) analysis on Merck Kieselgel 60 F254 plates or Fuji Silysia NH plates. Chromatographic purification was carried out on silica gel columns [(Merck Kieselgel 60, 70–230 mesh or 230–400 mesh, Merck) or (Chromatorex NH-DM 1020, 100–200 mesh)] or on Purif-Pack (SI: 60 μM or NH: 60 μM, Fuji Silysia Chemical, Ltd.). Melting points were determined on a BÜCHI B-545 melting point apparatus and were uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Bruker Ultra Shield-300 (300 MHz) instruments. Chemical shifts are given in parts per million (ppm) with tetramethylsilane as an internal standard. Abbreviations are used as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, dt = doublet of triplets, td = triplet of doublets, ddd = doublet of doublets of doublets, br = broad. Coupling constants (*J* values) are given in hertz (Hz). Low-resolution mass spectra (MS) were determined on a Waters Liquid Chromatography–Mass Spectrometer System (MS), using a CAPCELL PAK UG-120 ODS (Shiseido Co., Ltd.) column (2.0 mm i.d. × 50 mm) with aqueous CH_3CN (10–95%) containing 0.05% trifluoroacetic acid (TFA), and an HP-1100 (Agilent Technologies) apparatus for monitoring at 220 nm. All MS experiments were performed using electrospray ionization (ESI) in positive ion mode. Analytical HPLC was performed on a Shimadzu LC-VP instrument, equipped with CAPCELL PAK C18 UG120 S-3 μm, 2.0 × 50 mm column with a 4 min linear gradient from 90/10 to 5/95 and subsequently with a 1.5 min isocratic elution 5/95 A/B, where A = H_2O–0.1% TFA, B = CH_3CN–0.1% TFA, at a flow rate of 0.5 mL/min, with UV detection at 220 nm, at column temperature of 25 ˚C, or performed on a Waters Quattro micro API (Agilent HP1100, Gilson 215) instrument, equipped with CAPCELL PAK C18 UG120 S-3 μm, 1.5 × 35 mm column, by gradient elution: 0.00 min (A/B = 100/0), 2.00 min (A/B = 0/100), 3.00 min (A/B = 0/100), 3.01 min (A/B = 100/0), 3.30 min (A/B = 100/0) where A = 2% CH_3CN/H_2O with 5 mM NH_4OAc; B = 95% CH_3CN/H_2O with 5 mM NH_4OAc, at a flow rate of 0.5 mL/min, with UV detection at 220 nm, at column temperature of 40 ˚C. A part of compounds were assessed by the following method. The analytical HPLC system consisted of Prominence UFLC (Shimadzu Corporation, Japan), L-column2 ODS column (30 x 2.1 mm I.D., 2 μm) (Chemicals Evaluation and Research Institute, Japan) at column temperature of 50 ˚C and nano quantity analyte detector, QT-500 (Quant technologies LLC, MN, USA). The mobile phase A and B are a mixture of distillated water, 50 mmol/L NH_4OAc aqueous solution and

MeCN (8:1:1,v/v/v) and a mixture of MeCN and 50 mmol/L ammonium acetate aqueous solution (9:1,v/v), respectively. The flow rate maintained 0.5 mL/min. The mixture ratio of mobile phase A and B changed from 95/5 to 5/95 with a 2 min linear gradient and subsequently with a 1 min isocratic elution 5/95. Elemental analyses were carried out by Takeda Analytical Laboratories Limited, and were within 0.4% of the theoretical values unless otherwise noted. The purity of compounds was assessed by elemental analysis or analytical HPLC (>95%). Optical rotations were determined on a JASCO P-1030 polarimeter. Preparative purifications were performed using a Gilson pumping system in conjunction with a photodiode array detector (Hewlett Packard 1100 Series) and a Gilson 215 auto sampler. Separations were achieved using the following method, which utilized a YMC packed column (CombiPrep ODS-A, 5 μm, 20 mm i.d. × 50 mm) with a 1 min isocratic elution 10/90, a 3.7 min linear gradient from 10/90 to 0/100, and then a 2.7 min isocratic elution 0/100 A/B at a flow rate of 25 mL/min. Abbreviations of the solvents and reagents are used as follows: $CDCl_3$, deuterochloroform; DMSO-d_6, hexadeuterodimethyl sulfoxide; IPA, 2-propanol; Et_2O, diethyl ether; CH_2Cl_2, dichloromethane; CCl_4, carbon tetrachloride; H_2, hydrogen; NaH, sodium hydride; $NaBH_4$, sodium borohydride; $LiAlH_4$, lithium aluminum hydride; $AlCl_3$, aluminum chloride; $TiCl_4$, titanium (IV) chloride; NaOH, sodium hydroxide; KOH, potassium hydroxide; $LiOH \cdot H_2O$, lithium hydroxide monohydrate; HCl, hydrochloric acid; H_2SO_4, sulfuric acid; H_3PO_4, phosphoric acid; $HClO_4$, perchloric acid; NH_4Cl, ammonium chloride; $NaNH_2$, sodium amide; NH_3, ammonia; $NaHCO_3$, sodium hydrogen carbonate; $MgSO_4$, magnesium sulfate; Na_2SO_4, sodium sulfate; K_2CO_3, potassium carbonate; Na_2CO_3, sodium carbonate; $Na_2S_2O_3$, sodium thiosulfate; KF, potassium fluoride; Cs_2CO_3, cesium carbonate; K_3PO_4, potassium phosphate; KI, potassium iodide; NaCN, sodium cyanide; $POCl_3$, phosphorousoxy chloride; $SOCl_2$, thionyl chloride; $Pd(PPh_3)_4$, tetrakis(triphenylphosphine)palladium(0); $PdCl_2(dppf) \cdot CH_2Cl_2$, [1,1′-bis(diphenylphosphino) ferrocene]dichloropalladium(II) complex with CH_2Cl_2; $Pd_2(dba)_3$, tris(dibenzylidene-acetone)dipalladium(0); Br_2, bromine.

2-Methoxy-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-one (5c). Step 1: To a solution of triethyl 4-phosphonocrotonate (24.0 g, 95.9 mmol) in THF (100 mL) was added portionwise NaH (60% in mineral oil, 3.84 g, 96.0 mmol) at 0 °C, and the mixture was stirred under nitrogen atmosphere at 0 °C for 30 min. To the mixture was added dropwise a solution of 3-methoxybenzaldehyde (12.3 g, 90.0 mmol) in THF (100 mL) at 0 °C and the mixture was stirred at room temperature for 2 h. To the mixture was added DMF (50 mL) and the mixture was further stirred at room temperature for 18 h. The reaction mixture was concentrated, and the residue was diluted with AcOEt, washed sequentially with 1 M HCl aqueous solution and brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 10:90–30:70) to give ethyl (2*E*,4*E*)-5-(3-methoxyphenyl)penta-2,4-dienoate (7.70 g, 37%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.32 (t, *J* = 7.1 Hz, 3H), 3.84 (s, 3H), 4.23 (q, *J* = 7.1 Hz, 2H), 5.99 (d, *J* = 15.3 Hz, 1H), 6.85–6.88 (m, 3H), 6.98 (t, *J* = 1.5 Hz, 1H), 7.06 (d, *J* = 7.7 Hz, 1H), 7.25–7.30 (m, 1H), 7.44 (ddd, *J* = 15.3, 6.4, 3.8 Hz, 1H). MS *m/z* 233 $(M + H)^+$. Step 2: The obtained oil in step 1 was hydrogenated on 10% Pd/C (1.1 g, containing 50% water) in EtOH (100 mL) under H_2 atmosphere (balloon pressure) at room temperature. After reaction was completed, the catalyst was removed by filtration, and the filtrate was concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 5:95–20:80) to give ethyl 5-(3-methoxyphenyl)pentanoate (6.01 g, 77%) as a colorless oil. Step 3: To a solution of the obtained oil (6.01 g, 25.4 mmol) in step 2 in EtOH (50 mL) and THF (50 mL) was added 2 M NaOH aqueous solution (25 mL), and the mixture was stirred at room temperature for 3 h. To the mixture was added 1 M HCl aqueous solution, and the mixture was extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated to give 5-(3-methoxyphenyl)pentanoic acid (5.28 g, 99%) as a red-brown oil. ^{1}H NMR ($CDCl_3$) δ 1.66–1.70 (m, 4H), 2.36–2.41 (m, 2H), 2.59–2.64 (m, 2H), 3.80 (s, 3H), 6.72–6.78 (m, 3H), 7.17–7.22 (m, 1H). MS *m/z* 209 $(M + H)^+$. Step 4: A mixture of phosphorus (V) oxide (10 g) and methanesulfonic acid (70 mL) was stirred at 100 °C for 1 h. The solution was poured into the obtained oil (5.28 g, 25.4 mmol) in step 3 and the resulting mixture was stirred at 100 °C for 1 h. The reaction mixture was poured into ice water and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 0:100–30:70) to give **5c** (4.02 g, 83%) as a red-brown oil. ^{1}H NMR ($CDCl_3$) δ 1.75–1.93 (m, 4H), 2.67–2.74 (m, 2H), 2.89–2.93 (m, 2H), 3.85 (s, 3H), 6.70 (d, *J* = 2.5 Hz, 1H), 6.81 (dd, *J* = 8.7, 2.5 Hz, 1H), 7.79 (d, *J* = 8.7 Hz, 1H). MS *m/z* 191 $(M + H)^+$.

5-Benzyloxy-1-indanone (6a). Step 1: To a suspension of 5-methoxy-1-indanone (**5a**) (10.3 g, 63.5 mmol) in toluene (150 mL) was added portionwise $AlCl_3$ (16.9 g, 127 mmol) at 0 °C, and the mixture was stirred under nitrogen atmosphere at reflux for 4 h. The reaction mixture was allowed to cool to room temperature and poured into ice water. The mixture was extracted with AcOEt–THF. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated to give 5-hydroxy-1-indanone as a yellow solid. Step 2: The obtained solid in step 1 was suspended in acetone (120 mL). To the suspension were added benzyl bromide (10.9 g, 64.0 mmol) and K_2CO_3 (12.3 g, 88.9 mmol), and the mixture was stirred under nitrogen atmosphere at reflux for 1 h. The reaction mixture was concentrated, and to the residue were added AcOEt and water. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The resulting solid was washed with AcOEt to give **6a** (10.8 g) as colorless crystals. The second crop and third crop were similarly obtained (3.36 g) (washed with hexane–AcOEt). Total 14.2 g (94%). ^{1}H NMR ($CDCl_3$) δ 2.65–2.69 (m, 2H), 3.09 (t, *J* = 6.0 Hz, 2H), 5.15 (s, 2H), 6.97–7.00 (m, 2H), 7.32–7.46 (m, 5H), 7.68–7.72 (m, 1H). MS *m/z* 239 $(M + H)^+$.

2-(Benzyloxy)-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-one (6c). The title compound was prepared from **5c** by a similar to that described for **6a** in 91% yield as colorless prisms (hexane–AcOEt). ^{1}H NMR ($CDCl_3$) δ 1.76–1.93 (m, 4H), 2.71 (t, *J* = 6.0 Hz, 2H), 2.91 (t, *J* = 6.0 Hz, 2H), 5.11 (s, 2H), 6.79 (d, *J* = 2.5 Hz, 1H), 6.88 (dd, *J* = 8.7, 2.5 Hz, 1H), 7.31–7.45 (m, 5H), 7.78 (d, *J* = 8.7 Hz, 1H). MS *m/z* 267 $(M + H)^+$.

Ethyl (5-Hydroxy-2,3-dihydro-1*H*-inden-1-yl)acetate (7a). Step 1: To a solution of triethyl phosphonoacetate (15.7 g, 70.0 mmol) in toluene (50 mL) was added portionwise NaH (60% in mineral oil, 2.25 g, 56.3 mmol) at 0 °C, and the mixture was stirred under nitrogen atmosphere at 50 °C for 1 h. The reaction mixture was added dropwise to a suspension of **6a** (10.7 g, 44.9 mmol) in toluene (50 mL) under nitrogen atmosphere at 0 °C, and the reaction mixture was stirred at reflux for 6 h. The mixture was quenched with diluted HCl aqueous solution, and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 10:90–40:60) to give a yellow oil. Step 2: The obtained oil in step 1 was dissolved in EtOH (80 mL) and hydrogenated on 10% Pd/C (2.0 g, containing 50% water) under H_2 atmosphere (balloon pressure) at room temperature for 24 h. The catalyst was removed by filtration, and the filtrate was concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 10:90–40:60) to give **7a** (5.27 g, 54% in 2 steps) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.28 (t, *J* = 7.1 Hz, 3H), 1.69–1.81 (m, 1H), 2.32–2.44 (m, 2H), 2.71 (dd, *J* = 15.3, 5.8 Hz, 1H), 2.77–2.94 (m, 2H), 3.46–3.56 (m, 1H), 4.18 (q, *J* = 7.1 Hz, 2H), 4.71 (s, 1H), 6.62 (dd, *J* = 8.1, 2.2 Hz, 1H), 6.70 (d, *J* = 2.2 Hz, 1H), 7.02 (d, *J* = 8.1 Hz, 1H). MS *m/z* 221 $(M + H)^+$.

Ethyl (6-Hydroxy-1,2,3,4-tetrahydronaphthalen-1-yl)acetate (7b). Step 1: Triethyl phosphonoacetate (15.0 mL, 75.0 mmol) was added dropwise to a suspension of NaH (60% in mineral oil, 2.80 g, 70.0 mmol) in toluene (35 mL) under nitrogen atmosphere at 0 °C, and the mixture was stirred at 50 °C for 1 h. The mixture was cooled to 0 °C and a solution of 6-methoxy-1-tetralone (**5b**) (8.81 g, 50.0 mmol) in toluene (35 mL) was added dropwise. The resulting mixture was stirred at reflux for 6 h. The mixture was quenched with diluted HCl aqueous solution, and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 0:100–25:75) to give a colorless oil. Step 2: The obtained oil in step 1 was hydrogenated on 10% Pd/C (1.0 g, containing 50% water) in EtOH (100 mL) under H_2 atmosphere (balloon pressure) at room temperature for 22 h. The catalyst was removed by filtration, and the filtrate was concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 0:100–15:85) to give ethyl (6-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)acetate (6.68 g, 54% in 2 steps) as a colorless oil. Step 3: To a mixture of the obtained oil (6.68 g, 26.9 mmol) in step 2 and 1-dodecanethiol (7.73 mL, 32.3 mmol) in toluene (75 mL) was added portionwise $AlCl_3$ (10.8 g, 81.0 mmol) at 0 °C, and the resulting mixture was stirred under nitrogen atmosphere at room temperature for 18 h. The mixture was quenched with ice water, and extracted with AcOEt. The extract was washed subsequently with 2 M HCl aqueous solution and brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 5:95–40:60) to give **7b** (6.19 g, 98%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.27 (t, J = 7.1 Hz, 3H), 1.63–1.95 (m, 4H), 2.48 (dd, J = 15.1, 9.6 Hz, 1H), 2.62–2.79 (m, 3H), 3.23–3.32 (m, 1H), 4.17 (q, J = 7.1 Hz, 2H), 4.63 (s, 1H), 6.54 (d, J = 2.5 Hz, 1H), 6.61 (dd, J = 8.2, 2.5 Hz, 1H), 7.02 (d, J = 8.2 Hz, 1H). MS *m/z* 235 $(M + H)^+$.

Ethyl (2-Hydroxy-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-yl)acetate (7c). The title compound was prepared from **6c** by a similar to that described for **7a** in 89% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.22 (t, J = 7.2 Hz, 3H), 1.44–1.92 (m, 6H), 2.61–2.86 (m, 4H), 3.36–3.44 (m, 1H), 4.12 (q, J = 7.2 Hz, 2H), 4.66 (s, 1H), 6.54–6.59 (m, 2H), 6.95 (d, J = 7.9 Hz, 1H). MS *m/z* 249 $(M + H)^+$.

4-(Chloromethyl)-7-hydroxy-2*H*-chromen-2-one (9). Ethyl 4-chloroacetoacetate (14.0 g, 85.0 mmol) was dissolved in concentrated H_2SO_4 (30 mL) at 0 °C, and resorcinol (**8**) (8.81 g, 80.0 mmol) was added portionwise. The mixture was stirred at room temperature for 2 h. The reaction mixture was poured into ice water, and the resulting solid was collected by filtration, washed with water, and dried to give **9** (14.1 g, 84%) as a beige solid. ^{1}H NMR ($CDCl_3$) δ 4.63 (s, 2H), 6.42 (s, 1H), 6.88 (dd, J = 8.7, 2.5 Hz, 1H), 6.98 (d, J = 2.5 Hz, 1H), 7.57 (d, J = 8.7 Hz, 1H). MS *m/z* 211 $(M + H)^+$.

Methyl (6-Hydroxy-1-benzofuran-3-yl)acetate (7d). Step 1: A mixture of **9** (10.9 g, 51.8 mmol) and 1 M NaOH aqueous solution (500 mL) was stirred at reflux for 2 h. The reaction mixture was acidified with concentrated H_2SO_4 and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated to give (6-hydroxy-1-benzofuran-3-yl)acetic acid (8.27 g, 83%) as brown crystals. ^{1}H NMR (DMSO-d_6) δ 3.60 (s, 2H), 6.73 (dd, J = 8.5, 2.0 Hz, 1H), 6.87 (d, J = 2.0 Hz, 1H), 7.34 (d, J = 8.5 Hz, 1H), 7.66 (s, 1H), 9.52 (br s, 1H), 12.41 (br s, 1H). MS *m/z* 193 $(M + H)^+$. Step 2: (6-Hydroxy-1-benzofuran-3-yl)acetic acid (9.85 g, 51.3 mmol) was suspended in MeOH (45 mL), and to the suspension was added concentrated H_2SO_4 (5 mL), and the mixture was stirred at reflux for 4 h. After evaporation of the solvent, the residue was diluted with Et_2O, washed sequentially with water, saturated $NaHCO_3$ aqueous solution, and brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 10:90–50:50) to give a solid, which was washed with hexane–AcOEt to give **7d** (7.38 g, 70%) as pale-yellow crystals. ^{1}H NMR ($CDCl_3$) δ 3.67 (d, J = 0.9 Hz, 2H), 3.73 (s, 3H), 4.91 (s, 1H), 6.79 (dd, J = 8.3, 2.2 Hz, 1H), 6.95 (d, J = 2.2 Hz, 1H), 7.38 (d, J = 8.3 Hz, 1H), 7.52 (s, 1H). MS *m/z* 207 $(M + H)^+$.

Methyl (6-Hydroxy-2,3-dihydro-1-benzofuran-3-yl)acetate (7e). Compound **7d** (11.4 g, 55.3 mmol) was hydrogenated on 10% Pd/C (2 g, containing 50% water) in MeOH (100 mL) under H_2 atmosphere (balloon pressure) at room temperature for 18 h. The catalyst was removed by filtration, and the filtrate was concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 20:80–50:50) to give a solid. Recrystallization from hexane–AcOEt gave **7e** (8.74 g, 76%) as colorless prisms. mp 108–109 °C. ^{1}H NMR ($CDCl_3$) δ 2.55 (dd, J = 16.4, 9.1 Hz, 1H), 2.74 (dd, J = 16.4, 5.7 Hz, 1H), 3.72 (s, 3H), 3.74–3.84 (m, 1H), 4.26 (dd, J = 9.1, 5.7 Hz, 1H), 4.75 (t, J = 9.1 Hz, 1H), 4.82 (s, 1H), 6.31–6.34 (m, 2H), 6.97 (d, J = 8.7 Hz, 1H). MS *m/z* 209 $(M + H)^+$.

Ethyl 8-(Benzyloxy)-2,3-dihydro-1-benzoxepine-4-carboxylate (11). Step 1: A mixture of 2,4-dihydroxybenzaldehyde (**10**) (13.8 g, 100 mmol), benzyl chloride (20.1 mL, 175 mmol), and KF (11.6 g, 200 mmol) in CH_3CN (100 mL) was stirred at reflux for 20 h. The mixture was concentrated, diluted with water, and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 5:95–40:60) to give 4-(benzyloxy)-2-hydroxybenzaldehyde (9.76 g, 43%) as colorless crystals. Step 2: A mixture of the obtained crystals (9.76 g, 42.8 mmol) in step 1, ethyl 4-bromobutyrate (7.34 mL, 51.3 mmol), and Cs_2CO_3 (20.9 g, 64.1 mmol) in DMF (100 mL) was stirred at 80 °C for 4 days. After evaporation of the solvent, the residue was diluted with water, and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 5:95–40:60) to give a solid. Recrystallization from heptane–AcOEt gave

11 (5.74 g, 41%) as colorless crystals. ^{1}H NMR ($CDCl_3$) δ 1.34 (t, *J* = 7.2 Hz, 3H), 2.92–2.98 (m, 2H), 4.21–4.30 (m, 4H), 5.06 (s, 2H), 6.59 (d, *J* = 2.4 Hz, 1H), 6.66 (dd, *J* = 8.6, 2.4 Hz, 1H), 7.24 (d, *J* = 8.6 Hz, 1H), 7.30–7.45 (m, 5H), 7.54 (s, 1H). MS *m*/*z* 325 $(M + H)^+$.

Ethyl 8-Hydroxy-2,3,4,5-tetrahydro-1-benzoxepine-4-carboxylate (7f). The title compound was prepared from **11** by a similar to that described for **7e** in 100% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.25 (t, *J* = 7.1 Hz, 3H), 2.10–2.29 (m, 2H), 2.56–2.67 (m, 1H), 2.90–3.10 (m, 2H), 3.77–3.87 (m, 1H), 4.14 (q, *J* = 7.1 Hz, 2H), 4.23–4.33 (m, 1H), 4.85 (s, 1H), 6.43–6.51 (m, 2H), 7.00 (d, *J* = 8.0 Hz, 1H). MS *m*/*z* 237 $(M + H)^+$.

(2′,6′-Dimethylbiphenyl-3-yl)methanol (13). Step 1: 3-Bromobenzaldehyde (**12**) (18.5 g, 100 mmol) and (2,6-dimethylphenyl)boronic acid (21.0 g, 140 mmol) were dissolved in a mixture of 1 M Na_2CO_3 aqueous solution (200 mL), EtOH (100 mL), and toluene (200 mL). After argon substitution, $Pd(PPh_3)_4$ (5.78 g, 5.00 mmol) was added. The reaction mixture was stirred under argon atmosphere at 80 °C for 20 h. The reaction mixture was cooled, and water was added to the reaction mixture. The mixture was diluted with AcOEt, and the mixture was filtered through a pad of Celite. The organic layer of the filtrate was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 0:100–10:90) to give 2′,6′-dimethylbiphenyl-3-carbaldehyde (20.4 g, 97%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 2.02 (s, 6H), 7.11–7.23 (m, 3H), 7.42–7.46 (m, 1H), 7.61 (t, *J* = 7.6 Hz, 1H), 7.68–7.69 (m, 1H), 7.86–7.90 (m, 1H), 10.06 (s, 1H). MS *m/z* 211 $(M + H)^+$. Step 2: The obtained oil (18.5 g, 88.0 mmol) in step 1 was dissolved in a mixture of DME (100 mL) and THF (100 mL), and $NaBH_4$ (1.66 g, 44.0 mmol) was added at 0 °C. The mixture was stirred at 0 °C for 3 h and then at room temperature for 3 h. The reaction mixture was quenched with diluted HCl aqueous solution and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 10:90–50:50) to give **13** (15.6 g, 83%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.66 (t, *J* = 5.9 Hz, 1H), 2.03 (s, 6H), 4.74 (d, *J* = 5.9 Hz, 2H), 7.07–7.19 (m, 5H), 7.35 (d, *J* = 7.5 Hz, 1H), 7.43 (t, *J* = 7.5 Hz, 1H). MS *m/z* 195 $(M - 18 + H)^+$.

{5-[(2′,6′-Dimethylbiphenyl-3-yl)methoxy]-2,3-dihydro-1*H*-inden-1-yl}acetic Acid (14). Step 1: To a mixture of **7a** (0.529 g, 2.40 mmol), **13** (0.637 g, 3.00 mmol), and P(*n*-Bu)$_3$ (1.20 mL, 4.80 mmol) in toluene (40 mL) was added ADDP (1.21 g, 4.80 mmol) at 0 °C, and the mixture was stirred under nitrogen atmosphere at room temperature for 6 h. To the mixture were added ADDP (0.606 g, 2.40 mmol) and P(*n*-Bu)$_3$ (0.606 mL, 2.40 mmol). After stirred at room temperature for 15 h, ADDP (0.606 g, 2.40 mmol) and P(*n*-Bu)$_3$ (0.606 mL, 2.40 mmol) were added, and the mixture was stirred at room temperature for 6 h. Hexane (20 mL) was added, and the precipitate was removed by filtration. The filtrate was concentrated, and the residue was purified by silica gel column chromatography (AcOEt:hexane =

0:100–30:70) to give ethyl {5-[(2',6'-dimethylbiphenyl-3-yl)methoxy]-2,3-dihydro-1*H*-inden-1-yl}acetate (0.239 g, 24%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.27 (t, *J* = 7.1 Hz, 3H), 1.68–1.80 (m, 1H), 2.01 (s, 6H), 2.32–2.44 (m, 2H), 2.71 (dd, *J* = 15.3, 5.7 Hz, 1H), 2.78–2.96 (m, 2H), 3.47–3.57 (m, 1H), 4.17 (q, *J* = 7.1 Hz, 2H), 5.08 (s, 2H), 6.78 (dd, *J* = 8.2, 2.4 Hz, 1H), 6.84 (d, *J* = 2.4 Hz, 1H), 7.05–7.20 (m, 6H), 7.38–7.46 (m, 2H). MS *m/z* 415 $(M + H)^+$. Step 2: To a solution of the obtained oil (0.238 g, 0.574 mmol) in step 1 in EtOH (2 mL) and THF (2 mL) was added 2 M NaOH aqueous solution (1.00 mL, 2.00 mmol), and the mixture was stirred at room temperature for 7 h. The mixture was acidified with 1 M HCl aqueous solution, and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated to give a solid. Recrystallization from hexane–AcOEt gave **14** (0.118 g, 53%) as colorless prisms. mp 114 °C. ^{1}H NMR ($CDCl_3$) δ 1.71–1.83 (m, 1H), 2.01 (s, 6H), 2.36–2.51 (m, 2H), 2.76–2.96 (m, 3H), 3.49–3.58 (m, 1H), 5.09 (s, 2H), 6.79 (dd, *J* = 8.3, 2.5 Hz, 1H), 6.85 (s, 1H), 7.08–7.17 (m, 5H), 7.20 (s, 1H), 7.38–7.47 (m, 2H). MS *m/z* 387 $(M + H)^+$. HPLC purity (220 nm) 99.8%. Anal. Calcd for $C_{26}H_{26}O_3$: C, 80.80; H, 6.78. Found: C, 80.63; H, 6.97.

The following compounds **15**–**19** were also prepared from **13** and appropriate phenols **7b**–**f** by a similar to that described for **14**.

{6-[(2′,6′-Dimethylbiphenyl-3-yl)methoxy]-1,2,3,4-tetrahydronaphthalen-1-yl}acetic Acid (15). Step 1: Ethyl {6-[(2′,6′-dimethylbiphenyl-3-yl)methoxy]-1,2,3,4-tetrahydronaphthalen-1-yl}acetate in 23% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.27 (t, *J* = 7.2 Hz, 3H), 1.63–1.93 (m, 4H), 2.01 (s, 6H), 2.48 (dd, *J* = 15.2, 9.7 Hz, 1H), 2.62–2.74 (m, 3H), 3.25–3.33 (m, 1H), 4.16 (q, *J* = 7.2 Hz, 2H), 5.07 (s, 2H), 6.67 (d, *J* = 2.6 Hz, 1H), 6.76 (dd, *J* = 8.5, 2.6 Hz, 1H), 7.05–7.19 (m, 6H), 7.37–7.46 (m, 2H). MS *m/z* 429 $(M + H)^+$. Step 2: **15** in 57% yield as colorless prisms (hexane–AcOEt). mp 120 °C. ^{1}H NMR ($CDCl_3$) δ 1.67–1.98 (m, 4H), 2.01 (s, 6H), 2.55 (dd, *J* = 15.5, 9.9 Hz, 1H), 2.70–2.77 (m, 3H), 3.26–3.34 (m, 1H), 5.08 (s, 2H), 6.68 (d, *J* = 2.6 Hz, 1H), 6.78 (dd, *J* = 8.5, 2.6 Hz, 1H), 7.07–7.19 (m, 6H), 7.37–7.46 (m, 2H). MS *m/z* 401 $(M + H)^+$. HPLC purity (220 nm) 100%. Anal. Calcd for $C_{27}H_{28}O_3$: C, 80.97; H, 7.05. Found: C, 80.89; H, 7.27.

{2-[(2′,6′-Dimethylbiphenyl-3-yl)methoxy]-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-yl}acetic Acid (16). Step 1: Ethyl {2-[(2′,6′-dimethylbiphenyl-3-yl)methoxy]-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-yl}acetate in 72% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.22 (t, *J* = 7.1 Hz, 3H), 1.45–1.92 (m, 6H), 2.01 (s, 6H), 2.62–2.88 (m, 4H), 3.36–3.45 (m, 1H), 4.11 (q, *J* = 7.1 Hz, 2H), 5.08 (s, 2H), 6.70 (dd, *J* = 8.3, 2.7 Hz, 1H), 6.74 (d, *J* = 2.7 Hz, 1H), 6.99 (d, *J* = 8.3 Hz, 1H), 7.08–7.20 (m, 5H), 7.37–7.46 (m, 2H). MS *m/z* 443 $(M + H)^+$. Step 2: **16** in 60% yield as colorless crystals (hexane–AcOEt). mp 86–88 °C. ^{1}H NMR ($CDCl_3$) δ 1.44–1.92 (m, 6H), 2.01 (s, 6H), 2.68–2.89 (m, 4H), 3.36–3.44 (m, 1H), 5.08 (s, 2H), 6.70–6.75 (m, 2H), 7.00 (d, *J* = 8.3 Hz, 1H), 7.06–7.19 (m,

5H), 7.37–7.46 (m, 2H). MS *m/z* 415 $(M + H)^+$. HPLC purity (220 nm) 99.5%. Anal. Calcd for $C_{28}H_{30}O_3$: C, 81.13; H, 7.29. Found: C, 81.03; H, 7.53.

{6-[(2′,6′-Dimethylbiphenyl-3-yl)methoxy]-1-benzofuran-3-yl}acetic Acid (17). Step 1: Methyl {6-[(2′,6′-dimethylbiphenyl-3-yl)methoxy]-1-benzofuran-3-yl}acetate in 96% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 2.01 (s, 6H), 3.67 (d, *J* = 1.0 Hz, 2H), 3.72 (s, 3H), 5.15 (s, 2H), 6.97 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.07–7.19 (m, 5H), 7.23 (s, 1H), 7.40–7.48 (m, 3H), 7.53 (s, 1H). MS *m/z* 401 $(M + H)^+$. Step 2: **17** in 80% yield as colorless plates (hexane–AcOEt). mp 128–129 °C. ^{1}H NMR ($CDCl_3$) δ 2.01 (s, 6H), 3.71 (d, *J* = 0.8 Hz, 2H), 5.15 (s, 2H), 6.97 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.07–7.19 (m, 5H), 7.23 (s, 1H), 7.40–7.48 (m, 3H), 7.54 (s, 1H). MS *m/z* 387 $(M + H)^+$. HPLC purity (220 nm) 99.4%. Anal. Calcd for $C_{25}H_{22}O_4$: C, 77.70; H, 5.74. Found: C, 77.52; H, 5.49.

{6-[(2′,6′-Dimethylbiphenyl-3-yl)methoxy]-2,3-dihydro-1-benzofuran-3-yl}acetic Acid (18). Step 1: Methyl {6-[(2′,6′-dimethylbiphenyl-3-yl)methoxy]-2,3-dihydro-1-benzofuran-3-yl}acetate in 72% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 2.01 (s, 6H), 2.55 (dd, *J* = 16.5, 9.2 Hz, 1H), 2.75 (dd, *J* = 16.5, 6.0 Hz, 1H), 3.71 (s, 3H), 3.75–3.85 (m, 1H), 4.26 (dd, *J* = 9.2, 6.0 Hz, 1H), 4.75 (t, *J* = 9.2 Hz, 1H), 5.06 (s, 2H), 6.45–6.50 (m, 2H), 7.02 (d, *J* = 7.9 Hz, 1H), 7.08–7.19 (m, 5H), 7.37–7.46 (m, 2H). MS *m/z* 403 $(M + H)^+$. Step 2: **18** in 73% yield as colorless needles (hexane–AcOEt). mp 147–148 °C. ^{1}H NMR ($CDCl_3$) δ 2.01 (s, 6H), 2.61 (dd, *J* = 16.8, 9.2 Hz, 1H), 2.81 (dd, *J* = 16.8, 5.7 Hz, 1H), 3.76–3.86 (m, 1H), 4.29 (dd, *J* = 9.2, 5.7 Hz, 1H), 4.76 (t, *J* = 9.2 Hz, 1H), 5.07 (s, 2H), 6.46–6.51 (m, 2H), 7.04–7.19 (m, 6H), 7.37–7.46 (m, 2H). MS *m/z* 389 $(M + H)^+$. HPLC purity (220 nm) 99.4%. Anal. Calcd for $C_{25}H_{24}O_4$: C, 77.30; H, 6.23. Found: C, 77.08; H, 6.25.

8-[(2′,6′-Dimethylbiphenyl-3-yl)methoxy]-2,3,4,5-tetrahydro-1-benzoxepine-4-carboxylic Acid (19). Step 1: Ethyl 8-[(2′,6′-Dimethylbiphenyl-3-yl)methoxy]-2,3,4,5-tetrahydro-1-benzoxepine-4-carboxylate in 90% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.25 (t, *J* = 7.2 Hz, 3H), 2.01 (s, 6H), 2.15–2.29 (m, 2H), 2.57–2.65 (m, 1H), 2.94 (dd, *J* = 14.3, 2.3 Hz, 1H), 3.05 (dd, *J* = 14.3, 9.6 Hz, 1H), 3.76–3.84 (m, 1H), 4.13 (q, *J* = 7.2 Hz, 2H), 4.24–4.32 (m, 1H), 5.07 (s, 2H), 6.59–6.63 (m, 2H), 7.02–7.19 (m, 6H), 7.37–7.46 (m, 2H). MS *m/z* 431 $(M + H)^+$. Step 2: **19** in 76% yield as colorless crystals (hexane–AcOEt). mp 100–101 °C. ^{1}H NMR ($CDCl_3$) δ 2.01 (s, 6H), 2.17–2.29 (m, 2H), 2.63–2.72 (m, 1H), 2.96–3.11 (m, 2H), 3.79–3.85 (m, 1H), 4.25–4.32 (m, 1H), 5.07 (s, 2H), 6.60–6.64 (m, 2H), 7.04–7.19 (m, 6H), 7.37–7.46 (m, 2H). MS *m/z* 403 $(M + H)^+$. Anal. Calcd for $C_{26}H_{26}O_4$: C, 77.59; H, 6.51. Found: C, 77.53; H, 6.48.

2-Bromo-5-methoxybenzaldehyde (21). To a suspension of 3-methoxybenzaldehyde (20.4 g, 150 mmol) in AcOH (400 mL) was added dropwise a solution of Br_2 (7.68 mL, 150 mmol) in AcOH (50 mL) at room temperature, and the mixture was stirred at room temperature for 24 h, then added to water. The resulting crystals were collected by filtration, washed with water, and dried to give **21** (28.2 g, 88%) as colorless crystals. mp 71–72 °C.

^{1}H NMR ($CDCl_3$) δ 3.85 (s, 3H), 7.04 (dd, *J* = 8.9, 3.2 Hz, 1H), 7.42 (d, *J* = 3.2 Hz, 1H), 7.50–7.55 (m, 1H), 10.32 (s, 1H). HPLC purity (220 nm) >99%. Anal. Calcd for $C_8H_7BrO_2$: C, 44.68; H, 3.28. Found: C, 44.81; H, 3.41.

3-(2-Bromo-5-methoxyphenyl)propanenitrile (22). Step 1: A mixture of **21** (20.6 g, 95.8 mmol), cyanoacetic acid (8.96 g, 105 mmol), and NH_4OAc (1.11 g, 14.4 mmol) in toluene (55 mL) and pyridine (32 mL) was stirred with a Dean-Stark apparatus at reflux for 0.5 h. The mixture was cooled and diluted with toluene. The resulting crystals were collected by filtration and washed with toluene. The obtained ammonium salts were treated with 1 M HCl aqueous solution, and the resulting free acids were collected by filtration, washed with water, and dried. The product was washed with MeOH to give 3-(2-bromo-5-methoxyphenyl)-2-cyanoacrylic acid (21.5 g, 80%) as yellow crystals. ^{1}H NMR (DMSO-d_6) δ 3.82 (s, 3H), 7.15 (dd, *J* = 8.9, 3.0 Hz, 1H), 7.66 (d, *J* = 3.0 Hz, 1H), 7.74 (d, *J* = 8.9 Hz, 1H), 8.39 (s, 1H). HPLC purity (220 nm) >99%. Anal. Calcd for $C_{11}H_8BrNO_3$: C, 46.84; H, 2.86; N, 4.97. Found: C, 46.93; H, 2.85; N, 4.94. Step 2: To a suspension of the obtained crystals (21.0 g, 74.4 mmol) in step 1 in saturated $NaHCO_3$ aqueous solution (55 mL) and MeOH (270 mL) was added portionwise $NaBH_4$ (8.20 g, 217 mmol) at 0 °C, and the mixture was stirred under nitrogen atmosphere at room temperature for 4 h. After MeOH was evaporated, the residue was diluted with water, acidified with 6 M HCl aqueous solution, and extracted with Et_2O. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated to give 3-(2-bromo-5-methoxyphenyl)-2-cyanopropanoic acid (20.8 g, 98%) as colorless crystals. ^{1}H NMR ($CDCl_3$) δ 3.18 (dd, *J* = 13.7, 9.9 Hz, 1H), 3.52 (dd, *J* = 13.7, 5.8 Hz, 1H), 3.80 (s, 3H), 4.02 (dd, *J* = 9.9, 5.8 Hz, 1H), 6.76 (dd, *J* = 8.8, 3.0 Hz, 1H), 6.92 (d, *J* = 3.0 Hz, 1H), 7.47 (d, *J* = 8.8 Hz, 1H). HPLC purity (220 nm) >99%. Anal. Calcd for $C_{11}H_{10}BrNO_3$: C, 46.50; H, 3.55; N, 4.93. Found: C, 46.47; H, 3.50; N, 4.92. Step 3: A suspension of the obtained crystals (20.0 g, 70.4 mmol) in step 2 in DMA (40 mL) was stirred under nitrogen atmosphere at 180 °C for 2 h. After cooling, the mixture was concentrated, diluted with water, and extracted with Et_2O. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 0:100–25:75) to give **22** (14.9 g, 88%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 2.67 (t, *J* = 7.3 Hz, 2H), 3.04 (t, *J* = 7.3 Hz, 2H), 3.80 (s, 3H), 6.72 (dd, *J* = 8.9, 3.0 Hz, 1H), 6.85 (d, *J* = 3.0 Hz, 1H), 7.44 (d, *J* = 8.9 Hz, 1H). HPLC purity (220 nm) >99%.

3-Hydroxybicyclo[4.2.0]octa-1,3,5-triene-7-carbonitrile (23). Step 1: $NaNH_2$ (6.24 g, 160 mmol) was added to liquid NH_3 (ca. 500 mL) at −78 °C. After stirring at −33 °C for 0.5 h, **22** (9.60 g, 40.0 mmol) was added, and the mixture was stirred at −33 °C for 1 h. After removal of the solvent, the residue was then cooled down to −78 °C and quenched with NH_4Cl aqueous solution. The mixture was extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by

silica gel column chromatography (AcOEt:hexane = 0:100–25:75) to give 3-methoxybicyclo[4.2.0]octa-1,3,5-triene-7-carbonitrile (2.87 g, 48%) as a light green oil. ^{1}H NMR ($CDCl_3$) δ 3.49 (dd, *J* = 14.1, 2.7 Hz, 1H), 3.62 (dd, *J* = 14.1, 5.4 Hz, 1H), 3.79 (s, 3H), 4.17 (dd, *J* = 5.4, 2.7 Hz, 1H), 6.71 (d, *J* = 2.1 Hz, 1H), 6.84 (dd, *J* = 8.3, 2.1 Hz, 1H), 7.12 (d, *J* = 8.3 Hz, 1H). MS *m/z* 160 $(M + H)^+$. Step 2: To a mixture of the obtained oil (0.796 g, 5.00 mmol) in step 1 and dodecyl methyl sulfide (3.25 g, 15.0 mmol) in toluene (10 mL) was added portionwise $AlCl_3$ (2.00 g, 15.0 mmol) at 0 °C, and the mixture was stirred under nitrogen atmosphere at 0 °C for 3 h. The mixture was quenched with diluted HCl aqueous solution, and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 5:95–40:60) to give **23** (0.573 g, 79%) as colorless crystals. ^{1}H NMR ($CDCl_3$) δ 3.48 (dd, *J* = 14.3, 2.7 Hz, 1H), 3.61 (dd, *J* = 14.3, 5.7 Hz, 1H), 4.16 (dd, *J* = 5.7, 2.7 Hz, 1H), 5.02 (s, 1H), 6.65 (d, *J* = 2.1 Hz, 1H), 6.75 (dd, *J* = 8.1, 2.1 Hz, 1H), 7.07 (d, *J* = 8.1 Hz, 1H). MS *m/z* 146 $(M + H)^+$. Anal. Calcd for C_9H_7NO: C, 74.47; H, 4.86; N, 9.65. Found: C, 74.64; H, 4.86; N, 9.67.

3-[(2′,6′-Dimethylbiphenyl-3-yl)methoxy]bicyclo[4.2.0]octa-1,3,5-triene-7-carboxylic Acid (24). Step 1: 3-[(2′,6′-Dimethylbiphenyl-3-yl)methoxy]bicyclo[4.2.0]octa-1,3,5-triene-7-carbonitrile was prepared from **13** and **23** by a similar to that described for **14**-step 1 in 90% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 2.01 (s, 6H), 3.43–3.51 (m, 1H), 3.56–3.65 (m, 1H), 4.16 (dd, *J* = 5.6, 2.5 Hz, 1H), 5.09 (s, 2H), 6.76 (d, *J* = 1.7 Hz, 1H), 6.91 (dd, *J* = 8.3, 2.1 Hz, 1H), 7.07–7.20 (m, 6H), 7.36–7.41 (m, 1H), 7.42–7.48 (m, 1H). MS *m/z* 352 $(M + Na)^+$. Step 2: To a solution of the obtained oil (2.28 g, 6.72 mmol) in step 1 in EtOH (10 mL) was added KOH (0.943 g, 16.8 mmol), and the mixture was stirred at room temperature for 60 h. Water (3 mL) was added to the mixture, which was stirred at reflux for 9 h. After cooling, the mixture was acidified with 1 M HCl aqueous solution, and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated to give **24** (2.38 g, 99%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 2.01 (s, 6H), 3.41 (d, *J* = 4.1 Hz, 2H), 4.25 (t, *J* = 4.1 Hz, 1H), 5.08 (s, 2H), 6.76 (d, *J* = 2.1 Hz, 1H), 6.87 (dd, *J* = 8.1, 2.1 Hz, 1H), 7.04–7.21 (m, 6H), 7.36–7.47 (m, 2H). MS *m/z* 359 $(M + H)^+$.

{3-[(2′,6′-Dimethylbiphenyl-3-yl)methoxy]bicyclo[4.2.0]octa-1,3,5-trien-7-yl}acetic Acid (25). Step 1: To a mixture of **24** (2.38 g, 6.65 mmol) in THF (15 mL) was added portionwise $LiAlH_4$ (0.473 g, 9.98 mmol) at 0 °C, and the mixture was stirred under nitrogen atmosphere at room temperature for 1 h. The mixture was cooled to 0 °C and $Na_2SO_4 \cdot 10\ H_2O$ was added portionwise to the mixture. After stirring at room temperature overnight, the mixture was filtered through a pad of Celite, and the filtrate was concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 20:80–50:50) to give {3-[(2′,6′-dimethylbiphenyl-3-yl)methoxy]bicyclo[4.2.0]octa-1,3,5-trien-7-yl}methanol (2.01 g, 88%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.41 (t, *J* = 5.7 Hz, 1H), 2.01 (s, 6H), 2.86

(dd, J = 14.1, 2.3 Hz, 1H), 3.23 (dd, J = 14.1, 5.2 Hz, 1H), 3.59–3.67 (m, 1H), 3.80–3.93 (m, 2H), 5.08 (s, 2H), 6.76 (d, J = 2.0 Hz, 1H), 6.83 (dd, J = 8.0, 2.0 Hz, 1H), 7.01 (d, J = 8.0 Hz, 1H), 7.07–7.21 (m, 5H), 7.37–7.47 (m, 2H). MS m/z 327 $(M - 18 + H)^+$. Step 2: To a mixture of the obtained oil (2.01 g, 5.84 mmol) in step 1 in pyridine (15 mL) was added portionwise *p*-TsCl (2.00 g, 10.5 mmol) at room temperature, and the mixture was stirred under nitrogen atmosphere at room temperature for 14 h. The mixture was added to diluted HCl aqueous solution at 0 °C and extracted with Et_2O. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated to give {3-[(2′,6′-dimethylbiphenyl-3-yl) methoxy]bicyclo[4.2.0]octa-1,3,5-trien-7-yl}methyl 4-methylbenzenesulfonate (2.58 g, 89%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 2.00 (s, 6H), 2.44 (s, 3H), 2.75 (dd, J = 14.4, 2.3 Hz, 1H), 3.24 (dd, J = 14.4, 5.1 Hz, 1H), 3.64–3.73 (m, 1H), 4.11–4.19 (m, 1H), 4.22–4.30 (m, 1H), 5.06 (s, 2H), 6.71 (d, J = 2.0 Hz, 1H), 6.79 (dd, J = 8.1, 2.0 Hz, 1H), 6.91 (d, J = 8.1 Hz, 1H), 7.06–7.20 (m, 5H), 7.33 (d, J = 7.9 Hz, 2H), 7.36–7.40 (m, 1H), 7.41–7.47 (m, 1H), 7.75–7.81 (m, 2H). MS m/z 499 $(M + H)^+$. Step 3: To a suspension of NaCN (0.505 g, 10.3 mmol) in DMSO (7 mL) was added dropwise a solution of the obtained oil (2.58 g, 5.17 mmol) in step 2 in DMSO (15 mL) at room temperature, and the mixture was stirred under nitrogen atmosphere at room temperature for 48 h. The mixture was poured into $NaHCO_3$ aqueous solution at 0 °C, and extracted with Et_2O. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 0:100–25:75) to give {3-[(2′,6′-dimethylbiphenyl-3-yl)methoxy]bicyclo[4.2.0]octa-1,3,5-trien-7-yl}acetonitrile (1.65 g, 90%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 2.01 (s, 6H), 2.67–2.73 (m, 2H), 2.85 (dd, J = 14.4, 2.3 Hz, 1H), 3.43 (dd, J = 14.4, 5.1 Hz, 1H), 3.67–3.76 (m, 1H), 5.08 (s, 2H), 6.76 (d, J = 2.0 Hz, 1H), 6.86 (dd, J = 8.2, 2.0 Hz, 1H), 7.06–7.21 (m, 6H), 7.37–7.48 (m, 2H). MS m/z 354 $(M + H)^+$. Step 4: Compound **25** was prepared from the obtained oil in step 3 by a similar to that described for **24**-step 2 in 82% yield as a yellow viscous oil. ^{1}H NMR ($CDCl_3$) δ 2.01 (s, 6H), 2.72–2.84 (m, 3H), 3.39 (dd, J = 14.4, 5.3 Hz, 1H), 3.71–3.81 (m, 1H), 5.08 (s, 2H), 6.74 (d, J = 2.0 Hz, 1H), 6.82 (dd, J = 8.2, 2.0 Hz, 1H), 7.03 (d, J = 8.2 Hz, 1H), 7.07–7.21 (m, 5H), 7.37–7.48 (m, 2H). MS m/z 373 $(M + H)^+$. HPLC purity (220 nm) 100%.

Ethyl 2-Methyl-6-oxo-1,6-dihydropyridine-3-carboxylate (27). Step 1: To a mixture of ethyl acetoacetate (26.0 g, 200 mmol) and silica gel (2 g) was added dropwise 28% NH_3 aqueous solution (14.6 g, 240 mmol) at room temperature, and the mixture was stirred at room temperature for 18 h. The mixture was filtered, and the filtrate was diluted with water, and then extracted with AcOEt. The extract was washed with brine, dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 5:95–40:60) to give ethyl (2*Z*)-3-aminobut-2-enoate (21.5 g, ca. 80% purity, 67%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.26 (t, J = 7.1 Hz, 3H), 1.90 (s, 3H), 4.11 (q, J = 7.1 Hz, 2H), 4.52 (s, 1H). Step 2: A mixture of the obtained oil (21.5 g, ca. 133 mmol) in

step 1 and methyl propiolate (11.8 g, 140 mmol) in toluene (140 mL) was stirred under nitrogen atmosphere at reflux for 4 h. To the mixture was added methyl propiolate (5.64 g, 67.1 mmol), and the mixture was stirred under nitrogen atmosphere at reflux for 12 h. To the mixture was added again methyl propiolate (8.48 g, 101 mmol), and the mixture was stirred under nitrogen atmosphere at reflux for 20 h. After cooling, the mixture was concentrated to give crude 5-ethyl 1-methyl (2*E*,4*Z*)-4-(1-aminoethylidene)pent-2-enedioate as a orange solid. This product was used for the next reaction without further purification. ^{1}H NMR ($CDCl_3$) δ 1.36 (t, *J* = 7.2 Hz, 3H), 2.27 (s, 3H), 3.73 (s, 3H), 4.26 (q, *J* = 7.2 Hz, 2H), 5.43 (br s, 1H), 6.18 (d, *J* = 15.5 Hz, 1H), 7.65 (d, *J* = 15.5 Hz, 1H), 9.58 (br s, 1H). Step 3: A solution of the crude product in DMF (350 mL) was stirred under nitrogen atmosphere at reflux for 6 days. After evaporation of the solvent, the resulting solid was washed with toluene to give **27** (7.49 g) as yellow crystals. The mother liquor was purified by silica gel column chromatography (AcOEt:hexane = 50:50–100:0) to give the second crop (0.62 g) as yellow crystals. Total 8.11 g (34% in 2 steps). ^{1}H NMR (DMSO-d_6) δ 1.27 (t, *J* = 7.0 Hz, 3H), 2.52 (s, 3H), 4.20 (q, *J* = 7.0 Hz, 2H), 6.20 (d, *J* = 9.5 Hz, 1H), 7.81 (d, *J* = 9.5 Hz, 1H), 12.04 (br s, 1H). MS *m*/*z* 182 $(M + H)^+$.

Ethyl 6-Chloro-2-methylnicotinate (28). A mixture of **27** (8.09 g, 44.6 mmol) and $POCl_3$ (20.0 g, 130 mmol) was stirred under nitrogen atmosphere at 120 °C for 2 h. After cooling, the mixture was poured into ice water, basified with 8 M NaOH aqueous solution, and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 5:95–30:70) to give **28** (7.88 g, 89%) as colorless crystals. ^{1}H NMR ($CDCl_3$) δ 1.40 (t, *J* = 7.2 Hz, 3H), 2.82 (s, 3H), 4.38 (q, *J* = 7.2 Hz, 2H), 7.24 (d, *J* = 8.3 Hz, 1H), 8.16 (d, *J* = 8.3 Hz, 1H). MS *m*/*z* 200 $(M + H)^+$.

Ethyl 2-Chloro-5-oxo-6,7-dihydro-5*H*-cyclopenta[*b*]pyridine-6-carboxylate (29). Step 1: A mixture of **28** (7.88 g, 39.5 mmol), NBS (7.74 g, 43.5 mmol), and AIBN (64.9 mg, 0.395 mmol) in CCl_4 (80 mL) was stirred under nitrogen atmosphere at reflux for 4 h. The mixture was concentrated, and the residue was washed with Et_2O. The filtrate was concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 0:100–25:75) to give ethyl 2-(bromomethyl)-6-chloronicotinate (6.94 g, 63%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.43 (t, *J* = 7.1 Hz, 3H), 4.44 (q, *J* = 7.1 Hz, 2H), 4.97 (s, 2H), 7.35 (d, *J* = 8.3 Hz, 1H), 8.24 (d, *J* = 8.3 Hz, 1H). MS *m*/*z* 278 $(M + H)^+$. Step 2: To a solution of diethyl malonate (8.01 g, 50.0 mmol) in THF (100 mL) was added portionwise NaH (60% in mineral oil, 2.00 g, 50.0 mmol) at room temperature, and the mixture was stirred under nitrogen atmosphere at room temperature for 0.5 h. To the mixture was added the obtained oil (6.49 g, 24.9 mmol) in step 1, and the mixture was stirred under nitrogen atmosphere at room temperature for 12 h. The mixture was poured into ice water, neutralized with 1 M HCl aqueous solution, acidified with diluted citric acid aqueous

solution, and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 5:95–30:70) to give diethyl {[6-chloro-3-(ethoxy-carbonyl)pyridin-2-yl]methyl}malonate (6.58 g, 74%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.27 (t, *J* = 7.1 Hz, 6H), 1.40 (t, *J* = 7.1 Hz, 3H), 3.82 (d, *J* = 7.5 Hz, 2H), 4.14 (t, *J* = 7.5 Hz, 1H), 4.21 (q, *J* = 7.1 Hz, 4H), 4.39 (q, *J* = 7.1 Hz, 2H), 7.23 (d, *J* = 8.3 Hz, 1H), 8.17 (d, *J* = 8.3 Hz, 1H). MS *m*/*z* 358 $(M + H)^+$. Step 3: To a suspension of NaH (60% in mineral oil, 0.88 g, 22.0 mmol) in toluene (300 mL) was added dropwise a solution of the obtained oil (6.58 g, 18.4 mmol) in step 2 in toluene (100 mL) at room temperature, and the mixture was stirred under nitrogen atmosphere at reflux for 4 h. The mixture was poured into ice water, neutralized with 1 M HCl aqueous solution, acidified with diluted citric acid aqueous solution, and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated to give a solid, which was washed with MeOH to give **29** (1.58 g) as yellow crystals. The mother liquor was concentrated to give the second crop (3.06 g). Total 4.40 g (99%). ^{1}H NMR ($CDCl_3$) δ 1.38 (t, *J* = 7.1 Hz, 3H), 3.63 (s, 2H), 4.35 (q, *J* = 7.1 Hz, 2H), 7.35 (d, *J* = 8.1 Hz, 1H), 7.84 (d, *J* = 8.1 Hz, 1H), 10.49 (br s, 1H). MS *m*/*z* 286 $(M + H)^+$.

2-Hydroxy-6,7-dihydro-5*H*-cyclopenta[*b*]pyridin-5-one (30). A mixture of **29** (4.40 g, 18.4 mmol) and 85% H_3PO_4 (50 mL) was stirred under nitrogen atmosphere at 185 °C for 3 h. After cooling, the mixture was poured into ice water, and neutralized with 8 M NaOH aqueous solution and $NaHCO_3$. The mixture was concentrated, and diluted with EtOH. The insoluble material was removed by filtration, washed with EtOH, and the filtrate was concentrated. The resulting solid was washed with EtOH, and dried to give **30** (2.33 g, 85%) as a khaki solid. ^{1}H NMR (DMSO-d_6) δ 2.25–2.33 (m, 2H), 2.56–2.65 (m, 2H), 5.78 (d, *J* = 8.9 Hz, 1H), 7.19 (d, *J* = 8.9 Hz, 1H). MS *m*/*z* 150 $(M + H)^+$.

2-[(2′,6′-Dimethylbiphenyl-3-yl)methoxy]-6,7-dihydro-5*H*-cyclopenta[*b*]pyridin-5-ol (31). Step 1: To a solution of **13** (1.78 g, 8.40 mmol) and Et_3N (1.41 mL, 10.1 mmol) in THF (15 mL) was added portionwise MsCl (0.782 mL, 10.1 mmol) at 0 °C, and the mixture was stirred under nitrogen atmosphere at room temperature for 1 h. The mixture was diluted with THF, and the insoluble material was removed by filtration, and the filtrate was concentrated. The residue was dissolved in DMF (15 mL), and to the solution were added **30** (1.04 g, 7.00 mmol) and K_2CO_3 (1.16, 8.40 mmol) at room temperature. The mixture was stirred under nitrogen atmosphere at 80 °C for 13 h. The mixture was poured into water and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 30:70–80:20) to give a crude oil (0.36 g). Step 2: To a solution of the obtained oil in step 1 in MeOH (1 mL) and THF (2 mL) was added portionwise $NaBH_4$ (42 mg, 1.00 mol) at 0 °C, and the mixture was stirred under nitrogen atmosphere at 0 °C for 2 h.

The mixture was poured into diluted citric acid aqueous solution and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 20:80–60:40) to give **31** (0.147 g, 6% in 2 steps) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.72 (d, *J* = 6.8 Hz, 1H), 1.92–2.04 (m, 7H), 2.49–2.62 (m, 1H), 2.77–2.89 (m, 1H), 3.02–3.14 (m, 1H), 5.18–5.27 (m, 1H), 5.42 (s, 2H), 6.66 (d, *J* = 8.3 Hz, 1H), 7.07–7.19 (m, 4H), 7.24 (s, 1H), 7.40–7.46 (m, 2H), 7.60 (d, *J* = 8.3 Hz, 1H). MS *m*/*z* 346 $(M + H)^+$.

{2-[(2′,6′-Dimethylbiphenyl-3-yl)methoxy]-6,7-dihydro-5*H*-cyclopenta[*b*]pyridin-5-yl} acetic Acid (32). Step 1: To a solution of **31** (0.142 g, 0.411 mmol) in toluene (1 mL) were sequentially added $SOCl_2$ (0.073 mL, 1.00 mmol) and pyridine (0.0809 mL, 1.00 mmol) at room temperature, and the mixture was stirred under nitrogen atmosphere at room temperature for 1 h. The mixture was quenched with saturated $NaHCO_3$ aqueous solution and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated to give a brown oil. Step 2: To a solution of diethyl malonate (0.160 g, 1.00 mmol) in THF (3 mL) was added NaH (60% in mineral oil, 40 mg, 1.00 mmol) at room temperature and the mixture was stirred under nitrogen atmosphere at room temperature for 1 h. The mixture was added into the obtained oil in step 1 at room temperature, and the resulting mixture was stirred under nitrogen atmosphere at room temperature for 14 h. The mixture was diluted with citric acid aqueous solution and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 0:100–25:75) to give diethyl {2-[(2′,6′-dimethylbiphenyl-3-yl)methoxy]-6,7-dihydro-5*H*-cyclopenta[*b*]pyridin-5-yl}malonate (0.106 g, 53% in 2 steps) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.18–1.32 (m, 6H), 1.96–2.08 (m, 7H), 2.30–2.44 (m, 1H), 2.79–3.04 (m, 2H), 3.53 (d, *J* = 8.3 Hz, 1H), 3.82–3.91 (m, 1H), 4.14–4.25 (m, 4H), 5.38 (s, 2H), 6.55 (d, *J* = 8.3 Hz, 1H), 7.05–7.20 (m, 4H), 7.23 (s, 1H), 7.37–7.44 (m, 3H). MS *m*/*z* 488 $(M + H)^+$. Step 3: To a solution of the obtained oil (0.102 g, 0.209 mmol) in step 2 in EtOH (1 mL) and THF (2 mL) was added 2 M NaOH aqueous solution (0.4 mL, 0.800 mmol) at room temperature, and the mixture was stirred at 50 °C for 2.5 h. The mixture was acidified with diluted citric acid aqueous solution and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated to give a solid, which was suspended in toluene (4 mL), and the suspension was stirred under nitrogen atmosphere at reflux for 12 h. The mixture was concentrated, and the residue was purified by silica gel column chromatography (AcOEt:hexane = 10:90–80:20) to give crystals. Recrystallization from heptane–AcOEt gave **32** (31.0 mg, 38%) as colorless crystals. mp 136–137 °C. ^{1}H NMR ($CDCl_3$) δ 1.74–1.88 (m, 1H), 2.02 (s, 6H), 2.38–2.57 (m, 2H), 2.67–2.78 (m, 1H), 2.82–3.02 (m, 2H), 3.49–3.61 (m, 1H), 5.39 (s, 2H), 6.59 (d, *J* = 8.3 Hz, 1H), 7.06–7.19 (m, 4H), 7.24 (s, 1H), 7.39–7.46 (m, 3H). MS *m*/*z* 388 $(M + H)^+$. HPLC purity (220 nm) 100%.

5-[(2′,6′-Dimethylbiphenyl-3-yl)methoxy]-1*H*-indole (34). The title compound was prepared from **33** and **13** by a similar to that described for **14**-step 1 in 62% yield as a pale brown oil. ^{1}H NMR ($CDCl_3$) δ 2.02 (s, 6H), 5.15 (s, 2H), 6.42–6.48 (m, 1H), 6.93 (dd, *J* = 8.8, 2.6 Hz, 1H), 7.04–7.50 (m, 10H), 8.05 (br s, 1H). MS *m/z* 328 $(M + H)^+$.

{5-[(2′,6′-Dimethylbiphenyl-3-yl)methoxy]-1*H*-indole-1-yl}acetic Acid (35). Step 1: To a solution of **34** (0.95 g, 2.90 mmol) in THF (30 mL) and DMF (4 mL) was added NaH (60% in mineral oil, 0.12 g, 3.0 mmol) at 4 °C, and the mixture was stirred under nitrogen atmosphere at 4 °C for 20 min. To the mixture was added ethyl bromoacetate (0.36 mL, 3.25 mmol) at 4 °C, and the resulting mixture was stirred at room temperature for 2 days. The mixture was diluted with citric acid aqueous solution and extracted with AcOEt. The extract was washed with water and then brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 1:10–1:5) to give ethyl {5-[(2′,6′-dimethylbiphenyl-3-yl)methoxy]-1*H*-indole-1-yl}acetate (1.0 g, 83%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.25 (t, *J* = 7.0 Hz, 3H), 2.03 (s, 6H), 4.20 (q, *J* = 7.0 Hz, 2H), 4.80 (s, 2H), 5.14 (s, 2H), 6.45 (dd, *J* = 3.2, 0.8 Hz, 1H), 6.95 (dd, *J* = 8.8, 2.6 Hz, 1H), 7.04–7.48 (m, 10H). MS *m/z* 414 $(M + H)^+$. Step 2: To a solution of the obtained oil (0.27 g, 0.65 mmol) in step 1 in MeOH (10 mL) and THF (10 mL) was added a solution of KOH (85%, 130 mg, 1.97 mmol) in water (5 mL) at room temperature, and the mixture was stirred for 18 h. The mixture was acidified with diluted citric acid aqueous solution and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 1:2–2:1) to give **35** (0.19 g, 76%) as a pale yellow amorphous powder. ^{1}H NMR ($CDCl_3$) δ 2.02 (s, 6H), 4.84 (s, 2H), 5.14 (s, 2H), 6.46 (d, *J* = 3.2 Hz, 1H), 6.96 (dd, *J* = 8.8, 2.2 Hz, 1H), 7.00–7.48 (m, 10H). MS *m/z* 386 $(M + H)^+$. HPLC purity (220 nm) 97.4%.

3-(2-Methylnaphthalen-1-yl)benzaldehyde (41a). To a mixture of 1-bromo-2-methylnaphthalene (3.32 g, 15.0 mmol), (3-formylphenyl)boronic acid (**36**) (2.13 g, 15.0 mmol), and 1 M Na_2CO_3 aqueous solution (30 mL, 30.0 mmol) in EtOH (15 mL) and toluene (30 mL) was added $Pd(PPh_3)_4$ (0.867 g, 0.750 mmol), and the mixture was stirred under argon atmosphere at 80 °C for 24 h. After cooling, the mixture was partitioned between water and AcOEt. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 0:100–20:80) to give **41a** (2.39 g, 65%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 2.23 (s, 3H), 7.27–7.45 (m, 4H), 7.57 (dt, *J* = 7.6, 1.4 Hz, 1H), 7.69 (t, *J* = 7.6 Hz, 1H), 7.81–7.88 (m, 3H), 7.98 (dt, *J* = 7.6, 1.4 Hz, 1H), 10.09 (s, 1H). MS *m/z* 247 $(M + H)^+$.

3-(1-Benzothiophen-3-yl)benzaldehyde (41b). The title compound was prepared from **36** and 3-bromo-1-benzothiophene by a similar to that described for **41a** in 94% yield as a

pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 7.40–7.48 (m, 1H), 7.49 (s, 1H), 7.67 (t, *J* = 7.4 Hz, 1H), 7.83–8.14 (m, 5H). MS *m/z* 239 $(M + H)^+$.

3-(1-Benzothiophen-5-yl)benzaldehyde (41c). The title compound was prepared from **36** and 5-bromo-1-benzothiophene by a similar to that described for **41a** in 70% yield as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 7.41 (t, *J* = 5.6 Hz, 1H), 7.52 (t, *J* = 5.6 Hz, 1H), 7.60–8.04 (m, 5H), 8.08 (d, *J* = 1.6 Hz, 1H), 8.17–8.21 (m, 1H). 10.12 (s, 1H). MS *m/z* 239 $(M + H)^+$.

2′,6′-Dimethyl-6-methoxybiphenyl-3-carbaldehyde (41d). The title compound was prepared from (2-methoxy-5-formylphenyl)boronic acid (**39**) and 1-bromo-2,6-dimethylbenzene by a similar to that described for **41a** in 87% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.99 (s, 6H), 3.84 (s, 3H), 6.98–7.22 (m, 4H), 7.60 (d, *J* = 2.2 Hz, 1H), 7.91 (dd, *J* = 8.8, 2.2 Hz, 1H), 9.92 (s, 1H). MS *m/z* 241 $(M + H)^+$.

6-(Benzyloxy)-2′,6′-dimethylbiphenyl-3-carbaldehyde (41e). To a mixture of 4-(benzyloxy)-3-bromobenzaldehyde (**40**) (11.6 g, 39.8 mmol), 2,6-dimethylphenylboronic acid (6.60 g, 44.0 mmol), and K_3PO_4 (17.0 g, 80.0 mmol) in toluene (240 mL) and H_2O (60 mL) were added $Pd_2(dba)_3$ (0.549 g, 0.600 mmol) and SPhos (0.985 g, 2.40 mmol), and the mixture was stirred under argon atmosphere at 100 °C for 24 h. After cooling, the mixture diluted with water and AcOEt, and filtered through a pad of Celite, and the filtrate was concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 0:100–30:70) to give **41e** (12.6 g, quantitative) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 2.02 (s, 6H), 5.15 (s, 2H), 7.09–7.33 (m, 9H), 7.63 (d, *J* = 2.0 Hz, 1H), 7.85 (dd, *J* = 8.5, 2.0 Hz, 1H), 9.91 (s, 1H). MS *m/z* 317 $(M + H)^+$.

Ethyl 2′,4′-Dimethylbiphenyl-3-carboxylate (41f). The title compound was prepared from ethyl 3-bromobenzoate (**38**) and (2,4-dimethylphenyl)boronic acid by a similar to that described for **41a** in quantitative yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.39 (t, *J* = 7.0 Hz, 3H), 2.23 (s, 3H), 2.37 (s, 3H), 4.38 (q, *J* = 7.0 Hz, 2H), 7.02–7.54 (m, 5H), 8.00–8.05 (m, 2H).

2′,4′,6′-Trimethylbiphenyl-3-carbaldehyde (41g). The title compound was prepared from 3-bromobenzaldehyde (**37**) and (2,4,6-trimethylphenyl)boronic acid by a similar to that described for **41a** in 76% yield as a colorless oil. MS *m/z* 225 $(M + H)^+$.

[3-(2-Methylnaphthalen-1-yl)phenyl]methanol (42a). To a solution of **41a** (2.39 g, 9.70 mmol) in DME (10 mL) and THF (10 mL) was added portionwise $NaBH_4$ (0.189 g, 5.00 mmol) at 0 °C, and the mixture was stirred under nitrogen atmosphere at 0 °C for 3 h. To the mixture was added HCl aqueous solution, and the mixture was extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 5:95–30:70) to give **42a** (1.96 g, 81%) as a colorless viscous oil. ^{1}H NMR ($CDCl_3$) δ 1.66 (t, *J* = 5.9 Hz, 1H), 2.03 (s, 6H), 4.74 (d, *J* = 5.9, 2H Hz), 7.07–7.19 (m, 5H), 7.35 (d, *J* = 7.5 Hz, 1H), 7.43 (t, *J* = 7.5 Hz, 1H). MS *m/z* 231 $(M - 18 + H)^+$.

[3-(1-Benzothiophen-3-yl)phenyl]methanol (42b). To a solution of **41b** (2.1 g, 8.81 mmol) in dry THF (30 mL) was added $LiAlH_4$ (0.37 g, 9.75 mmol) at 0 °C, and the resulting mixture was stirred at room temperature for 2 h. The mixture was cooled to 0 °C, $Na_2SO_4 \cdot 10\ H_2O$ (3.0 g, 5.74 mmol) was added carefully and the mixture was stirred at room temperature for 1 h. The insoluble material was removed by filtration, and the filtrate was concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 1:5–1:3) to give **42b** (2.0 g, 95%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.72 (t, *J* = 5.8 Hz, 1H), 4.80 (d, *J* = 5.8 Hz, 2H), 7.35–7.64 (m, 7H), 7.88–7.98 (m, 2H). MS *m/z* 264 $(M + Na)^+$.

[3-(1-Benzothiophen-5-yl)phenyl]methanol (42c). The title compound was prepared from **41c** by a similar to that described for **42a** in 99% yield as colorless prisms. ^{1}H NMR ($CDCl_3$) δ 1.73 (t, *J* = 6.0 Hz, 1H), 4.79 (d, *J* = 6.0 Hz, 2H), 7.35–7.63 (m, 6H), 7.68 (s, 1H), 7.94 (d, *J* = 8.1 Hz, 1H), 8.04 (d, *J* = 1.8 Hz, 1H). MS *m/z* 264 $(M + Na)^+$.

(2′,6′-Dimethyl-6-methoxybiphenyl-3-yl)methanol (42d). The title compound was prepared from **41d** by a similar to that described for **42a** in 88% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 2.01 (s, 6H), 3.74 (s, 3H), 4.65 (d, *J* = 5.2 Hz, 2H), 6.97 (d, *J* = 8.4 Hz, 1H), 7.03 (d, *J* = 2.2 Hz, 1H), 7.06–7.24 (m, 3H), 7.35 (dd, *J* = 8.4, 2.6 Hz, 1H). MS *m/z* 225 $(M - 18 + H)^+$.

(6-Benzyloxy-2′,6′-dimethylbiphenyl-3-yl)methanol (42e). The title compound was prepared from **41e** by a similar to that described for **42a** in 99% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 2.03 (s, 6H), 4.64 (s, 2H), 5.02 (s, 2H), 6.99 (d, *J* = 8.3 Hz, 1H), 7.05–7.32 (m, 10H). MS *m/z* 301 $(M - 18 + H)^+$.

(2′,4′-Dimethylbiphenyl-3-yl)methanol (42f). The title compound was prepared from **41f** by a similar to that described for **42b** in 96% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 2.24 (s, 3H), 2.36 (s, 3H), 4.73 (d, *J* = 6.0 Hz, 2H), 7.00–7.45 (m, 7H).

(2′,4′,6′-Trimethylbiphenyl-3-yl)methanol (42g). The title compound was prepared from **41g** by a similar to that described for **42a** in 70% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 2.00 (s, 6H), 2.33 (s, 3H), 4.73 (d, *J* = 6.2 Hz, 2H), 6.94 (s, 2H), 7.00–7.42 (m, 4H). MS *m/z* 250 $(M + Na)^+$.

4′-Hydroxy-2′,6′-dimethylbiphenyl-3-carbaldehyde (44). The title compound was prepared from 4-bromo-3,5-dimethylphenol (**43**) and **36** by a similar to that described for **41a** in 83% as pale yellow crystals. ^{1}H NMR ($CDCl_3$) δ 1.97 (s, 6H), 4.69 (s, 1H), 6.62 (s, 2H), 7.42 (dt, *J* = 7.7, 1.4 Hz, 1H), 7.59 (t, *J* = 7.6 Hz, 1H), 7.66 (t, *J* = 1.7 Hz, 1H), 7.86 (dt, *J* = 7.6, 1.5 Hz, 1H), 10.05 (s, 1H). MS *m/z* 227 $(M + H)^+$.

[4′-(Benzyloxy)-2′,6′-dimethylbiphenyl-3-yl]methanol (42h). Step 1: A mixture of **44** (2.26 g, 10.0 mmol), benzyl bromide (3.42 g, 20.0 mmol), and K_2CO_3 (2.76 g, 20.0 mmol) in DMF (10 mL) was stirred at 70 °C for 2 h. The mixture was diluted with water and extracted with AcOEt. The organic layer was washed with brine, dried over anhydrous

$MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 0:100–10:90) to give 4′-(benzyloxy)-2′,6′-dimethylbiphenyl-3-carbaldehyde (2.90 g, 92%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 2.00 (s, 6H), 5.09 (s, 2H), 6.77 (s, 2H), 7.31–7.49 (m, 6H), 7.59 (t, *J* = 7.5 Hz, 1H), 7.66–7.68 (m, 1H), 7.84–7.89 (m, 1H), 10.05 (s, 1H). MS *m/z* 317 $(M + H)^+$. Step 2: Compound **42h** was prepared from the obtained oil by a similar to that described for **42a** in 95% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.65 (t, *J* = 5.9 Hz, 1H), 2.01 (s, 6H), 4.73 (d, *J* = 5.9 Hz, 2H), 5.07 (s, 2H), 6.75 (s, 2H), 7.07 (d, *J* = 7.3 Hz, 1H), 7.13 (s, 1H), 7.30–7.48 (m, 7H). MS *m/z* 319 $(M + H)^+$.

[4′-(2-Ethoxyethoxy)-2′,6′-dimethylbiphenyl-3-yl]methanol (42i). Step 1: 4′-(2-Ethoxyethoxy)-2′,6′-dimethylbiphenyl-3-carbaldehyde was prepared from **44** and 2-chloroethyl ethyl ether by a similar to that described for **42h**-step 1 in 89% as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.26 (t, *J* = 7.0 Hz, 3H), 1.99 (s, 6H), 3.62 (q, *J* = 7.0 Hz, 2H), 3.81 (t, *J* = 4.9 Hz, 2H), 4.15 (t, *J* = 4.9 Hz, 2H), 6.71 (s, 2H), 7.42 (dt, *J* = 7.5, 1.5 Hz, 1H), 7.58 (t, *J* = 7.5 Hz, 1H), 7.66 (t, *J* = 1.5 Hz, 1H), 7.86 (dt, *J* = 7.5, 1.5 Hz, 1H), 10.05 (s, 1H). MS *m/z* 299 $(M + H)^+$. Step 2: Compound **42i** was prepared from the obtained oil by a similar to that described for **42a** in 98% yield as colorless crystals. ^{1}H NMR ($CDCl_3$) δ 1.25 (t, *J* = 7.1 Hz, 3H), 1.66 (t, *J* = 5.9 Hz, 1H), 2.00 (s, 6H), 3.62 (q, *J* = 7.1 Hz, 2H), 3.80 (t, *J* = 5.1 Hz, 2H), 4.14 (t, *J* = 5.1 Hz, 2H), 4.73 (d, *J* = 5.9 Hz, 2H), 6.69 (s, 2H), 7.06 (d, *J* = 7.3 Hz, 1H), 7.12 (s, 1H), 7.33 (d, *J* = 7.3 Hz, 1H), 7.40 (t, *J* = 7.3 Hz, 1H). MS *m/z* 301 $(M + H)^+$.

The following compounds **45**–**53** were also prepared from **7e** and appropriate alcohols **42a**–**i** by a similar to that described for **14**.

(6-{[3-(2-Methylnaphthalen-1-yl)benzyl]oxy}-2,3-dihydro-1-benzofuran-3-yl)acetic Acid (45). Step 1: Methyl (6-{[3-(2-methylnaphthalen-1-yl)benzyl]oxy}-2,3-dihydro-1-benzofuran-3-yl)acetate in 91% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 2.22 (s, 3H), 2.55 (dd, *J* = 16.5, 9.3 Hz, 1H), 2.74 (dd, *J* = 16.5, 5.4 Hz, 1H), 3.71 (s, 3H), 3.75–3.85 (m, 1H), 4.26 (dd, *J* = 9.3, 6.0 Hz, 1H), 4.75 (t, *J* = 9.3 Hz, 1H), 5.09 (s, 2H), 6.47–6.51 (m, 2H), 7.02 (d, *J* = 7.9 Hz, 1H), 7.21–7.25 (m, 1H), 7.28–7.34 (m, 2H), 7.37–7.42 (m, 3H), 7.47–7.54 (m, 2H), 7.76–7.84 (m, 2H). MS *m/z* 439 $(M + H)^+$. Step 2: **45** in 55% yield as colorless needles (hexane–AcOEt). mp 115–116 °C. ^{1}H NMR ($CDCl_3$) δ 2.23 (s, 3H), 2.61 (dd, *J* = 16.8, 9.2 Hz, 1H), 2.81 (dd, *J* = 16.8, 5.4 Hz, 1H), 3.76–3.86 (m, 1H), 4.29 (dd, *J* = 9.2, 6.0 Hz, 1H), 4.76 (t, *J* = 9.2 Hz, 1H), 5.10 (s, 2H), 6.48–6.52 (m, 2H), 7.05 (d, *J* = 8.1 Hz, 1H), 7.21–7.25 (m, 1H), 7.28–7.34 (m, 2H), 7.37–7.42 (m, 3H), 7.47–7.54 (m, 2H), 7.78 (d, *J* = 8.5 Hz, 1H), 7.83 (d, *J* = 7.7 Hz, 1H). MS *m/z* 425 $(M + H)^+$. HPLC purity (220 nm) 99.6%. Anal. Calcd for $C_{28}H_{24}O_4$: C, 79.22; H, 5.70. Found: C, 79.02; H, 6.01.

(6-{[3-(1-Benzothiophen-3-yl)benzyl]oxy}-2,3-dihydro-1-benzofuran-3-yl)acetic Acid (46). Step 1: Methyl (6-{[3-(1-benzothiophen-3-yl)benzyl]oxy}-2,3-dihydro-1-benzofuran-3-yl)acetate in 72% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 2.56 (dd, *J* = 16.4, 9.2 Hz, 1H), 2.75 (d, *J* = 16.4, 5.4 Hz, 1H,), 3.71 (s, 3H), 3.76–3.86 (m, 1H), 4.27 (dd, *J* = 9.2, 6.0 Hz,

1H), 4.76 (t, *J* = 9.2 Hz, 1H), 5.10 (s, 2H), 6.49–6.53 (m, 2H), 7.04 (d, *J* = 7.9 Hz, 1H), 7.35–7.56 (m, 6H), 7.63 (s, 1H), 7.84–7.94 (m, 2H). MS *m/z* 431 $(M + H)^+$. Step 2: **46** in 69% yield as colorless needles (hexane–AcOEt). mp 126–128 °C. ^{1}H NMR ($CDCl_3$) δ 2.62 (dd, *J* = 16.8, 9.3 Hz, 1H), 2.81 (dd, *J* = 16.8, 5.4 Hz, 1H), 3.77–3.87 (m, 1H), 4.29 (dd, *J* = 9.3, 6.0 Hz, 1H), 4.77 (t, *J* = 9.3 Hz, 1H), 5.11 (s, 2H), 6.49–6.54 (m, 2H), 7.07 (d, *J* = 8.1 Hz, 1H), 7.36–7.57 (m, 6H), 7.64 (s, 1H), 7.85–7.95 (m, 2H). MS *m/z* 417 $(M + H)^+$. HPLC purity (220 nm) 100%. Anal. Calcd for $C_{25}H_{20}O_4S$: C, 72.09; H, 4.84. Found: C, 71.92; H, 4.82.

(6-{[3-(1-Benzothiophen-5-yl)benzyl]oxy}-2,3-dihydro-1-benzofuran-3-yl)acetic Acid (47). Step 1: Methyl (6-{[3-(1-benzothiophen-5-yl)benzyl]oxy}-2,3-dihydro-1-benzofuran-3-yl)acetate in 74% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 2.56 (dd, *J* = 16.4, 9.2 Hz, 1H), 2.75 (dd, *J* = 16.4, 5.7 Hz, 1H), 3.71 (s, 3H), 3.76–3.86 (m, 1H), 4.27 (dd, *J* = 9.2, 6.1 Hz, 1H), 4.76 (t, *J* = 9.2 Hz, 1H), 5.09 (s, 2H), 6.50–6.54 (m, 2H), 7.04 (d, *J* = 8.1 Hz, 1H), 7.38–7.50 (m, 4H), 7.57–7.63 (m, 2H), 7.70 (s, 1H), 7.94 (d, *J* = 8.3 Hz, 1H), 8.03 (d, *J* = 1.7 Hz, 1H). MS *m/z* 431 $(M + H)^+$. Step 2: **47** in 84% yield as colorless plates (hexane–AcOEt). mp 139–140 °C. ^{1}H NMR ($CDCl_3$) δ 2.62 (dd, *J* = 16.8, 9.3 Hz, 1H), 2.82 (dd, *J* = 16.8, 5.4 Hz, 1H), 3.82 (m, 1H), 4.29 (dd, *J* = 9.3, 6.0 Hz, 1H), 4.77 (t, *J* = 9.3 Hz, 1H), 5.10 (s, 2H), 6.50–6.55 (m, 2H), 7.07 (d, *J* = 8.1 Hz, 1H), 7.38–7.50 (m, 4H), 7.58–7.63 (m, 2H), 7.71 (s, 1H), 7.94 (d, *J* = 8.3 Hz, 1H), 8.03 (d, *J* = 1.5 Hz, 1H). MS *m/z* 417 $(M + H)^+$. HPLC purity (220 nm) 99.8%. Anal. Calcd for $C_{25}H_{20}O_4S$: C, 72.09; H, 4.84. Found: C, 71.95; H, 5.01.

{6-[(6-Methoxy-2′,6′-dimethylbiphenyl-3-yl)methoxy]-2,3-dihydro-1-benzofuran-3-yl} acetic Acid (48). Step 1: Methyl {6-[(6-methoxy-2′,6′-dimethylbiphenyl-3-yl)methoxy]-2,3-dihydro-1-benzofuran-3-yl}acetate in 73% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 2.00 (s, 6H), 2.55 (dd, *J* = 16.4, 9.2 Hz, 1H), 2.74 (dd, *J* = 16.4, 5.4 Hz, 1H), 3.71 (s, 3H), 3.74 (s, 3H), 3.77–3.85 (m, 1H), 4.25 (dd, *J* = 9.2, 6.0 Hz, 1H), 4.74 (t, *J* = 9.2 Hz, 1H), 4.97 (s, 2H), 6.45–6.49 (m, 2H), 6.97–7.03 (m, 2H), 7.07–7.18 (m, 4H), 7.39 (dd, *J* = 8.4, 2.2 Hz, 1H). MS *m/z* 433 $(M + H)^+$. Step 2: **48** in 58% yield as colorless prisms (hexane–AcOEt). mp 138–140 °C. ^{1}H NMR ($CDCl_3$) δ 2.00 (s, 6H), 2.61 (dd, *J* = 16.8, 9.2 Hz, 1H), 2.80 (dd, *J* = 16.8, 5.4 Hz, 1H), 3.74 (s, 3H), 3.77–3.85 (m, 1H), 4.28 (dd, *J* = 9.2, 6.0 Hz, 1H), 4.75 (t, *J* = 9.2 Hz, 1H), 4.98 (s, 2H), 6.45–6.50 (m, 2H), 6.97–7.19 (m, 6H), 7.39 (dd, *J* = 8.5, 2.3 Hz, 1H). Anal. Calcd for $C_{26}H_{26}O_5 \cdot 0.25\ H_2O$: C, 73.83; H, 6.31. Found: C, 73.73; H, 6.41.

(6-{[6-(Benzyloxy)-2′,6′-dimethylbiphenyl-3-yl]methoxy}-2,3-dihydro-1-benzofuran-3-yl)acetic Acid (49). Step 1: Methyl (6-{[6-(benzyloxy)-2′,6′-dimethylbiphenyl-3-yl]methoxy}-2,3-dihydro-1-benzofuran-3-yl)acetate in 50% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 2.03 (s, 6H), 2.55 (dd, *J* = 16.5, 9.1 Hz, 1H), 2.74 (dd, *J* = 16.5, 5.4 Hz, 1H), 3.71 (s, 3H), 3.74–3.86 (m, 1H), 4.25 (dd, *J* = 9.1, 6.1 Hz, 1H), 4.74 (t, *J* = 9.1 Hz, 1H), 4.92–5.08 (m, 4H), 6.43–6.50 (m, 2H), 6.96–7.04 (m, 2H), 7.08–7.45 (m, 10H). MS *m/z* 509 $(M + H)^+$.

Step 2: **49** in 76% yield as colorless prisms (heptane–AcOEt). mp 102–104 °C. ^{1}H NMR ($CDCl_3$) δ 2.03 (s, 6H), 2.56–2.67 (m, 1H), 2.76–2.86 (m, 1H,), 3.75–3.86 (m, 1H), 4.28 (dd, *J* = 9.1, 6.0 Hz, 1H), 4.75 (t, *J* = 9.1 Hz, 1H), 4.92–5.09 (m, 4H), 6.43–6.52 (m, 2H), 6.96–7.46 (m, 12H). MS *m/z* 495 $(M + H)^+$. Anal. Calcd for $C_{32}H_{30}O_5$: C, 77.01; H, 6.16. Found: C, 77.10; H, 6.07.

{6-[(2′,4′-Dimethylbiphenyl-3-yl)methoxy]-2,3-dihydro-1-benzofuran-3-yl}acetic Acid (50). Step 1: Methyl {6-[(2′,4′-dimethylbiphenyl-3-yl)methoxy-2,3-dihydro-1-benzofuran-3-yl]acetate in 67% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 2.22 (s, 3H), 2.36 (s, 3H), 2.55 (dd, *J* = 16.5, 9.3 Hz, 1H), 2.75 (dd, *J* = 16.5, 5.4 Hz, 1H), 3.71 (s, 3H), 3.75–3.85 (m, 1H), 4.26 (dd, *J* = 9.3, 6.0 Hz, 1H), 4.75 (t, *J* = 9.3 Hz, 1H), 5.05 (s, 2H), 6.47–6.51 (m, 2H), 7.01–7.14 (m, 4H), 7.25–7.28 (m, 1H), 7.36–7.44 (m, 3H). MS *m/z* 403 $(M + H)^+$. Step 2: **50** in 92% yield as colorless prisms (hexane–AcOEt). mp 167–168 °C. ^{1}H NMR ($CDCl_3$) δ 2.23 (s, 3H), 2.36 (s, 3H), 2.62 (dd, *J* = 16.8, 9.2 Hz, 1H), 2.81 (dd, *J* = 16.8, 5.4 Hz, 1H), 3.76-3.86 (m, 1H), 4.29 (dd, *J* = 9.2, 6.0 Hz, 1H), 4.76 (t, *J* = 9.2 Hz, 1H), 5.06 (s, 2H), 6.47–6.53 (m, 2H), 7.05–7.15 (m, 4H), 7.25–7.28 (m, 1H), 7.36–7.44 (m, 3H). MS *m/z* 389 $(M + H)^+$. HPLC purity (220 nm) 99.8%. Anal. Calcd for $C_{25}H_{24}O_4 \cdot 0.25\ H_2O$: C, 76.41; H, 6.28. Found: C, 76.61; H, 6.41.

{6-[(2′,4′,6′-Trimethylbiphenyl-3-yl)methoxy]-2,3-dihydro-1-benzofuran-3-yl}acetic Acid (51). Step 1: Methyl {6-[(2′,4′,6′-trimethylbiphenyl-3-yl)methoxy]-2,3-dihydro-1-benzofuran-3-yl}acetate in 78% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.98 (s, 6H), 2.32 (s, 3H), 2.55 (dd, *J* = 16.4, 9.2 Hz, 1H), 2.74 (dd, *J* = 16.4, 5.4 Hz, 1H), 3.71 (s, 3H), 3.75–3.85 (m, 1H), 4.26 (dd, *J* = 9.2, 6.0 Hz, 1H), 4.74 (t, *J* = 9.2 Hz, 1H), 5.05 (s, 2H), 6.45–6.50 (m, 2H), 6.93 (s, 2H), 7.01 (d, *J* = 8.1 Hz, 1H), 7.07–7.10 (m, 1H), 7.17 (s, 1H), 7.36–7.45 (m, 2H). MS *m/z* 417 $(M + H)^+$. Step 2: **51** in 75% yield as colorless plates (hexane–AcOEt). mp 158 °C. ^{1}H NMR ($CDCl_3$) δ 1.98 (s, 6H), 2.32 (s, 3H), 2.61 (dd, *J* = 16.8, 9.2 Hz, 1H), 2.80 (dd, *J* = 16.8, 5.4 Hz, 1H), 3.75–3.85 (m, 1H), 4.28 (dd, *J* = 9.2, 6.1 Hz, 1H), 4.76 (t, *J* = 9.2 Hz, 1H), 5.06 (s, 2H), 6.46–6.51 (m, 2H), 6.93 (s, 2H), 7.03–7.10 (m, 2H), 7.18 (s, 1H), 7.36–7.45 (m, 2H). MS *m/z* 403 $(M + H)^+$. HPLC purity (220 nm) 100%. Anal. Calcd for $C_{26}H_{26}O_4$: C, 77.59; H, 6.51. Found: C, 77.51; H, 6.61.

(6-{[4′-(Benzyloxy)-2′,6′-dimethylbiphenyl-3-yl]methoxy}-2,3-dihydro-1-benzofuran-3-yl)acetic Acid (52). Step 1: Methyl (6-{[4′-(benzyloxy)-2′,6′-dimethylbiphenyl-3-yl] methoxy}-2,3-dihydro-1-benzofuran-3-yl)acetate in 93% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.99 (s, 6H), 2.55 (dd, *J* = 16.5, 9.1 Hz, 1H), 2.75 (dd, *J* = 16.5, 5.4 Hz, 1H), 3.71 (s, 3H), 3.75–3.85 (m, 1H), 4.26 (dd, *J* = 9.1, 6.1 Hz, 1H), 4.75 (t, *J* = 9.1 Hz, 1H), 5.05 (s, 2H), 5.07 (s, 2H), 6.45–6.50 (m, 2H), 6.75 (s, 2H), 7.01 (d, *J* = 8.1 Hz, 1H), 7.08 (dt, *J* = 7.0, 1.5 Hz, 1H), 7.17 (s, 1H), 7.30–7.48 (m, 7H). MS *m/z* 509 $(M + H)^+$. Step 2: **52** in 91% yield as colorless prisms (hexane–AcOEt). ^{1}H NMR ($CDCl_3$) δ 1.99 (s, 6H), 2.61 (dd, *J* = 16.8, 9.2 Hz, 1H), 2.81 (dd, *J* = 16.8, 5.4 Hz, 1H), 3.75–3.86 (m, 1H), 4.28 (dd, *J* = 9.2, 6.0

Hz, 1H), 4.76 (t, *J* = 9.2 Hz, 1H), 5.06 (s, 2H), 5.07 (s, 2H), 6.46–6.51 (m, 2H), 6.75 (s, 2H), 7.03–7.10 (m, 2H), 7.17 (s, 1H), 7.30–7.48 (m, 7H). MS *m/z* 495 $(M + H)^+$. HPLC purity (220 nm) 98.8%. Anal. Calcd for $C_{32}H_{30}O_5$: C, 77.71; H, 6.11. Found: C, 77.59; H, 6.28.

(6-{[4′-(2-Ethoxyethoxy)-2′,6′-dimethylbiphenyl-3-yl]methoxy}-2,3-dihydro-1-benzofuran-3-yl)acetic Acid (53). Step 1: Methyl (6-{[4′-(2-ethoxyethoxy)-2′,6′-dimethylbiphenyl-3-yl]methoxy}-2,3-dihydro-1-benzofuran-3-yl)acetate in 89% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.25 (t, *J* = 7.1 Hz, 3H), 1.98 (s, 6H), 2.55 (dd, *J* = 16.5, 9.2 Hz, 1H), 2.75 (dd, *J* = 16.5, 5.4 Hz, 1H), 3.62 (q, *J* = 7.1 Hz, 2H), 3.71 (s, 3H), 3.75–3.85 (m, 3H), 4.14 (t, *J* = 5.1 Hz, 2H), 4.26 (dd, *J* = 9.2, 6.0 Hz, 1H), 4.75 (t, *J* = 9.2 Hz, 1H), 5.05 (s, 2H), 6.45–6.50 (m, 2H), 6.68 (s, 2H), 7.01 (d, *J* = 8.1 Hz, 1H), 7.08 (dt, *J* = 7.0, 1.6 Hz, 1H), 7.16 (s, 1H), 7.35–7.44 (m, 2H). MS *m/z* 491 $(M + H)^+$. Step 2: **53** in 59% yield as colorless prisms (hexane–AcOEt). mp 72 °C. ^{1}H NMR ($CDCl_3$) δ 1.25 (t, *J* = 7.1 Hz, 3H), 1.98 (s, 6H), 2.61 (dd, *J* = 16.8, 9.2 Hz, 1H), 2.80 (dd, *J* = 16.8, 5.4 Hz, 1H), 3.62 (q, *J* = 7.1 Hz, 2H), 3.75–3.85 (m, 3H), 4.14 (t, *J* = 5.0 Hz, 2H), 4.28 (dd, *J* = 9.2, 6.0 Hz, 1H), 4.75 (t, *J* = 9.2 Hz, 1H), 5.05 (s, 2H), 6.45–6.51 (m, 2H), 6.68 (s, 2H), 7.03–7.10 (m, 2H), 7.16 (s, 1H), 7.34–7.44 (m, 2H). MS *m/z* 477 $(M + H)^+$. HPLC purity (220 nm) 100%. Anal. Calcd for $C_{29}H_{32}O_6$: C, 73.09; H, 6.77. Found: C, 73.06; H, 6.73.

Ethyl 6-Methoxy-1-oxo-2,3-dihydro-1*H*-indene-2-carboxylate (55). To a solution of diethyl carbonate (14.8 g, 125 mmol) in toluene (100 mL) was added portionwise NaH (60% in mineral oil, 7.51 g, 188 mmol) at room temperature and the mixture was stirred at 120 °C for 30 min. To the mixture was added a solution of 6-methoxy-1-indanone (**54**) (10.2 g, 62.6 mmol) in toluene (100 mL), and the resulting mixture was stirred at 120 °C for 3 h. After cooling to room temperature, 1 M HCl aqueous solution was added to the mixture, and the mixture was extracted with AcOEt. The extract was washed with water and then brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 5:95–40:60) to give **55** (7.42 g, 51%) as a yellow powder. ^{1}H NMR ($CDCl_3$) δ 1.31 (t, *J* = 7.2 Hz, 3H), 3.25–3.35 (m, 1H), 3.42–3.51 (m, 1H), 3.74 (dd, *J* = 8.0, 3.9 Hz, 1H), 3.83 (s, 3H), 4.25 (q, *J* = 7.2 Hz, 2H), 7.14–7.28 (m, 2H), 7.39 (d, *J* = 8.3 Hz, 1H). MS *m/z* 235 $(M + H)^+$.

Ethyl 5-Hydroxy-2,3-dihydro-1*H*-indene-2-carboxylate (56a). Step 1: To a solution of **55** (7.42 g, 31.7 mmol) in TFA (100 mL) was added triethylsilane (11.1 g, 95.1 mmol) at 0 °C, and the mixture was stirred at 0 °C for 2 h. To the mixture was added triethylsilane (11.1 g, 95.1 mmol), and the mixture was stirred at room temperature for 2 h. The mixture was concentrated, and the residue was poured into saturated $NaHCO_3$ aqueous solution, and extracted with AcOEt. The extract was washed with water and then brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 3:97–40:60) to give ethyl 5-methoxy-2,3-dihydro-1*H*-indene-2-carboxylate (5.81 g) as a colorless oil. Step 2: To an ice-cooled solution of the

obtained oil (5.81 g, 26.4 mmol) in step 1 in dichloromethane (50 mL) were added sequentially $AlCl_3$ (10.5 g, 79.2 mmol) and 1-octanethiol (7.72 g, 52.8 mmol), and the mixture was stirred at 0 °C for 0.5 h, and then at room temperature for 3 h. The mixture was poured into ice water and extracted with AcOEt. The extract was washed with water and then brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 8:92–60:40) to give **56a** (2.08 g, 32% in 2 steps) as colorless crystals. mp 57–58 °C. ^{1}H NMR ($CDCl_3$) δ 1.28 (t, *J* = 7.2 Hz, 3H), 3.06–3.24 (m, 4H), 3.23–3.39 (m, 1H), 4.18 (q, *J* = 7.2 Hz, 2H), 5.02 (s, 1H), 6.63 (dd, *J* = 8.1, 2.5 Hz, 1H), 6.68 (d, *J* = 2.5 Hz, 1H), 7.04 (d, *J* = 8.1 Hz, 1H). MS *m/z* 207 $(M + H)^+$.

Ethyl 6-Methoxy-1-oxo-1,2,3,4-tetrahydronaphthalene-2-carboxylate (58). The title compound was prepared from 6-methoxy-1-tetralone (**57**) by a similar to that described for **55** in 70% yield as a yellow oil. MS *m/z* 271 $(M + Na)^+$.

Ethyl 6-Hydroxy-1,2,3,4-tetrahydronaphthalene-2-carboxylate (56c). The title compound was prepared from **58** by a similar to that described for **56a** in 67% yield as colorless crystals. mp 78–79 °C. ^{1}H NMR ($CDCl_3$) δ 1.28 (t, *J* = 7.2 Hz 3H), 1.74–1.90 (m, 1H), 2.11–2.22 (m, 1H), 2.62–2.75 (m, 1H), 2.76–3.00 (m, 4H), 4.18 (q, *J* = 7.2 Hz, 2H), 4.90 (s, 1H), 6.56 (d, *J* = 2.5 Hz, 1H), 6.61 (dd, *J* = 8.3, 2.5 Hz, 1H), 6.96 (d, *J* = 8.3 Hz, 1H). MS *m/z* 220 $(M + H)^+$. Anal. Calcd for $C_{13}H_{16}O_3$: C, 70.89; H, 7.32. Found: C, 70.87; H, 7.20.

5-(Benzyloxy)-2-benzofuran-1(3*H*)-one (60). Step 1: To a stirred suspension of 5-aminophthalide (**59**) (5.00 g, 33.5 mmol) in 5% H_2SO_4 aqueous solution (50 mL) was added sodium nitrite (2.54 g, 36.9 mmol) in water (2.5 mL) at 0 °C. The mixture was stirred at 0 °C for 15 min, at room temperature for 15 min, and then at reflux for 1 h. The mixture was cooled to room temperature. The resulting crystals were collected by filtration, washed with water and dried to give reddish crystals (4.47 g). Step 2: The obtained crystals in step 1 were suspended in DMF (100 mL), and then K_2CO_3 (4.52 g, 32.7 mmol) and benzyl bromide (3.9 mL, 32.7 mmol) were added. The mixture was stirred at 60 °C for 5 h. The mixture was quenched with saturated NH_4Cl aqueous solution (50 mL) and extracted with AcOEt. The extract was washed sequentially with water and brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 15:85–50:50) to give **60** (1.66 g, 21% in 2 steps) as colorless crystals. mp 119–120 °C. ^{1}H NMR ($CDCl_3$) δ 5.16 (s, 2H), 5.24 (s, 2H), 6.98 (d, *J* = 1.5 Hz, 1H), 7.12 (dd, *J* = 8.6, 1.5 Hz, 1H), 7.32–7.47 (m, 5H), 7.83 (d, *J* = 8.6 Hz, 1H). MS *m/z* 241 $(M + H)^+$.

***tert*-Butyl (5-Hydroxy-1,3-dihydro-2-benzofuran-1-yl)acetate (56e).** Step 1: To a solution of diisopropylamine (1.23 mL, 8.79 mmol) in THF (45 mL) was added dropwise 1.6 M *n*-BuLi in hexane (5.49 mL, 8.79 mmol) under nitrogen atmosphere at −78 °C, and the mixture was stirred at −78 °C for 30 min. To the mixture was added tert-butyl acetate (1.18

mL, 8.79 mmol) at −78 °C, and the mixture was stirred at −78 °C for 30 min. To the mixture was added dropwise a solution of **60** (1.66 g, 6.91 mmol) in THF (95 mL) at −78 °C, and the mixture was stirred at −78 °C for 1.5 h, and then allowed to warm to room temperature for 2 h. The mixture was quenched with saturated NH_4Cl aqueous solution and extracted with AcOEt. The extract was washed sequentially with water and brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 15:85–60:40) to afford *tert*-butyl [5-(benzyloxy)-1-hydroxy-1,3-dihydro-2-benzofuran-1-yl]acetate (1.66 g, crude) as colorless crystals. Step 2: To a solution of the obtained crystals (1.50 g) in step 1 in CH_2Cl_2 (50 mL) were added triethylsilane (4 mL) and TFA (8 mL) at 0 °C, and the mixture was stirred at room temperature for 1 h. The mixture was concentrated and azeotroped with toluene twice. The residue was purified by silica gel column chromatography (AcOEt:hexane = 10:90–50:50) to afford *tert*-butyl [5-(benzyloxy)-1,3-dihydro-2-benzofuran-1-yl]acetate (0.381 g, 16%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 9H), 2.61–2.71 (m, 2H), 4.93–5.19 (m, 4H), 5.56 (t, J = 6.0 Hz, 1H), 6.82 (s, 1H), 6.88 (dd, J = 8.3, 2.3 Hz, 1H), 7.10 (d, J = 8.3 Hz, 1H), 7.29–7.48 (m, 5H). MS *m/z* 363 $(M + Na)^+$. Step 3: A mixture of the obtained oil (0.381 g, 1.12 mmol) in step 2 and 10% Pd/C (75 mg, containing 50% water) in EtOH (11 mL) was stirred under H_2 atmosphere (balloon pressure) at room temperature for 2 h. The mixture was diluted with AcOEt and passed through a pad of Celite, and the filtrate was concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 30:70–90:10) to afford **56e** (0.240 g, 86%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 9H), 2.57–2.72 (m, 2H), 4.89–5.15 (m, 3H), 5.54 (t, J = 6.2 Hz, 1H), 6.58–6.84 (m, 2H), 7.05 (d, J = 8.1 Hz, 1H). MS *m/z* 273 $(M + Na)^+$.

The following compounds **61**–**64** were also prepared from **42i** and appropriate phenols **56a**–**d** by a similar to that described for **14**.

5-{[4′-(2-Ethoxyethoxy)-2′,6′-dimethylbiphenyl-3-yl]methoxy}-2,3-dihydro-1*H*-indene-2-carboxylic Acid (61). Step 1: Ethyl 5-{[4′-(2-ethoxyethoxy)-2′,6′-dimethylbiphenyl-3-yl]methoxy}-2,3-dihydro-1*H*-indene-2-carboxylate in 41% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.22–1.32 (m, 3H), 1.99 (s, 6H), 3.07–3.23 (m, 4H), 3.23–3.40 (m, 1H), 3.62 (q, J = 7.0 Hz, 2H), 3.78–3.83 (m, 2H), 4.08–4.23 (m, 4H), 5.07 (s, 2H), 6.69 (s, 2H), 6.75–6.85 (m, 2H), 7.05–7.11 (m, 2H), 7.17 (br s, 1H), 7.35–7.46 (m, 2H). MS *m/z* 489 $(M + H)^+$. Step 2: **61** in 90% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.23–1.28 (m, 3H), 1.98 (s, 6H), 3.10–3.28 (m, 4H), 3.30–3.45 (m, 1H), 3.62 (q, J = 7.0 Hz, 2H), 3.77–3.84 (m, 2H), 4.07–4.18 (m, 2H), 5.07 (s, 2H), 6.69 (s, 2H), 6.75–6.87 (m, 2H), 7.05–7.12 (m, 2H), 7.17 (s, 1H), 7.34–7.48 (m, 2H). MS *m/z* 461 $(M + H)^+$. HPLC purity (220 nm) 99.9%.

(5-{[4′-(2-Ethoxyethoxy)-2′,6′-dimethylbiphenyl-3-yl]methoxy}-2,3-dihydro-1*H*-indene-2-yl)acetic Acid (62). Step 1: Methyl (5-{[4′-(2-ethoxyethoxy)-2′,6′-dimethyl-biphenyl-3-yl]methoxy}-2,3-dihydro-1*H*-indene-2-yl)acetate in 70% yield as a colorless oil.

^{1}H NMR ($CDCl_3$) δ 1.25 (t, *J* = 7.0 Hz, 3H), 1.98 (s, 6H), 2.48 (d, *J* = 7.3 Hz, 2H), 2.51–2.64 (m, 2H), 2.80–2.95 (m, 1H), 3.01–3.13 (m, 2H), 3.62 (q, *J* = 7.0 Hz, 2H), 3.69 (s, 3H), 3.80 (t, *J* = 5.0 Hz, 2H), 4.14 (t, *J* = 5.0 Hz, 2H), 5.07 (s, 2H), 6.69 (s, 2H), 6.76 (dd, *J* = 8.2, 2.4 Hz, 1H), 6.80–6.83 (m, 1H), 7.04–7.10 (m, 2H), 7.17 (s, 1H), 7.35–7.45 (m, 2H). MS *m/z* 489 $(M + H)^+$. Step 2: **62** in 78% yield as colorless crystals (hexane–AcOEt). mp 83–84 °C. ^{1}H NMR ($CDCl_3$) δ 1.25 (t, *J* = 7.0 Hz, 3H), 1.98 (s, 6H), 2.50–2.68 (m, 4H), 2.81–2.97 (m, 1H), 3.05–3.17 (m, 2H), 3.62 (q, *J* = 7.0 Hz, 2H), 3.77–3.84 (m, 2H), 4.11–4.17 (m, 2H), 5.07 (s, 2H), 6.69 (s, 2H), 6.77 (d, *J* = 8.1 Hz, 1H), 6.82 (s, 1H), 7.07 (d, *J* = 7.2 Hz, 2H), 7.17 (s, 1H), 7.35–7.45 (m, 2H). MS *m/z* 475 $(M + H)^+$. HPLC purity (220 nm) 100%. Anal. Calcd for $C_{30}H_{34}O_5$: C, 75.92; H, 7.22. Found: C, 75.82; H, 7.15.

6-{[4′-(2-Ethoxyethoxy)-2′,6′-dimethylbiphenyl-3-yl]methoxy}-1,2,3,4-tetrahydronaphthalene-2-carboxylic Acid (63). Step 1: Ethyl 6-{[4′-(2-ethoxyethoxy)-2′,6′-dimethylbiphenyl-3-yl]methoxy}-1,2,3,4-tetrahydronaphthalene-2-carboxylate in 85% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.21–1.32 (m, 6H), 1.73–1.90 (m, 1H), 1.99 (s, 6H), 2.10–2.25 (m, 1H), 2.61–2.74 (m, 1H), 2.76–3.02 (m, 4H), 3.62 (q, *J* = 7.0 Hz, 2H), 3.77–3.83 (m, 2H), 4.07–4.23 (m, 4H), 5.06 (s, 2H), 6.69 (br s, 3H), 6.76 (dd, *J* = 8.4, 2.7 Hz, 1H), 7.00 (d, *J* = 8.4 Hz, 1H), 7.05–7.11 (m, 1H), 7.17 (s, 1H), 7.35–7.46 (m, 2H). MS *m/z* 503 $(M + H)^+$. Step 2: **63** in 57% yield as colorless crystals (hexane–AcOEt). mp 118–119 °C. ^{1}H NMR ($CDCl_3$) δ 1.25 (t, *J* = 7.1 Hz, 3H), 1.73–1.96 (m, 1H), 1.98 (s, 6H), 2.16–2.28 (m, 1H), 2.66–3.07 (m, 5H), 3.62 (q, *J* = 7.1 Hz, 2H), 3.77–3.84 (m, 2H), 4.10–4.17 (m, 2H), 5.06 (s, 2H), 6.67–6.71 (m, 3H), 6.77 (dd, *J* = 8.4, 2.5 Hz, 1H), 7.01 (d, *J* = 8.4 Hz, 1H), 7.04–7.10 (m, 1H), 7.17 (s, 1H), 7.35–7.46 (m, 2H). MS *m/z* 475 $(M + H)^+$. HPLC purity (220 nm) 98.3%. Anal. Calcd for $C_{30}H_{34}O_5 \cdot 0.25\ H_2O$: C, 75.21; H, 7.26. Found: C, 75.35; H, 7.05.

(6-{[4′-(2-Ethoxyethoxy)-2′,6′-dimethylbiphenyl-3-yl]methoxy}-1,2,3,4-tetrahydronaphthalen-2-yl)acetic Acid (64). Step 1: Methyl (6-{[4′-(2-ethoxyethoxy)-2′,6′-dimethylbiphenyl-3-yl]methoxy}-1,2,3,4-tetrahydronaphthalen-2-yl)acetate in 57% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.21–1.30 (m, 3H), 1.36–1.53 (m, 1H), 1.87–2.03 (m, 7H), 2.16–2.33 (m, 1H), 2.33–2.49 (m, 3H), 2.74–2.88 (m, 3H), 3.62 (q, *J* = 7.0 Hz, 2H), 3.70 (s, 3H), 3.77–3.83 (m, 2H), 4.08–4.17 (m, 2H), 5.06 (s, 2H), 6.69 (s, 3H), 6.71–6.77 (m, 1H), 6.95 (d, *J* = 8.5 Hz, 1H), 7.05–7.10 (m, 1H), 7.17 (s, 1H), 7.34–7.46 (m, 2H). MS *m/z* 525 $(M + Na)^+$. Step 2: **64** in 91% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.20–1.31 (m, 3H), 1.39–1.57 (m, 1H), 1.92–2.03 (m, 7H), 2.18–2.35 (m, 1H), 2.37–2.52 (m, 3H), 2.75–2.93 (m, 3H), 3.62 (q, *J* = 7.1 Hz, 2H), 3.78–3.83 (m, 2H), 4.08–4.18 (m, 2H), 5.06 (s, 2H), 6.69 (s, 3H), 6.71–6.79 (m, 1H), 6.96 (d, *J* = 8.3 Hz, 1H), 7.03–7.12 (m, 1H), 7.17 (s, 1H), 7.32–7.48 (m, 2H). MS *m/z* 489 $(M + H)^+$. HPLC purity (220 nm) 99.7%.

(5-{[4′-(2-Ethoxyethoxy)-2′,6′-dimethylbiphenyl-3-yl]methoxy}-1,3-dihydro-2-benzofuran-1-yl)acetic Acid (65). Step 1: *tert*-Butyl (5-{[4′-(2-ethoxyethoxy)-2′,6′-dimethylbiphenyl-3-yl]methoxy}-1,3-dihydro-2-benzofuran-1-yl)acetate was prepared from **42i** and **56e**

by a similar to that described for **14**-step 1 in 75% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.26 (t, *J* = 7.0 Hz, 3H), 1.45 (s, 9H), 1.98 (s, 6H), 2.65 (dd, *J* = 5.7, 1.9 Hz, 2H), 3.62 (q, *J* = 7.0 Hz, 2H), 3.80 (t, *J* = 5.1 Hz, 2H), 4.12 (t, *J* = 5.1 Hz, 2H), 4.94–5.14 (m, 4H), 5.55 (t, *J* = 5.7 Hz, 1H), 6.69 (s, 2H), 6.77–6.93 (m, 2H), 7.08 (d, *J* = 8.5 Hz, 2H), 7.17 (s, 1H), 7.33–7.49 (m, 2H). MS *m/z* 555 $(M + Na)^+$. Step 2: To a solution of the obtained oil (0.380 g, 0.713 mmol) in step 1 in toluene (10 mL) was added dropwise TFA (5 mL) at 0 °C, and the mixture was stirred at 0 °C for 0.5 h, and allowed to warm to room temperature for 5 h. The mixture was concentrated and azeotroped with toluene (50 mL) twice. The resultant residue was purified by silica gel column chromatography (AcOEt:hexane = 30:70–90:10) to afford crystals, which was washed with hexane–AcOEt to afford **65** (0.246 g, 72%) as colorless crystals. mp 73–74 °C. ^{1}H NMR ($CDCl_3$) δ 1.26 (t, *J* = 7.0 Hz, 3H), 1.98 (s, 6H), 2.74 (dd, *J* = 16.0, 4.2 Hz, 1H), 2.85 (dd, *J* = 16.0, 4.2 Hz, 1H), 3.62 (q, *J* = 7.0 Hz, 2H), 3.80 (t, *J* = 5.1 Hz, 2H), 4.12 (t, *J* = 5.1 Hz, 2H), 4.97–5.21 (m, 4H), 5.53–5.65 (m, 1H), 6.69 (s, 2H), 6.82 (d, *J* = 1.9 Hz, 1H), 6.86–6.96 (m, 1H), 7.05–7.21 (m, 3H), 7.34–7.48 (m, 2H). MS *m/z* 499 $(M + Na)^+$. HPLC purity (220 nm) 100%. Anal. Calcd for $C_{29}H_{32}O_6$: C, 73.09; H, 6.77. Found: C, 72.74; H, 6.72.

Ethyl (5-Hydroxy-2-oxo-2,3-dihydro-1*H*-indol-1-yl)acetate (67). Step 1: To a solution of 5-methoxyisatin (**66**) (10.9 g, 61.5 mmol) in DMF (60 mL) was added portionwise NaH (60% in mineral oil, 2.95 g, 73.8 mmol) at 0 °C, and the mixture was stirred at 0 °C for 1 h. To the mixture was added ethyl bromoacetate (8.87 mL, 80.0 mmol) at 0 °C, and the mixture was stirred at room temperature for 2 h. The mixture was diluted with water, and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 30:70–40:60), and washed with hexane–AcOEt to give ethyl (5-methoxy-2,3-dioxo-2,3-dihydro-1*H*-indol-1-yl)acetate (12.6 g, 78%) as vermilion crystals. mp 85–86 °C. ^{1}H NMR ($CDCl_3$) δ 1.28 (t, *J* = 7.2 Hz, 3H), 3.81 (s, 3H), 4.24 (q, *J* = 7.2 Hz, 2H), 4.46 (s, 2H), 6.71 (d, *J* = 8.5 Hz, 1H), 7.15 (dd, *J* = 8.5, 2.8 Hz, 1H), 7.19 (d, *J* = 2.8 Hz, 1H). MS *m/z* 264 $(M + H)^+$. HPLC purity (220 nm) >96%. Anal. Calcd for $C_{13}H_{13}NO_5$: C, 59.31; H, 4.98; N, 5.32. Found: C, 59.16; H, 5.01; N, 5.42. Step 2: The obtained crystals (2.63 g, 10.0 mmol) in step 1 was hydrogenated on 10% Pd/C (1.25 g, containing 50% water) in 70% perchloric acid (2 mL) and AcOH (100 mL) under H_2 atmosphere (balloon pressure) at 50 °C for 20 h. The catalyst was removed by filtration, and the filtrate was concentrated. The residue was esterified with $SOCl_2$ (1.45 mL, 20.0 mmol) in EtOH (50 mL) at room temperature for 20 h. The mixture was concentrated, and the residue was diluted with AcOEt, washed with saturated $NaHCO_3$ aqueous solution and then brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 20:80–60:40) to give ethyl (5-methoxy-2-oxo-2,3-dihydro-1*H*-indol-1-yl)acetate (0.309 g) as slightly purple needles. The second crop (0.589

g) was similarly obtained. Total 0.898 g (36%). mp 95–96 °C. ^{1}H NMR ($CDCl_3$) δ 1.27 (t, *J* = 7.2 Hz, 3H), 3.58 (s, 2H), 3.78 (s, 3H), 4.22 (q, *J* = 7.2 Hz, 2H), 4.44 (s, 2H), 6.61 (d, *J* = 8.5 Hz, 1H), 6.78 (dd, *J* = 8.5, 2.4 Hz, 1H), 6.90 (d, *J* = 2.4 Hz, 1H). MS *m/z* 250 $(M + H)^+$. HPLC purity (220 nm) >98%. Anal. Calcd for $C_{13}H_{15}NO_4$: C, 62.64; H, 6.07; N, 5.62. Found: C, 62.74; H, 6.04; N, 5.54. Step 3: To a solution of the obtained needles (0.838 g, 3.36 mmol) in step 2 in CH_2Cl_2 (20 mL) was added portionwise $AlCl_3$ (2.24 g, 16.8 mmol) at 0 °C, and then 1-octanethiol (2.92 mL) was added. The mixture was stirred under nitrogen atmosphere at 0 °C for 3.5 h. The mixture was concentrated, and the residue was quenched with 1 M HCl aqueous solution, and extracted with AcOEt–THF. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated to give crystals. Recrystallization from hexane–AcOEt gave **67** (0.637 g, 81%) as colorless prisms. mp 185 °C (decomp.). ^{1}H NMR ($CDCl_3$) δ 1.28 (t, *J* = 7.2 Hz, 3H), 3.55 (s, 2H), 4.23 (q, *J* = 7.2 Hz, 2H), 4.44 (s, 2H), 5.05 (br s, 1H), 6.54 (d, *J* = 8.4 Hz, 1H), 6.70 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.77 (d, *J* = 2.2 Hz, 1H). MS *m/z* 236 $(M + H)^+$. HPLC purity (220 nm) >99%. Anal. Calcd for $C_{12}H_{13}NO_4 \cdot 0.2\ H_2O$: C, 60.35; H, 5.65; N, 5.86. Found: C, 60.42; H, 5.51; N, 5.86.

(5-{[4′-(2-Ethoxyethoxy)-2′,6′-dimethylbiphenyl-3-yl]methoxy}-2-oxo-2,3-dihydro-1*H*-indol-1-yl)acetic Acid (68). Step 1: Ethyl (5-{[4′-(2-ethoxyethoxy)-2′,6′-dimethylbiphenyl-3-yl]methoxy}-2-oxo-2,3-dihydro-1H-indol-1-yl)acetate was prepared from **67** and **42i** by a similar to that described for **14**-step 1 in 22% yield as a brown oil. ^{1}H NMR ($CDCl_3$) d: 1.23–1.30 (m, 6H), 1.98 (s, 6H), 3.60–3.70 (m, 4H), 3.80–3.86 (m, 2H), 4.14 (t, *J* = 4.9 Hz, 2H), 4.22 (q, *J* = 7.1 Hz, 2H), 4.45 (s, 2H), 5.07 (s, 2H), 6.62 (d, *J* = 8.5 Hz, 1H), 6.68 (s, 2H), 6.86 (dd, *J* = 8.5, 2.4 Hz, 1H), 6.97 (d, *J* = 2.4 Hz, 1H), 7.09 (d, *J* = 7.3 Hz, 1H), 7.17 (s, 1H), 7.35–7.40 (m, 1H), 7.43 (t, *J* = 7.3 Hz, 1H). MS *m/z* 518 $(M + H)^+$. HPLC purity (220 nm) >99%. Step 2: A mixture of the obtained oil (1.18 g, 2.28 mmol) in step 1 and 60% perchloric acid (0.500 mL, 4.97 mmol) in AcOH (15 mL) was stirred at 50 °C for 6 h. The mixture was concentrated, diluted with water, and extracted with AcOEt. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 30:70–80:20) to give **68** (0.200 g, 18%) as a brown oil. ^{1}H NMR ($CDCl_3$) δ 1.25 (t, *J* = 7.0 Hz, 3H), 1.97 (s, 6H), 3.58 (s, 2H), 3.63 (q, *J* = 7.0 Hz, 2H), 3.81 (t, *J* = 4.9 Hz, 2H), 4.14 (t, *J* = 4.9 Hz, 2H), 4.49 (s, 2H), 5.07 (s, 2H), 6.63 (d, *J* = 8.6 Hz, 1H), 6.68 (s, 2H), 6.86 (dd, *J* = 8.6, 2.4 Hz, 1H), 6.95 (d, *J* = 2.1 Hz, 1H), 7.08 (d, *J* = 7.4 Hz, 1H), 7.15 (s, 1H), 7.34–7.39 (m, 1H), 7.42 (t, *J* = 7.4 Hz, 1H). MS *m/z* 490 $(M + H)^+$. HPLC purity (220 nm) >99%.

***N*-[4-(Choromethyl)-2-oxo-2*H*-chromen-7-yl]-2-nitrobenzenesulfonamide (70).** Step 1: To a mixture of 3-aminophenol (5.46 g, 50.0 mmol) and pyridine (75 mL) was added portionwise 2-nitrobenzenesulfonyl chloride (11.6 g, 52.5 mmol) at room temperature, and the mixture was stirred under nitrogen atmosphere at room temperature for 72 h. After

evaporation of the solvent, the residue was diluted with AcOEt, washed with saturated $NaHCO_3$ aqueous solution and then brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 20:80–60:40) to give *N*-(3-hydroxyphenyl)-2-nitrobenzenesulfonamide (11.3 g, 77%) as a brown oil. ^{1}H NMR ($CDCl_3$) δ 5.48 (br s, 1H), 6.62–6.68 (m, 1H), 6.71 (ddd, *J* = 8.1, 2.2, 0.7 Hz, 1H), 6.79 (t, *J* = 2.2 Hz, 1H), 7.11 (t, *J* = 8.1 Hz, 1H), 7.16–7.31 (br s, 1H), 7.60 (td, *J* = 7.8, 1.3 Hz, 1H), 7.70 (td, *J* = 7.8, 1.3 Hz, 1H), 7.87 (td, *J* = 7.8, 1.3 Hz, 2H). MS *m/z* 295 $(M + H)^+$. Step 2: Compound **70** was prepared from the obtained oil in step 1 by a similar to that described for **9** in 51% yield as a beige powder. ^{1}H NMR (DMSO-d_6) δ 4.94 (s, 2H), 6.56 (s, 1H), 7.10 (d, *J* = 2.1 Hz, 1H), 7.15 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.76 (d, *J* = 8.7 Hz, 1H), 7.83–7.93 (m, 2H), 8.01–8.05 (m, 1H), 8.09–8.13 (m, 1H), 11.49 (s, 1H). MS *m/z* 282 $(M + H)^+$.

Methyl (6-{[(2-Nitrophenyl)sulfonyl]amino}-1-benzofuran-3-yl)acetate (71). Step 1: A mixture of **70** (14.3 g, 36.2 mmol) and 1 M NaOH aqueous solution (120 mL) was stirred at room temperature for 24 h. The mixture was acidified with 1 M HCl aqueous solution and extracted with AcOEt–THF. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated to give a solid (13.6 g, quantitative). Step 2: To a suspension of the obtained solid (2.84 g, 7.55 mmol) in MeOH (8 mL) was added dropwise $SOCl_2$ (2 mL, 27.4 mmol) at 0 °C, and the mixture was stirred at room temperature for 2.5 h. The mixture was concentrated, and the residue was diluted with water and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 20:80–60:40) to give crystals. Recrystallization from hexane–AcOEt gave **71** (1.82 g, 62%) as yellow prisms. ^{1}H NMR ($CDCl_3$) δ 3.66 (d, *J* = 0.9 Hz, 2H), 3.72 (s, 3H), 7.05 (dd, *J* = 8.4, 1.8 Hz, 1H), 7.35 (s, 1H), 7.39–7.45 (m, 2H), 7.55 (td, *J* = 7.7, 1.5 Hz, 1H), 7.61 (t, *J* = 0.9 Hz, 1H), 7.68 (td, *J* = 7.7, 1.5 Hz, 1H), 7.81 (dd, *J* = 7.7, 1.5 Hz, 1H), 7.87 (dd, *J* = 7.7, 1.5 Hz, 1H). MS *m/z* 391 $(M + H)^+$.

Methyl [6-({[4′-(2-Ethoxyethoxy)-2′,6′-dimethylbiphenyl-3-yl]methyl}amino)-1-benzofuran-3-yl]acetate (72). Step 1: To a mixture of **71** (0.781 g, 2.00 mmol), **42i** (0.661 g, 2.00 mmol) and PPh_3 (1.05 g, 4.00 mmol) in toluene (40 mL) was added DEAD (40% toluene solution, 1.81 mL, 4.00 mmol), and the mixture was stirred at room temperature for 20 h. After evaporation of the solvent, the residue was purified by silica gel column chromatography (AcOEt:hexane = 20:80–60:40) to give methyl (6-{{[4′-(2-ethoxyethoxy)-2′,6′-dimethylbiphenyl-3-yl]methyl}[(2-nitrophenyl)sulfonyl]amino}-1-benzofuran-3-yl)acetate as a brown oil. MS m/z 673 $(M + H)^+$. Step 2: To a solution of the obtained oil in step 1 and mercaptoacetic acid (0.278 mL, 4.00 mmol) in DMF (2 mL) was added lithium hydroxide hydrate (0.336 g, 8.00 mmol), and the mixture was stirred at room temperature for 22 h. The mixture was diluted with AcOEt, washed sequentially with saturated $NaHCO_3$

aqueous solution and brine, dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 5:95–30:70) to give **72** (0.743 g, 76% in 2 steps) as a brown oil. ^{1}H NMR ($CDCl_3$) δ 1.25 (t, *J* = 7.1 Hz, 3H), 1.97 (s, 6H), 3.57–3.66 (m, 4H), 3.71 (s, 3H), 3.77–3.82 (m, 2H), 4.10–4.15 (m, 2H), 4.20 (s, 1H), 4.40 (s, 2H), 6.62 (dd, *J* = 8.5, 2.1 Hz, 1H), 6.67 (s, 2H), 6.69 (d, *J* = 1.9 Hz, 1H), 7.04 (dt, *J* = 7.0, 1.6 Hz, 1H), 7.14 (s, 1H), 7.27–7.42 (m, 4H). MS *m/z* 488 $(M + H)^+$.

[6-({[4′-(2-Ethoxyethoxy)-2′,6′-dimethylbiphenyl-3-yl]methyl}amino)-2,3-dihydro-1-benzofuran-3-yl]acetic Acid Hydrochloride (73). Step 1: Compound **72** (0.494 g, 1.01 mmol) was hydrogenated on 10% Pd/C (0.25 g, containing 50% water) in MeOH (5 mL) and THF (2 mL) under H_2 atmosphere (balloon pressure) at room temperature for 16 h. The catalyst was removed by filtration, and the filtrate was concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 5:95–30:70) to give methyl [6-({[4′-(2-ethoxyethoxy)-2′,6′-dimethylbiphenyl-3-yl]methyl}amino)-2,3-dihydro-1-benzofuran-3-yl]acetate (0.325 g, 66%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.25 (t, *J* = 7.0 Hz, 3H), 1.97 (s, 6H), 2.47–2.57 (m, 1H), 2.67–2.77 (m, 1H), 3.61 (q, *J* = 7.0 Hz, 2H), 3.69–3.82 (m, 6H), 4.06–4.17 (m, 3H), 4.21 (dd, *J* = 9.0, 5.9 Hz, 1H), 4.32 (s, 2H), 4.69 (t, *J* = 9.0 Hz, 1H), 6.09–6.21 (m, 2H), 6.67 (s, 2H), 6.91 (d, *J* = 7.9 Hz, 1H), 7.03 (dt, *J* = 7.4, 1.4 Hz, 1H), 7.10 (s, 1H), 7.28–7.33 (m, 1H), 7.37 (t, *J* = 7.4 Hz, 1H). MS *m/z* 490 $(M + H)^+$. Step 2: To a solution of the obtained oil (0.325 g, 0.664 mmol) in step 1 in MeOH (3 mL) and THF (3 mL) was added 2 M NaOH aqueous solution (1 mL), and the mixture was stirred at room temperature for 70 h. The mixture was acidified with diluted citric acid aqueous solution and extracted with AcOEt. The extract was washed with brine, dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 30:70–100:0) to give a pale yellow oil (0.320 g). This oil was dissolved in AcOEt (1.5 mL) and treated with 4 M HCl in AcOEt (0.5 mL) and the mixture was diluted with Et_2O to give **73** (0.288 g, 85%) as colorless crystals. mp 140 °C. ^{1}H NMR ($CDCl_3$) δ 1.25 (t, *J* = 7.1 Hz, 3H), 1.81 (s, 6H), 2.44–2.55 (m, 1H), 2.59–2.69 (m, 1H), 3.58–3.75 (m, 3H), 3.79 (t, *J* = 6.1 Hz, 2H), 4.10 (t, *J* = 6.1 Hz, 2H), 4.23 (dd, *J* = 9.2, 6.5 Hz, 1H), 4.46 (s, 2H), 4.64 (t, *J* = 9.2 Hz, 1H), 6.61 (s, 2H), 6.68 (d, *J* = 1.3 Hz, 1H), 6.75–6.83 (m, 2H), 7.00 (d, *J* = 7.9 Hz, 1H), 7.07 (d, *J* = 7.7 Hz, 1H), 7.39 (t, *J* = 7.7 Hz, 1H), 7.60 (d, *J* = 7.0 Hz, 1H), 11.70 (br s, 1H). MS *m/z* 476 (free form, $M + H)^+$. HPLC purity (220 nm) 99.8%. Anal. Calcd for $C_{29}H_{33}NO_5 \cdot HCl$: C, 68.02; H, 6.69; N, 2.74. Found: C, 67.77; H, 6.72; N, 2.50.

Ca Influx Activity of CHO Cells Expressing Human GPR40 (FLIPR Assay). CHO dhfr cells stably expressing human GPR40 (accession no. NM_005303) were plated and incubated overnight in 5% CO_2 at 37 °C. Then, cells were incubated in loading buffer (recording medium containing 2.5 μg/mL fluorescent calcium indicator Fluo 4-AM (Molecular Devices), 2.5 mmol/L probenecid (Dojindo) and 0.1% fatty acid-free BSA (Sigma)) for 60 min at 37 ºC. Various concentrations of test compounds or γ-linolenic acid

(Sigma) were added into the cells and increase of the intracellular Ca^{2+} concentration after addition were monitored by FLIPR Tetra system (Molecular Devices) for 90 seconds. The agonistic activities of test compounds and γ-linolenic acid on human GPR40 were expressed as [(A-B)/(C-B)] X 100 (increase of the intracellular Ca^{2+} concentration (A) in test compounds-treated cells, (B) in vehicle-treated cells and (C) in 10 μM γ-linolenic acid-treated cells). EC_{50} value of each compound was obtained with Prism 5 software (GraphPad).

Preparation of CHO Membrane for GPR40 Receptor Binding Assay. Cell lines stably expressing human GPR40 and rat GPR40 were used for the experiments. Each cell was cultured in Minimum Essential Medium Alpha (MEM-Alpha, Invitrogen) supplemented with 10% dialyzed Fetal-Bovine-Serum (dialyzed FBS, Thermo Trace Ltd.), 100 unit/mL penicillin and 100 unit/mL streptomycin in 5% CO_2/95% air atmosphere at 37 °C. Cells were harvested at confluence in Dulbecco's Phosphate-Buffered-Saline (D-PBS, Invitrogen) containing 1 mM EDTA and centrifuged. Cells were homogenized in ice-cold membrane preparation buffer (50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.5 mM PMSF (Wako), 20 μg/mL leupeptin, 0.1 μg/mL pepstatin A, 100 μg/mL Phosphoramidon, Peptide Institute, Inc.) and centrifuged (700 x g, 10 min, 4 °C). The supernatant was filtered through 40 μm Cell Strainer (BD Falcon) and ultracentrifuged (100,000 x g, 1 h, 4 °C) with Optima™ L-100 XP Ultracentrifuge (Beckman Coulter). The precipitation was suspended in the same buffer, and the protein concentration was determined with the BCA Protein assay reagent (Pierce) following membrane solubilization with 0.1% SDS and 0.1 M NaOH aqueous solution. The membrane suspension was stored at −80 °C until receptor binding assay.

GPR40 Receptor Binding Assay. The frozen cell membranes were resuspended in ice-cold assay buffer (25 mmol/L Tris-HCl (pH7.5), 5 mmol/L EDTA, 0.5 mmol/L PMSF, 20 μg/mL leupeptin, 0.1 μg/mL pepstatin A, 0.05% CHAPS (Wako), 0.2% fatty-acid-free BSA (Sigma)), and used for receptor binding assay. To determine the *Kd* values of 3-[4-({2',6'-dimethyl-6-[(4-[^{3}H])phenylmethoxy]biphenyl-3-yl}methoxy)phenyl] propanoic acid (Amersham Biosciences) for human and rat GPR40, binding assays were performed in the presence of various concentrations of the labeled ligand. After incubation at room temperature for 90 min, the membranes were harvested GF/C filter plates (MILLIPORE), and washed with ice-cold 50 mmol/L Tris-HCl (pH7.5) using FilterMate Harvester (PerkinElmer). The membrane-associated radioactivities were counted using TopCount liquid scintillation counter (PerkinElmer). Non-specific binding was defined as binding in the presence of 10 μmol/L of the unlabeled ligand. To determine the binding affinities of test compounds to human and rat GPR40, binding assays were performed in the presence of both various concentrations of test compounds and 2 nmol/L or 6 nmol/L of the labeled ligand. The 50% inhibitory concentrations (IC_{50} values) of test compounds for the labeled ligand were calculated using non-linear regression analysis in GraphPad Prism 3.0 (GraphPad Software). *Ki* values were converted as $Ki = IC_{50} / \{1 + (\text{the concentration of the labeled ligand}) / Kd\}$.

Homology Modeling and Ligand Docking. A homology model of GPR40 was constructed using the crystal structure of bovine rhodopsin (PDB code 1GZM),[74] which obtained from the RCSB Protein Data Bank, as a structural template. An alignment of the amino acid sequences between human/rat GPR40 and rhodopsin was created using Clustal X (version 2.0.11)[75] and manually revised. Procedures of homology modeling were performed in MOE (version 2008.10).[76] The CL2 loop on the extra cellular domain was excluded except Cys170 forming disulfide bond due to the difficulty of estimation. In the previous step, compound **18** was docked into the obtained receptor model using the program GOLD (version 4.1).[77] Then, the resultant docking modes with receptor models, replacing compound **18** with **15** or **19**, were subjected energy minimization with MOE after connecting each residual substituent. In the energy minimization process, the MMFF94s force field was used and the dielectric constant was set to 2*r, where r is the distance between two interacting atoms.

Pharmacokinetic Analysis in Rat Cassette Dosing. Test compounds were administered as a cassette dosing to non-fasted rats. After oral and intravenous administration, blood samples were collected. The blood samples were centrifuged to obtain the plasma fraction. The plasma samples were deproteinized with MeCN containing an internal standard. After centrifugation, the supernatant was diluted and centrifuged again. The compound concentrations in the supernatant were measured by LC/MS/MS.

Oral Glucose Tolerance Test (OGTT). The care and use of the animals and the experimental protocols used in this research were approved by the Experimental Animal Care and Use Committee of Takeda Pharmaceutical Company Limited. Female Wistar fatty (WF) rats were obtained from Takeda Rabics, Ltd (Hikari, Japan). They were fed a commercial diet CE-2 (Clea Japan Co.) and tap water ad libitum. Female WF rats (17–19 weeks old) were fasted overnight and orally given vehicle (0.5% methylcellulose) or compounds. All animals were received an oral glucose load (1 g/kg) one or four hours after drug administration. Blood samples were collected from tail vein before drug administration (pre), and just before glucose load (time 0), and 10, 30, 60 and 120 minutes after glucose load. Plasma glucose and plasma insulin levels were measured by Autoanalyzer 7080 (Hitachi, Japan) and radioimmunoassay (Millipore, USA), respectively. In the dose-dependent study, statistical significances versus vehicle control were assessed by one-tailed Williams test. Differences between two groups were analyzed by Aspin-Welch test.

Methyl [(3*S*)-6-Hydroxy-2,3-dihydro-1-benzofuran-3-yl]acetate (7g) and Methyl [(3*R*)-6-Hydroxy-2,3-dihydro-1-benzofuran-3-yl]acetate (7h). Methyl (6-hydroxy-2,3-dihydro-1-benzofuran-3-yl)acetate (**7e**) was optically resolved using normal phase preparative HPLC [column: CHIRALPAK AD, 50 mmID × 500 mmL; mobile phase: hexane/EtOH (88/12) (v/v) by isocratic elution; flow rate: 60 mL/min; detection: UV 220 nm; temperature: 30 ˚C]. Retention times of the two enantiomers were 16.3 and 18.7 min. The (*S*)-isomer **7g** (retention time 16.3 min) was thus obtained with a 99.7% ee [column: CHIRALPAK AD-H, 4.6 mmID × 250 mmL; mobile phase: hexane/IPA (80/20) (v/v) by isocratic elution; flow rate: 0.5 mL/min; detection: UV 220 nm; temperature: 30 ˚C]. $[\alpha]_D$ +5.4° (c 0.92, $CHCl_3$). The (*R*)-isomer **7h** (retention time 18.7 min) was thus obtained with a 99.1% ee [column: CHIRALPAK AD-H, 4.6 mmID × 250 mmL; mobile phase: hexane/IPA (80/20) (v/v) by isocratic elution; flow rate: 0.5 mL/min; detection: UV 220 nm; temperature: 30 ˚C]. $[\alpha]_D$ −6.4° (c 0.94, $CHCl_3$).

[(3*S*)-6-{[4′-(2-Ethoxyethoxy)-2′,6′-dimethylbiphenyl-3-yl]methoxy}-2,3-dihydro-1-benzofuran-3-yl]acetic Acid (75). Step 1: To a mixture of **7g** (0.250 g, 1.20 mmol), **74a** (0.360 g, 1.20 mmol), and P(*n*-Bu)$_3$ (0.388 g, 1.92 mmol) in toluene (20 mL) was added gradually ADDP (0.484 g, 1.92 mmol), and the mixture was stirred under nitrogen atmosphere at room temperature for 20 h. Hexane (10 mL) was added and the insoluble material was removed by filtration. The filtrate was concentrated, and the residue was purified by silica gel column chromatography (AcOEt:hexane = 5:95–40:60) to give methyl [(3*S*)-(6-{[4′-(2-ethoxyethoxy)-2′,6′-dimethylbiphenyl-3-yl]methoxy}-2,3-dihydro-1-benzofuran-3-yl)acetate (0.456 g, 77%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.25 (t, *J* = 7.0 Hz, 3H), 1.98 (s, 6H), 2.50–2.61 (m, 1H), 2.70–2.79 (m, 1H), 3.62 (q, *J* = 7.0 Hz, 2H), 3.71 (s, 3H), 3.75–3.86 (m, 3H), 4.11–4.16 (m, 2H), 4.26 (dd, *J* = 9.1, 6.1 Hz, 1H), 4.75 (t, *J* = 9.1 Hz, 1H), 5.05 (s, 2H), 6.44–6.51 (m, 2H), 6.69 (s, 2H), 7.01 (d, *J* = 8.1 Hz, 1H), 7.08 (dt, *J* = 7.1, 1.5 Hz, 1H), 7.16 (s, 1H), 7.34–7.45 (m, 2H). MS *m/z* 491 $(M + H)^+$. HPLC purity (220 nm) 100.0%. Step 2: To a solution of the obtained ester (0.451 g, 0.919 mmol) in MeOH (2 mL) and THF (4 mL) was added 2 M NaOH aqueous solution (0.750 mL, 1.50 mmol) at room temperature, and the mixture was stirred at 50 °C for 2 h. The mixture was diluted with water, acidified with 10% citric acid aqueous solution, and extracted with AcOEt. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 30:70–80:20) to give an oil, which was treated with hexane to give crystals. Recrystallization from hexane–AcOEt gave **75** (0.315 g, 72%) as colorless crystals. mp 58–59 °C. $[\alpha]_D$ +6.5° (c 0.30, CH_3CN). 99.7% ee [column: CHIRALPAK AD, 4.6 mmID × 250 mmL; mobile

phase: hexane/IPA/TFA (85/15/0.1) (v/v/v) by isocratic elution; flow rate: 0.5 mL/min; detection: UV 220 nm; temperature: room temperature]. ^{1}H NMR ($CDCl_3$) δ 1.25 (t, *J* = 7.0 Hz, 3H), 1.98 (s, 6H), 2.55–2.66 (m, 1H), 2.76–2.85 (m, 1H), 3.62 (q, *J* = 7.0 Hz, 2H), 3.75–3.86 (m, 3H), 4.14 (t, *J* = 5.0 Hz, 2H), 4.28 (dd, *J* = 9.1, 6.0 Hz, 1H), 4.75 (t, *J* = 9.1 Hz, 1H), 5.05 (s, 2H), 6.44–6.52 (m, 2H), 6.68 (s, 2H), 7.02–7.10 (m, 2H), 7.16 (s, 1H), 7.34–7.45 (m, 2H). MS *m/z* 477 $(M + H)^+$. HPLC purity (220 nm) 100.0%. Anal. Calcd for $C_{29}H_{32}O_6$: C, 73.09; H, 6.77. Found: C, 73.02; H, 6.73.

The following compounds **76–82** were also prepared from appropriate phenols **7e**, **g** or **h** and alcohols **74a–c** by a method similar to that described for **75**.

[(3*R*)-6-{[4′-(2-Ethoxyethoxy)-2′,6′-dimethylbiphenyl-3-yl]methoxy}-2,3-dihydro-1-benzofuran-3-yl]acetic Acid (76). Step 1: Methyl [(3*R*)-6-{[4′-(2-ethoxyethoxy)-2′,6′-dimethylbiphenyl-3-yl]methoxy}-2,3-dihydro-1-benzofuran-3-yl]acetate in 89% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.25 (t, *J* = 7.1 Hz, 3H), 1.98 (s, 6H), 2.50–2.61 (m, 1H), 2.69–2.79 (m, 1H), 3.62 (q, *J* = 7.1 Hz, 2H), 3.71 (s, 3H), 3.74–3.85 (m, 3H), 4.11–4.16 (m, 2H), 4.26 (dd, *J* = 9.1, 6.1 Hz, 1H), 4.75 (t, *J* = 9.1 Hz, 1H), 5.05 (s, 2H), 6.44–6.51 (m, 2H), 6.68 (s, 2H), 7.01 (d, *J* = 8.1 Hz, 1H), 7.08 (dt, *J* = 7.2, 1.6 Hz, 1H), 7.16 (s, 1H), 7.34–7.45 (m, 2H). MS *m/z* 491 $(M + H)^+$. HPLC purity (220 nm) 100.0%. Step 2: **76** in 80% yield as colorless crystals (hexane–AcOEt). mp 59–60 °C. $[\alpha]_D$ −7.5° (c 0.31, CH_3CN). 99.8% ee [column: CHIRALPAK AD, 4.6 mmID × 250 mmL; mobile phase: hexane/IPA/TFA (85/15/0.1) (v/v/v) by isocratic elution; flow rate: 0.5 mL/min; detection: UV 220 nm; temperature: room temperature]. ^{1}H NMR ($CDCl_3$) δ 1.25 (t, *J* = 7.0 Hz, 3H), 1.98 (s, 6H), 2.55–2.67 (m, 1H), 2.76–2.85 (m, 1H), 3.62 (q, *J* = 7.0 Hz, 2H), 3.75–3.86 (m, 3H), 4.14 (t, *J* = 5.0 Hz, 2H), 4.28 (dd, *J* = 9.1, 6.1 Hz, 1H), 4.75 (t, *J* = 9.1 Hz, 1H), 5.05 (s, 2H), 6.44–6.52 (m, 2H), 6.68 (s, 2H), 7.02–7.11 (m, 2H), 7.16 (s, 1H), 7.34–7.45 (m, 2H). MS *m/z* 477 $(M + H)^+$. HPLC purity (220 nm) 100.0%. Anal. Calcd for $C_{29}H_{32}O_6$: C, 73.09; H, 6.77. Found: C, 73.02; H, 6.77.

[6-({2′,6′-Dimethyl-4′-[(3-methyloxetan-3-yl)methoxy]biphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetic Acid (77). Step 1: Methyl [6-({2′,6′-dimethyl-4′-[(3-methyloxetan-3-yl)methoxy]biphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetate in 89% yield as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 3H), 2.00 (s, 6H), 2.49–2.61 (m, 1H), 2.69–2.80 (m, 1H), 3.71 (s, 3H), 3.74–3.86 (m, 1H), 4.04 (s, 2H), 4.26 (dd, *J* = 9.1, 6.1 Hz, 1H), 4.47 (d, *J* = 5.9 Hz, 2H), 4.64 (d, *J* = 5.9 Hz, 2H), 4.75 (t, *J* = 9.1 Hz, 1H), 5.06 (s, 2H), 6.44–6.51 (m, 2H), 6.70 (s, 2H), 7.02 (d, *J* = 8.1 Hz, 1H), 7.08 (dt, *J* = 7.1, 1.5 Hz, 1H), 7.17 (s, 1H), 7.35–7.46 (m, 2H). MS *m/z* 503 $(M + H)^+$. Step 2: **77** in 65% yield as colorless crystals (hexane–AcOEt). mp 150–151 °C. ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 3H), 2.00 (s, 6H), 2.55–2.67 (m, 1H), 2.75–2.86 (m, 1H), 3.75–3.87 (m, 1H), 4.04 (s, 2H), 4.28 (dd, *J* = 9.1, 6.0 Hz, 1H), 4.48 (d, *J* = 5.9 Hz, 2H), 4.65 (d, *J* = 5.9 Hz, 2H), 4.76 (t, *J* = 9.1 Hz, 1H), 5.06 (s, 2H), 6.44–6.52 (m, 2H), 6.70 (s, 2H), 7.01–7.11 (m, 2H), 7.17 (s, 1H), 7.34–7.46 (m, 2H).

MS *m/z* 489 $(M + H)^+$. HPLC purity (220 nm) 99.9%. Anal. Calcd for $C_{30}H_{32}O_6$: C, 73.75; H, 6.60. Found: C, 73.53; H, 6.61.

[(3*S*)-6-({2′,6′-Dimethyl-4′-[(3-methyloxetan-3-yl)methoxy]biphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetic Acid (78). Step 1: Methyl [(3*S*)-6-({2′,6′-dimethyl-4′-[(3-methyloxetan-3-yl)methoxy]biphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl] acetate in 95% yield as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 3H), 2.00 (s, 6H), 2.50–2.60 (m, 1H), 2.70–2.79 (m, 1H), 3.72 (s, 3H), 3.74–3.86 (m, 1H), 4.04 (s, 2H), 4.26 (dd, *J* = 9.1, 6.0 Hz, 1H), 4.47 (d, *J* = 5.8 Hz, 2H), 4.64 (d, *J* = 5.8 Hz, 2H), 4.75 (t, *J* = 9.1 Hz, 1H), 5.06 (s, 2H), 6.44–6.51 (m, 2H), 6.70 (s, 2H), 7.02 (d, *J* = 8.1 Hz, 1H), 7.05–7.11 (m, 1H), 7.17 (s, 1H), 7.35–7.46 (m, 2H). Step 2: **78** in 66% yield as colorless crystals (hexane–AcOEt). mp 140–142 °C. $[\alpha]_D$ +5.6° (c 0.30, CH_3CN). 99.8% ee [column: CHIRALPAK OD, 4.6 mmID × 250 mmL; mobile phase: hexane/IPA/TFA (80/20/0.1) (v/v/v) by isocratic elution; flow rate: 0.5 mL/min; detection: UV 220 nm; temperature: 30 °C]. ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 3H), 2.00 (s, 6H), 2.56–2.67 (m, 1H), 2.76–2.85 (m, 1H), 3.75–3.86 (m, 1H), 4.04 (s, 2H), 4.29 (dd, *J* = 9.1, 6.0 Hz, 1H), 4.48 (d, *J* = 5.9 Hz, 2H), 4.65 (d, *J* = 5.9 Hz, 2H), 4.76 (t, *J* = 9.1 Hz, 1H), 5.06 (s, 2H), 6.44–6.52 (m, 2H), 6.70 (s, 2H), 7.02–7.11 (m, 2H), 7.17 (s, 1H), 7.35–7.46 (m, 2H). MS *m/z* 489 $(M + H)^+$. HPLC purity (220 nm) 98.0%. Anal. Calcd for $C_{30}H_{32}O_6$: C, 73.75; H, 6.60. Found: C, 73.50; H, 6.73.

[(3*R*)-6-({2′,6′-Dimethyl-4′-[(3-methyloxetan-3-yl)methoxy]biphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetic Acid (79). Step 1: Methyl [(3*R*)-6-({2′,6′-dimethyl-4′-[(3-methyloxetan-3-yl)methoxy]biphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl] acetate in 90% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 3H), 2.00 (s, 6H), 2.50–2.61 (m, 1H), 2.70–2.80 (m, 1H), 3.72 (s, 3H), 3.74–3.86 (m, 1H), 4.04 (s, 2H), 4.26 (dd, *J* = 9.0, 6.1 Hz, 1H), 4.47 (d, *J* = 5.8 Hz, 2H), 4.64 (d, *J* = 5.8 Hz, 2H), 4.75 (t, *J* = 9.0 Hz, 1H), 5.06 (s, 2H), 6.44–6.51 (m, 2H), 6.70 (s, 2H), 7.02 (d, *J* = 7.9 Hz, 1H), 7.08 (dt, *J* = 7.1, 1.6 Hz, 1H), 7.17 (s, 1H), 7.35–7.46 (m, 2H). Step 2: **79** in 56% yield as colorless crystals (hexane–AcOEt). mp 136–138 °C. $[\alpha]_D$ −5.6° (c 0.31, CH_3CN). 99.4% ee [column: CHIRALPAK OD, 4.6 mmID × 250 mmL; mobile phase: hexane/IPA/TFA (80/20/0.1) (v/v/v) by isocratic elution; flow rate: 0.5 mL/min; detection: UV 220 nm; temperature: 30 °C]. ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 3H), 2.00 (s, 6H), 2.55–2.67 (m, 1H), 2.75–2.86 (m, 1H), 3.75–3.87 (m, 1H), 4.04 (s, 2H), 4.29 (dd, *J* = 9.2, 6.0 Hz, 1H), 4.48 (d, *J* = 5.8 Hz, 2H), 4.65 (d, *J* = 6.0 Hz, 2H), 4.76 (t, *J* = 9.0 Hz, 1H), 5.06 (s, 2H), 6.44–6.52 (m, 2H), 6.71 (s, 2H), 7.02–7.11 (m, 2H), 7.17 (s, 1H), 7.35–7.46 (m, 2H). MS *m/z* 489 $(M + H)^+$. Anal. Calcd for $C_{30}H_{32}O_6$: C, 73.75; H, 6.60. Found: C, 73.58; H, 6.77.

[6-({4′-[(1,1-Dioxidotetrahydro-2*H*-thiopyran-4-yl)oxy]-2′,6′-dimethylbiphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetic Acid (80). Step 1: Methyl [6-({4′-[(1,1-dioxidotetrahydro-2*H*-thiopyran-4-yl)oxy]-2′,6′-dimethylbiphenyl-3-yl}-methoxy)-2,3-dihy-

dro-1-benzofuran-3-yl]acetate in 93% yield as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.99 (s, 6H), 2.31–2.60 (m, 5H), 2.70–2.79 (m, 1H), 2.89–3.00 (m, 2H), 3.39–3.52 (m, 2H), 3.72 (s, 3H), 3.75–3.86 (m, 1H), 4.26 (dd, *J* = 9.1, 6.1 Hz, 1H), 4.64–4.70 (m, 1H), 4.75 (t, *J* = 9.1 Hz, 1H), 5.06 (s, 2H), 6.44–6.50 (m, 2H), 6.67 (s, 2H), 7.02 (d, *J* = 7.9 Hz, 1H), 7.07 (dt, *J* = 7.1, 1.5 Hz, 1H), 7.16 (s, 1H), 7.35–7.46 (m, 2H). MS *m/z* 551 $(M + H)^+$. HPLC purity (220 nm) 99.6%. Step 2: **80** in 80% yield as colorless crystals (hexane–AcOEt). mp 159–161 °C. ^{1}H NMR ($CDCl_3$) δ 1.99 (s, 6H), 2.31–2.56 (m, 4H), 2.56–2.67 (m, 1H), 2.76–2.85 (m, 1H), 2.90–3.00 (m, 2H), 3.39–3.52 (m, 2H), 3.75–3.87 (m, 1H), 4.29 (dd, *J* = 9.1, 6.0 Hz, 1H), 4.64–4.70 (m, 1H), 4.76 (t, *J* = 9.1 Hz, 1H), 5.06 (s, 2H), 6.44–6.52 (m, 2H), 6.67 (s, 2H), 7.03–7.10 (m, 2H), 7.16 (s, 1H), 7.35–7.46 (m, 2H). MS *m/z* 537 $(M + H)^+$. HPLC purity (220 nm) 100.0%. Anal. Calcd for $C_{30}H_{32}O_7S$: C, 67.14; H, 6.01. Found: C, 66.97; H, 6.12.

[(3*S*)-6-({4′-[(1,1-Dioxidotetrahydro-2*H*-thiopyran-4-yl)oxy]-2′,6′-dimethylbiphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetic Acid (81). Step 1: Methyl [(3*S*)-6-({4′-[(1,1-dioxidotetrahydro-2*H*-thiopyran-4-yl)oxy]-2′,6′-dimethylbiphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetate in 79% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.99 (s, 6H), 2.31–2.60 (m, 5H), 2.70–2.79 (m, 1H), 2.89–3.00 (m, 2H), 3.39–3.52 (m, 2H), 3.72 (s, 3H), 3.75–3.86 (m, 1H), 4.26 (dd, *J* = 9.1, 6.0 Hz, 1H), 4.64–4.69 (m, 1H), 4.75 (t, *J* = 9.1 Hz, 1H), 5.06 (s, 2H), 6.44–6.50 (m, 2H), 6.67 (s, 2H), 7.02 (d, *J* = 7.9 Hz, 1H), 7.04–7.09 (m, 1H), 7.16 (s, 1H), 7.35–7.46 (m, 2H). MS *m/z* 551 $(M + H)^+$. Step 2: **81** in 85% yield as colorless crystals (heptane–AcOEt). mp 154–155 °C. $[\alpha]_D$ +6.1° (c 0.30, CH_3CN). ^{1}H NMR ($CDCl_3$) δ 1.99 (s, 6H), 2.30–2.44 (m, 2H), 2.45–2.56 (m, 2H), 2.56–2.67 (m, 1H), 2.75–2.86 (m, 1H), 2.89–3.00 (m, 2H), 3.38–3.52 (m, 2H), 3.75–3.87 (m, 1H), 4.29 (dd, *J* = 9.1, 6.1 Hz, 1H), 4.63–4.70 (m, 1H), 4.76 (t, *J* = 9.1 Hz, 1H), 5.06 (s, 2H), 6.44–6.52 (m, 2H), 6.67 (s, 2H), 7.02–7.10 (m, 2H), 7.16 (s, 1H), 7.35–7.46 (m, 2H). MS *m/z* 537 $(M + H)^+$. HPLC purity (220 nm) 99.8%. Anal. Calcd for $C_{30}H_{32}O_7S$: C, 67.14; H, 6.01. Found: C, 67.10; H, 6.06.

[(3*R*)-6-({4′-[(1,1-Dioxidotetrahydro-2*H*-thiopyran-4-yl)oxy]-2′,6′-dimethylbiphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetic Acid (82). Step 1: Methyl [(3*R*)-6-({4′-[(1,1-dioxidotetrahydro-2*H*-thiopyran-4-yl)oxy]-2′,6′-dimethylbiphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetate in 86% yield as a colorless foam. ^{1}H NMR ($CDCl_3$) δ 1.99 (s, 6H), 2.30–2.61 (m, 5H), 2.70–2.79 (m, 1H), 2.89–2.99 (m, 2H), 3.38–3.52 (m, 2H), 3.72 (s, 3H), 3.74–3.86 (m, 1H), 4.26 (dd, *J* = 9.1, 6.0 Hz, 1H), 4.63–4.69 (m, 1H), 4.75 (t, *J* = 9.1 Hz, 1H), 5.06 (s, 2H), 6.42–6.50 (m, 2H), 6.67 (s, 2H), 6.99–7.10 (m, 2H), 7.16 (s, 1H), 7.35–7.46 (m, 2H). Step 2: **82** in 92% yield as colorless crystals (heptane–AcOEt). mp 156–157 °C. $[\alpha]_D$ −4.4° (c 0.30, CH_3CN). ^{1}H NMR ($CDCl_3$) δ 1.99 (s, 6H), 2.30–2.44 (m, 2H), 2.45–2.67 (m, 3H), 2.74–2.86 (m, 1H), 2.89–3.00 (m, 2H), 3.38–3.52 (m, 2H), 3.75–3.87 (m, 1H), 4.29 (dd, *J* = 9.1, 6.0 Hz, 1H), 4.63–4.69 (m, 1H),

4.76 (t, *J* = 9.1 Hz, 1H), 5.06 (s, 2H), 6.44–6.52 (m, 2H), 6.67 (s, 2H), 7.02–7.10 (m, 2H), 7.16 (s, 1H), 7.35–7.46 (m, 2H). MS *m/z* 537 $(M + H)^+$. HPLC purity (220 nm) 99.8%. Anal. Calcd for $C_{30}H_{32}O_7S$: C, 67.14; H, 6.01. Found: C, 66.94; H, 6.02.

[(3*S*)-6-({4′-[(4-Hydroxy-1,1-dioxidotetrahydro-2*H*-thiopyran-4-yl)methoxy]-2′,6′-dimethylbiphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetic Acid (83). The title compound was prepared from **7g** and **74d** by a method similar to that described for **75** except for step 2. Step 1: Methyl [(3*S*)-6-({4′-[(4-hydroxytetrahydro-2*H*-thiopyran-4-yl)methoxy]-2′,6′-dimethylbiphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetate in 90% yield as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.77–1.89 (m, 2H), 1.99 (s, 6H), 2.06–2.15 (m, 2H), 2.18 (s, 1H), 2.42–2.60 (m, 3H), 2.70–2.79 (m, 1H), 3.04–3.17 (m, 2H), 3.72 (s, 3H), 3.74–3.86 (m, 3H), 4.26 (dd, *J* = 9.1, 6.0 Hz, 1H), 4.75 (t, *J* = 9.1 Hz, 1H), 5.05 (s, 2H), 6.44–6.51 (m, 2H), 6.67 (s, 2H), 7.01 (d, *J* = 7.9 Hz, 1H), 7.04–7.09 (m, 1H), 7.15 (s, 1H), 7.35–7.45 (m, 2H). MS *m/z* 531 $(M - 18 + H)^+$. Step 2: To a solution of the obtained oil (0.586 g, 1.07 mmol) in AcOEt (5 mL) was added gradually *m*-CPBA (0.568 g, 2.14 mmol) at 0 °C, and the mixture was stirred at 0 °C for 2 h. The mixture was quenched with $Na_2S_2O_3$ aqueous solution and saturated $NaHCO_3$ aqueous solution, and extracted with AcOEt. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 40:60–80:20) to give methyl [(3*S*)-6-({4′-[(4-hydroxy-1,1-dioxidotetrahydro-2*H*-thiopyran-4-yl)methoxy]-2′,6′-dimethylbiphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetate (0.489 g, 79%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 2.00 (s, 6H), 2.16–2.33 (m, 4H), 2.46 (s, 1H), 2.50–2.61 (m, 1H), 2.69–2.80 (m, 1H), 2.90–3.01 (m, 2H), 3.43–3.57 (m, 2H), 3.72 (s, 3H), 3.74–3.86 (m, 1H), 3.88 (s, 2H), 4.26 (dd, *J* = 9.1, 6.1 Hz, 1H), 4.75 (t, *J* = 9.1 Hz, 1H), 5.06 (s, 2H), 6.43–6.51 (m, 2H), 6.67 (s, 2H), 6.99–7.10 (m, 2H), 7.15 (s, 1H), 7.35–7.47 (m, 2H). MS *m/z* 581 $(M + H)^+$. Step 3: **83** in 76% yield as colorless crystals (hexane–AcOEt). mp 198–201 °C. $[\alpha]_D$ +5.1° (c 0.30, CH_3CN). ^{1}H NMR ($CDCl_3$) δ 2.00 (s, 6H), 2.17–2.33 (m, 4H), 2.56–2.67 (m, 1H), 2.76–2.85 (m, 1H), 2.90–3.01 (m, 2H), 3.43–3.56 (m, 2H), 3.75–3.86 (m, 1H), 3.88 (s, 2H), 4.29 (dd, *J* = 9.1, 6.0 Hz, 1H), 4.76 (t, *J* = 9.1 Hz, 1H), 5.06 (s, 2H), 6.44–6.52 (m, 2H), 6.67 (s, 2H), 7.02–7.09 (m, 2H), 7.15 (s, 1H), 7.35–7.46 (m, 2H). MS *m/z* 567 $(M + H)^+$. HPLC purity (220 nm) 99.4%. Anal. Calcd for $C_{31}H_{34}O_8S$: C, 65.71; H, 6.05. Found: C, 65.69; H, 6.03.

[(3*S*)-6-({4′-[2-(Ethylsulfonyl)ethoxy]-2′,6′-dimethylbiphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetic Acid (84). The title compound was prepared from **7g** and **74e** by a method similar to that described for **75** except for step 3. Step 1: Methyl [(3*S*)-6-({4′-[2-(ethylsulfanyl)ethoxy]-2′,6′-dimethylbiphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetate in 60% yield as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.31 (t, *J* = 7.4 Hz, 3H), 1.99 (s, 6H), 2.50–2.79 (m, 4H), 2.92 (t, *J* = 7.0 Hz, 2H), 3.71 (s, 3H), 3.74–3.86 (m, 1H), 4.15 (t, *J* = 7.0 Hz, 2H), 4.26 (dd, *J* = 9.1, 6.1 Hz, 1H), 4.75 (t, *J* = 9.1 Hz, 1H), 5.05

(s, 2H), 6.44–6.51 (m, 2H), 6.66 (s, 2H), 7.01 (d, *J* = 7.9 Hz, 1H), 7.05–7.10 (m, 1H), 7.16 (s, 1H), 7.34–7.45 (m, 2H). MS *m/z* 507 $(M + H)^+$. Step 2: [(3*S*)-6-({4′-[2-(Ethylsulfanyl) ethoxy]-2′,6′-dimethylbiphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetic acid in 89% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.31 (t, *J* = 7.4 Hz, 3H), 1.99 (s, 6H), 2.56–2.71 (m, 3H), 2.76–2.86 (m, 1H), 2.92 (t, *J* = 7.0 Hz, 2H), 3.75–3.87 (m, 1H), 4.15 (t, *J* = 6.8 Hz, 2H), 4.28 (dd, *J* = 9.1, 6.1 Hz, 1H), 4.76 (t, *J* = 9.1 Hz, 1H), 5.05 (s, 2H), 6.44–6.52 (m, 2H), 6.66 (s, 2H), 7.02–7.10 (m, 2H), 7.16 (s, 1H), 7.34–7.45 (m, 2H). MS *m/z* 493 $(M + H)^+$. Step 3: To a solution of the obtained oil (0.304 g, 0.617 mmol) in MeOH (10 mL) was added dropwise a solution of Oxone® (0.569 g, 0.926 mmol) in water (5 mL) at 0 °C, and the mixture was stirred at 0 °C to room temperature for 12 h. MeOH was evaporated. The residue was diluted with water, and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by preparative HPLC to give crystals. Recrystallization from heptane–AcOEt gave **84** (0.237 g, 73%) as colorless crystals. mp 130–131 °C. $[\alpha]_D$ +6.6° (c 0.30, CH_3CN). ^{1}H NMR ($CDCl_3$) δ 1.47 (t, *J* = 7.5 Hz, 3H), 1.99 (s, 6H), 2.55–2.67 (m, 1H), 2.75–2.86 (m, 1H), 3.19 (q, *J* = 7.5 Hz, 2H), 3.42 (t, *J* = 5.4 Hz, 2H), 3.75–3.87 (m, 1H), 4.29 (dd, *J* = 9.1, 6.0 Hz, 1H), 4.44 (t, *J* = 9.1 Hz, 2H), 4.76 (t, *J* = 5.4 Hz, 1H), 5.06 (s, 2H), 6.44–6.52 (m, 2H), 6.64 (s, 2H), 7.02–7.09 (m, 2H), 7.15 (s, 1H), 7.35–7.46 (m, 2H). MS *m/z* 525 $(M + H)^+$. HPLC purity (220 nm) 99.8%. Anal. Calcd for $C_{29}H_{32}O_7S$: C, 66.39; H, 6.15. Found: C, 66.35; H, 6.15.

[(3*S*)-6-({2′,6′-Dimethyl-4′-[3-(methylsulfonyl)propoxy]biphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetic Acid Hemihydrate (85). Step 1: To a mixture of **7g** (0.208 g, 1.00 mmol), **74f** (0.348 g, 1.00 mmol), and P(*n*-Bu)$_3$ (0.324 g, 1.60 mmol) in toluene (15 mL) was added portionwise ADDP (0.404 g, 1.60 mmol), and the mixture was stirred under nitrogen atmosphere at room temperature for 1.5 h. Hexane (8 mL) was added, and the insoluble material was removed by filtration. The filtrate was concentrated, and the residue was purified by silica gel column chromatography (AcOEt:hexane = 40:60–80:20) to give methyl [(3*S*)-6-({2′,6′-dimethyl-4′-[3-(methylsulfonyl)propoxy]biphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetate (0.442 g, 82%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.99 (s, 6H), 2.30–2.41 (m, 2H), 2.49–2.61 (m, 1H), 2.69–2.79 (m, 1H), 2.97 (s, 3H), 3.23–3.31 (m, 2H), 3.71 (s, 3H), 3.74–3.86 (m, 1H), 4.08–4.13 (m, 2H), 4.26 (dd, *J* = 9.1, 6.1 Hz, 1H), 4.75 (t, *J* = 9.1 Hz, 1H), 5.05 (s, 2H), 6.43–6.51 (m, 2H), 6.64 (s, 2H), 7.01 (d, *J* = 8.0 Hz, 1H), 7.07 (dt, *J* = 7.1, 1.6 Hz, 1H), 7.15 (s, 1H), 7.34–7.46 (m, 2H). MS *m/z* 539 $(M + H)^+$. Step 2: To a solution of the obtained oil (11.2 g, 20.8 mmol) in MeOH (40 mL) and THF (80 mL) was added 2 M NaOH aqueous solution (20.0 mL, 40.0 mmol), and the mixture was stirred at 50 °C for 2 h. The mixture was concentrated, diluted with water, acidified with 1 M HCl aqueous solution, and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated to give crystals, which were washed with heptane–AcOEt. Recrystallization from EtOH–H_2O gave **85** (9.31 g, 85%) as colorless

crystals. mp 127–129 °C. $[\alpha]_D$ +5.3° (c 0.3085, CH_3CN). 99.6% ee [column, CHRALPAK AD-3 (NC002), 4.6 mmID × 250 mmL; mobile phase, hexane/IPA/TFA = 500/500/1 (v/v/v) by isocratic elution; flow rate, 0.5 mL/min; detection, UV 220 nm; column temperature, 30 °C]. ^{1}H NMR ($CDCl_3$) δ 1.99 (s, 6H), 2.29–2.41 (m, 2H), 2.61 (dd, *J* = 16.9, 9.2 Hz, 1H), 2.81 (dd, *J* = 16.9, 5.5 Hz, 1H), 2.97 (s, 3H), 3.23–3.31 (m, 2H), 3.75–3.87 (m, 1H), 4.13 (t, *J* = 5.8 Hz, 2H), 4.28 (dd, *J* = 9.1, 6.0 Hz, 1H), 4.76 (t, *J* = 9.1 Hz, 1H), 5.06 (s, 2H), 6.44–6.52 (m, 2H), 6.64 (s, 2H), 7.02–7.10 (m, 2H), 7.16 (s, 1H), 7.35–7.46 (m, 2H). MS *m/z* 525 $(M + H)^+$. HPLC purity (220 nm) 100.0%. Anal. Calcd for $C_{29}H_{32}O_7S \cdot 0.5\ H_2O$: C, 65.27; H, 6.23. Found: C, 65.23; H, 6.15.

Methyl {(3*S*)-6-[(4′-Hydroxy-2′,6′-dimethylbiphenyl-3-yl)methoxy]-2,3-dihydro-1-benzofuran-3-yl}acetate (86a). Step 1: Methyl {(3*S*)-6-[(4′-{[*tert*-butyl(dimethyl)silyl]oxy}-2′,6′-dimethylbiphenyl-3-yl)methoxy]-2,3-dihydro-1-benzofuran-3-yl}acetate was prepared from **7g** and **74g** by a method similar to that described for **75**-step 1 in 85% yield as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 0.23 (s, 6H), 1.00 (s, 9H), 1.95 (s, 6H), 2.55 (dd, *J* = 16.5, 9.3 Hz, 1H), 2.75 (dd, *J* = 16.5, 5.5 Hz, 1H), 3.71 (s, 3H), 3.74–3.88 (m, 1H), 4.26 (dd, *J* = 9.2, 6.0 Hz, 1H), 4.75 (t, *J* = 9.2 Hz, 1H), 5.05 (s, 2H), 6.44–6.51 (m, 2H), 6.57 (s, 2H), 7.01 (d, *J* = 7.9 Hz, 1H), 7.06–7.10 (m, 1H), 7.17 (s, 1H), 7.33–7.44 (m, 2H). MS *m/z* 533 $(M + H)^+$. Step 2: To a solution of the obtained solid (2.27 g, 4.26 mmol) in THF (25 mL) was added 1 M TBAF in THF (4.7 mL, 4.7 mmol) at room temperature and the mixture was stirred under nitrogen atmosphere at room temperature for 1 h. The mixture was concentrated and the residue was partitioned between water and AcOEt. The organic layer was separated, washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 20:80–60:40) to give **86a** (1.67 g, 94%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.97 (s, 6H), 2.55 (dd, *J* = 16.5, 9.8 Hz, 1H), 2.75 (dd, *J* = 16.5, 4.8 Hz, 1H), 3.72 (s, 3H), 3.74–3.86 (m, 1H), 4.26 (dd, *J* = 9.0, 6.2 Hz, 1H), 4.63 (s, 1H), 4.75 (t, *J* = 9.0 Hz, 1H), 5.05 (s, 2H), 6.43–6.50 (m, 2H), 6.59 (s, 2H), 7.01 (d, *J* = 8.1 Hz, 1H), 7.04–7.11 (m, 1H), 7.16 (s, 1H), 7.34–7.46 (m, 2H). MS *m/z* 419 $(M + H)^+$.

Methyl {(3*S*)-6-[(3′-Chloro-4′-hydroxy-2′,6′-dimethylbiphenyl-3-yl)methoxy]-2,3-dihydro-1-benzofuran-3-yl}acetate (86b). The title compound was prepared from **7g** and **74h** by a method similar to that described for **86a**. Step 1: Methyl {(3*S*)-6-[(4′-{[*tert*-butyl(dimethyl)silyl]oxy}-3′-chloro-2′,6′-dimethylbiphenyl-3-yl)methoxy]-2,3-dihydro-1-benzofuran-3-yl}acetate in 77% yield as colorless crystals. ^{1}H NMR ($CDCl_3$) δ 0.26 (s, 6H), 1.06 (s, 9H), 1.92 (s, 3H), 2.04 (s, 3H), 2.50–2.61 (m, 1H), 2.70–2.79 (m, 1H), 3.71 (s, 3H), 3.75–3.86 (m, 1H), 4.26 (dd, *J* = 9.1, 6.0 Hz, 1H), 4.75 (t, *J* = 9.1 Hz, 1H), 5.05 (s, 2H), 6.44–6.51 (m, 2H), 6.65 (s, 1H), 6.99–7.07 (m, 2H), 7.14 (s, 1H), 7.36–7.46 (m, 2H). MS *m/z* 567 $(M + H)^+$. Step 2: **86b** in 88% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.94 (s, 3H), 2.04 (s, 3H), 2.49–2.61 (m, 1H), 2.69–2.80 (m, 1H), 3.71 (s, 3H), 3.74–3.86 (m, 1H), 4.26 (dd, *J* = 9.1,

6.0 Hz, 1H), 4.75 (t, *J* = 9.1 Hz, 1H), 5.06 (s, 2H), 5.55 (s, 1H), 6.43–6.51 (m, 2H), 6.81 (s, 1H), 6.99–7.07 (m, 2H), 7.13 (s, 1H), 7.36–7.47 (m, 2H). MS *m/z* 453 $(M + H)^+$.

[(3*S*)-6-({2′,6′-Dimethyl-4′-[3-(2-oxopyrrolidin-1-yl)propoxy]biphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetic Acid (87). Step 1: To a mixture of **86a** (0.325 g, 0.777 mmol), 1-(3-hydroxypropyl)pyrrolidin-2-one (0.167 g, 1.16 mmol), and P(*n*-Bu)$_3$ (0.314 g, 1.55 mmol) in toluene (15 mL) was added ADDP (0.392 g, 1.55 mmol), and the mixture was stirred at room temperature for 15 h. Hexane (15 mL) was added, and the insoluble material was removed by filtration. The filtrate was concentrated and the residue was purified by silica gel column chromatography (AcOEt:hexane = 30:70–100:0) to afford methyl [(3*S*)-6-({2′,6′-dimethyl-4′-[3-(2-oxopyrrolidin-1-yl)propoxy]biphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetate (0.420 g, crude) as a colorless oil. The crude product was used for next reaction without further purification. MS *m/z* 544 $(M + H)^+$. Step 2: Compound **87** was prepared from the obtained oil by a method similar to that described for **75**-step 2 in 42% yield (from **86a**) as colorless crystals (heptane–MeCN). mp 90–92 °C. $[\alpha]_D$ +5.2° (c 0.32, CH_3CN). ^{1}H NMR ($CDCl_3$) δ 1.94–1.99 (m, 6H), 1.99–2.10 (m, 4H), 2.41 (t, *J* = 8.1 Hz, 2H), 2.59 (dd, *J* = 16.5, 9.3 Hz, 1H), 2.78 (dd, *J* = 16.5, 5.4 Hz, 1H), 3.42–3.52 (m, 4H), 3.73–3.85 (m, 1H), 4.00 (t, *J* = 6.3 Hz, 2H), 4.28 (dd, *J* = 9.2, 5.8 Hz, 1H), 4.74 (t, *J* = 9.2 Hz, 1H), 5.06 (s, 2H), 6.43–6.50 (m, 2H), 6.64 (s, 2H), 7.02–7.09 (m, 2H), 7.13 (s, 1H), 7.33–7.44 (m, 2H). MS *m/z* 530 $(M + H)^+$. HPLC purity (220 nm) 99.7%. Anal. Calcd for $C_{32}H_{35}NO_6 \cdot 0.5\ H_2O$: C, 71.36; H, 6.74; N, 2.60. Found: C, 71.39; H, 6.60; N, 2.61.

[(3*S*)-6-({2′,6′-Dimethyl-4′-[(1-methylpiperidin-4-yl)oxy]biphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetic Acid Hydrochloride (88). Step 1: To a mixture of **86a** (0.350 g, 0.836 mmol), 1-methylpiperidin-4-ol (0.143 g, 1.25 mmol), and PPh_3 (0.351 g, 1.34 mmol) in toluene (15 mL) was added 40% DEAD in toluene (0.582 g, 1.34 mmol), and the mixture was stirred at room temperature for 15 h. Then, 1-methylpiperidin-4-ol (0.098 g, 0.851 mmol), PPh_3 (0.197 g, 1.05 mmol), and 40% DEAD in toluene (0.328 g, 0.750 mmol) were added to the mixture. After stirred at room temperature for 8 h, hexane (15 mL) was added to the mixture, and the insoluble material was removed by filtration. The filtrate was concentrated, and the residue was purified by basic silica gel column chromatography (AcOEt:hexane = 20:80–50:50) to afford methyl [(3*S*)-6-({2′,6′-dimethyl-4′-[(1-methylpiperidin-4-yl)oxy]biphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetate (0.270 g, 63%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.80–1.93 (m, 2H), 1.95–2.10 (m, 8H), 2.24–2.36 (m, 5H), 2.55 (dd, *J* =16.5, 9.3 Hz, 1H), 2.66–2.80 (m, 3H), 3.71 (s, 3H), 3.74–3.87 (m, 1H), 4.21–4.39 (m, 2H), 4.75 (t, *J* = 9.0 Hz, 1H), 5.05 (s, 2H), 6.44–6.51 (m, 2H), 6.66 (s, 2H), 7.01 (d, *J* = 7.9 Hz, 1H), 7.06–7.11 (m, 1H), 7.17 (s, 1H), 7.33–7.45 (m, 2H). MS *m/z* 516 $(M + H)^+$. Step 2: To a stirred solution of the obtained oil (0.270 g, 0.52 mmol) in MeOH (4 mL) and THF (8 mL) was added 2 M NaOH aqueous solution (2.0 mL, 4.0 mmol).

The mixture was stirred at room temperature for 15 h. Then, the mixture was neutralized with saturated NH_4Cl aqueous solution. To the mixture was added sodium chloride and the mixture was extracted with AcOEt–THF–CH_2Cl_2. The extract was dried over anhydrous $MgSO_4$, and concentrated. The resultant residue was treated with 4 M HCl in AcOEt (5 mL, 20 mmol). Then, hexane (20 mL) was added to the mixture and the resulting crystals were collected by filtration to afford **88** (91 mg, 32%) as colorless crystals. mp 165–167 °C. MS *m/z* 502 $(M + H)^+$ as a free form. ^{1}H NMR (DMSO-d_6) δ 1.82–2.30 (m, 4H), 1.92 (s, 6H), 2.40–2.56 (m, 1H), 2.61–2.84 (m, 4H), 3.03–3.46 (m, 5H), 3.59–3.72 (m, 1H), 4.18 (dd, *J* = 9.1, 6.9 Hz, 1H), 4.67 (t, *J* = 9.1 Hz, 1H), 5.10 (s, 2H), 6.40–6.53 (m, 2H), 6.77 (s, 2H), 7.02–7.15 (m, 3H), 7.35–7.49 (m, 2H). HPLC purity (220 nm) 99.0%.

The following compounds **89** and **90** were also prepared from **86a** and the appropriate alcohols by a method similar to that described for **87**.

[(3*S*)-6-({4′-[(2,4-Dimethyl-1,3-thiazol-5-yl)methoxy]-2′,6′-dimethylbiphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetic Acid (89). Step 1: Methyl [(3*S*)-6-({4′-[(2,4-dimethyl-1,3-thiazol-5-yl)methoxy]-2′,6′-dimethylbiphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetate as a crude product (a colorless oil). MS *m/z* 544 $(M + H)^+$. Step 2: **89** in 24% yield (from **86a**) as colorless crystals (hexane–AcOEt). mp 158–159 °C. ^{1}H NMR ($CDCl_3$) δ 1.91 (s, 6H), 2.34 (s, 3H), 2.59 (s, 3H), 2.61–2.80 (m, 2H), 3.56–3.74 (m, 1H), 4.18 (dd, *J* = 9.0, 6.8 Hz, 1H), 4.68 (t, *J* = 9.0 Hz, 1H), 5.09 (s, 2H), 5.20 (s, 2H), 6.42–6.56 (m, 2H), 6.77 (s, 2H), 7.02–7.16 (m, 3H), 7.35–7.49 (m, 2H). MS *m/z* 530 $(M + H)^+$. HPLC purity (220 nm) 99.1%.

[(3*S*)-6-{[4′-(Imidazo[1,2-*a*]pyridin-5-ylmethoxy)-2′,6′-dimethylbiphenyl-3-yl]methoxy}-2,3-dihydro-1-benzofuran-3-yl]acetic Acid (90). Step 1: Methyl [(3*S*)-6-{[4′-(imidazo[1,2-*a*]pyridin-5-ylmethoxy)-2′,6′-dimethylbiphenyl-3-yl]methoxy}-2,3-dihydro-1-benzofuran-3-yl]acetate as a crude product (a colorless oil). MS *m/z* 549 $(M + H)^+$. Step 2: **90** in 16% yield (from **86a**) as colorless crystals (hexane–AcOEt). mp 204–205 °C. ^{1}H NMR (DMSO-d_6) δ 1.93 (s, 6H), 2.38 (dd, *J* = 16.5, 9.0 Hz, 1H), 2.62 (dd, *J* = 16.5, 5.5 Hz, 1H), 3.56–3.71 (m, 1H), 4.16 (dd, *J* = 9.0, 6.9 Hz, 1H), 4.66 (t, *J* = 9.0 Hz, 1H), 5.09 (s, 2H), 5.46 (s, 2H), 6.41–6.49 (m, 2H), 6.90 (s, 2H), 7.03–7.12 (m, 2H), 7.12–7.17 (m, 2H), 7.31 (dd, *J* = 9.0, 6.8 Hz, 1H), 7.35–7.48 (m, 2H), 7.60–7.71 (m, 2H), 7.93 (s, 1H). MS *m/z* 535 $(M + H)^+$. HPLC purity (220 nm) 99.3%.

The following compounds **91**–**95** were also prepared from **7g** and appropriate alcohols **74i**–**m** by a method similar to that described for **75**.

[(3*S*)-6-({2′,6′-Diethyl-4′-[3-(methylsulfonyl)propoxy]biphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetic Acid (91). Step 1: Methyl [(3*S*)-6-({2′,6′-diethyl-4′-[3-(methylsulfonyl)propoxy]biphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetate in 93% yield as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 0.98 (t, *J* = 7.5 Hz, 6H), 2.22–2.43 (m, 6H), 2.49–2.60 (m, 1H), 2.70–2.78 (m, 1H), 2.97 (s, 3H), 3.25–3.33 (m, 2H), 3.71 (s, 3H),

3.74–3.85 (m, 1H), 4.12–4.18 (m, 2H), 4.25 (dd, *J* = 9.0, 6.1 Hz, 1H), 4.74 (t, *J* = 9.0 Hz, 1H), 5.06 (s, 2H), 6.43–6.49 (m, 2H), 6.66 (s, 2H), 7.00 (d, *J* = 8.1 Hz, 1H), 7.07–7.11 (m, 1H), 7.18 (s, 1H), 7.36–7.44 (m, 2H). MS *m/z* 567 $(M + H)^+$. Step 2: **91** in 81% yield as colorless crystals (heptane–AcOEt). mp 87–89 °C. $[\alpha]_D$ +5.5° (c 0.30, CH_3CN). ^1H NMR ($CDCl_3$) δ 0.98 (t, *J* = 7.5 Hz, 6H), 2.22–2.42 (m, 6H), 2.55–2.66 (m, 1H), 2.75–2.85 (m, 1H), 2.97 (s, 3H), 3.25–3.33 (m, 2H), 3.74–3.86 (m, 1H), 4.15 (t, *J* = 5.7 Hz, 2H), 4.28 (dd, *J* = 9.1, 6.1 Hz, 1H), 4.75 (t, *J* = 9.1 Hz, 1H), 5.07 (s, 2H), 6.43–6.51 (m, 2H), 6.66 (s, 2H), 7.04 (d, *J* = 8.3 Hz, 1H), 7.06–7.12 (m, 1H), 7.18 (s, 1H), 7.35–7.45 (m, 2H). MS *m/z* 553 $(M + H)^+$. HPLC purity (220 nm) 99.9%. Anal. Calcd for $C_{29}H_{32}O_8S \cdot 0.15$ heptane: C, 67.81; H, 6.82. Found: C, 67.88; H, 6.84.

[(3*S*)-6-({4′-[(1,1-Dioxidotetrahydro-2*H*-thiopyran-4-yl)oxy]-2′,3′,5′,6′-tetramethylbiphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetic Acid (92). Step 1: Methyl [(3*S*)-6-({4′-[(1,1-dioxidotetrahydro-2*H*-thiopyran-4-yl)oxy]-2′,3′,5′,6′-tetramethylbiphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetate in 81% yield as a colorless amorphous powder. ^1H NMR ($CDCl_3$) δ 1.87 (s, 6H), 2.20 (s, 6H), 2.30–2.60 (m, 5H), 2.70–2.79 (m, 1H), 2.95–3.08 (m, 2H), 3.31–3.43 (m, 2H), 3.72 (s, 3H), 3.75–3.86 (m, 1H), 3.94–4.03 (m, 1H), 4.26 (dd, *J* = 9.1, 6.0 Hz, 1H), 4.75 (t, *J* = 9.1 Hz, 1H), 5.05 (s, 2H), 6.44–6.51 (m, 2H), 6.99–7.06 (m, 2H), 7.12 (s, 1H), 7.35–7.45 (m, 2H). MS *m/z* 579 $(M + H)^+$. Step 2: **92** in 64% yield as colorless crystals (heptane–AcOEt). mp 143–145 °C. $[\alpha]_D$ +2.8° (c 0.30, $CHCl_3$). ^1H NMR ($CDCl_3$) δ 1.87 (s, 6H), 2.20 (s, 6H), 2.29–2.55 (m, 4H), 2.55–2.67 (m, 1H), 2.75–2.85 (m, 1H), 2.95–3.08 (m, 2H), 3.31–3.44 (m, 2H), 3.74–3.87 (m, 1H), 3.94–4.04 (m, 1H), 4.28 (dd, *J* = 9.1, 6.1 Hz, 1H), 4.76 (t, *J* = 9.1 Hz, 1H), 5.05 (s, 2H), 7.00–7.08 (m, 2H), 7.12 (s, 1H), 7.35–7.46 (m, 2H). MS *m/z* 565 $(M + H)^+$. HPLC purity (220 nm) 99.6%. Anal. Calcd for $C_{32}H_{36}O_7S$: C, 68.06; H, 6.43. Found: C, 67.80; H, 6.40.

[(3*S*)-6-({2′,3′,5′,6′-Tetramethyl-4′-[3-(methylsulfonyl)propoxy]biphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetic Acid (93). Step 1: Methyl [(3*S*)-6-({2′,3′,5′,6′-tetramethyl-4′-[3-(methylsulfonyl)propoxy]biphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetate in 82% yield as a colorless oil. ^1H NMR ($CDCl_3$) δ 1.87 (s, 6H), 2.19 (s, 6H), 2.32–2.43 (m, 2H), 2.50–2.61 (m, 1H), 2.70–2.79 (m, 1H), 3.00 (s, 3H), 3.35–3.43 (m, 2H), 3.71 (s, 3H), 3.74–3.90 (m, 3H), 4.26 (dd, *J* = 9.1, 6.0 Hz, 1H), 4.75 (t, *J* = 9.1 Hz, 1H), 5.05 (s, 2H), 6.43–6.51 (m, 2H), 6.99–7.07 (m, 2H), 7.13 (s, 1H), 7.35–7.45 (m, 2H). MS *m/z* 567 $(M + H)^+$. Step 2: **93** in 94% yield as colorless crystals (heptane–AcOEt). mp 160–162 °C. $[\alpha]_D$ +6.3° (c 0.30, CH_3CN). ^1H NMR ($CDCl_3$) δ 1.87 (s, 6H), 2.19 (s, 6H), 2.32–2.43 (m, 2H), 2.56–2.66 (m, 1H), 2.75–2.85 (m, 1H), 3.00 (s, 3H), 3.35–3.43 (m, 2H), 3.75–3.89 (m, 3H), 4.28 (dd, *J* = 9.1, 6.0 Hz, 1H), 4.76 (t, *J* = 9.1 Hz, 1H), 5.05 (s, 2H), 6.44–6.52 (m, 2H), 7.01–7.07 (m, 2H), 7.13 (s, 1H), 7.35–7.45 (m, 2H). MS *m/z* 553 $(M + H)^+$. HPLC purity (220 nm) 99.3%. Anal. Calcd for $C_{31}H_{36}O_7S$: C, 67.37; H, 6.57. Found: C, 67.39; H, 6.64.

[(3*S*)-6-({4′-[(1,1-Dioxidotetrahydro-2*H*-thiopyran-4-yl)oxy]-3′-fluoro-2′,6′-dimethylbiphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetic Acid (94). Step 1: Methyl [(3*S*)-6-({4′-[(1,1-dioxidotetrahydro-2*H*-thiopyran-4-yl)oxy]-3′-fluoro-2′,6′-dimethylbiphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetate as a crude product (a colorless oil). MS *m/z* 569 $(M + H)^+$. Step 2: **94** in 15% yield (from **111c**) as colorless crystals (hexane–diisopropyl ether). mp 112–113 °C. ^{1}H NMR ($CDCl_3$) δ 1.89–1.97 (m, 6H), 2.29–2.45 (m, 2H), 2.46–2.56 (m, 2H), 2.61 (dd, *J* = 16.8, 9.0 Hz, 1H), 2.81 (dd, *J* = 16.8, 5.7 Hz, 1H), 2.90–3.01 (m, 2H), 3.46–3.59 (m, 2H), 3.75–3.86 (m, 1H), 4.29 (dd, *J* = 9.2, 6.0 Hz, 1H), 4.56–4.64 (m, 1H), 4.76 (t, *J* = 9.2 Hz, 1H), 5.06 (s, 2H), 6.44–6.51 (m, 2H), 6.74 (d, *J* = 8.3 Hz, 1H), 7.02–7.08 (m, 2H), 7.14 (s, 1H), 7.37–7.48 (m, 2H). MS *m/z* 535 $(M + H)^+$. HPLC purity (220 nm) 99.4%.

[6-({3′-Fluoro-2′,6′-dimethyl-4′-[3-(methylsulfonyl)propoxy]biphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetic Acid (95). Step 1: Methyl [(3*S*)-6-({3′-fluoro-2′,6′-dimethyl-4′-[3-(methylsulfonyl)propoxy]biphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetate in 77% yield as colorless crystals (heptane–AcOEt). mp 101–103 °C. ^{1}H NMR ($CDCl_3$) δ 1.90–1.93 (m, 3H), 1.96 (s, 3H), 2.33–2.44 (m, 2H), 2.55 (dd, *J* = 16.5, 5.7 Hz, 1H), 2.75 (dd, *J* = 16.5, 9.0 Hz, 1H), 2.98 (s, 3H), 3.28–3.35 (m, 2H), 3.72 (s, 3H), 3.74–3.86 (m, 1H), 4.17–4.29 (m, 3H), 4.75 (t, *J* = 9.0 Hz, 1H), 5.06 (s, 2H), 6.44–6.51 (m, 2H), 6.70 (d, *J* = 8.3 Hz, 1H), 6.99–7.07 (m, 2H), 7.13 (s, 1H), 7.36–7.46 (m, 2H). MS *m/z* 557 $(M + H)^+$. Anal. Calcd for $C_{30}H_{33}FO_7S$: C, 64.73; H, 5.98. Found: C, 64.75; H, 5.90. Step 2: **95** in 90% yield as colorless crystals (heptane–AcOEt). mp 115–117 °C. $[\alpha]_D$ +5.9° (c 0.30, CH_3CN). ^{1}H NMR ($CDCl_3$) δ 1.89–1.98 (m, 6H), 2.32–2.44 (m, 2H), 2.60 (dd, *J* = 16.8, 9.0 Hz, 1H), 2.80 (dd, *J* = 16.8, 5.4 Hz, 1H), 2.98 (s, 3H), 3.27–3.35 (m, 2H), 3.73–3.86 (m, 1H), 4.20 (t, *J* = 5.7 Hz, 2H), 4.28 (dd, *J* = 9.2, 6.0 Hz, 1H), 4.75 (t, *J* = 9.2 Hz, 1H), 5.06 (s, 2H), 6.44–6.51 (m, 2H), 6.70 (d, *J* = 8.1 Hz, 1H), 7.02–7.08 (m, 2H), 7.13 (s, 1H), 7.37–7.46 (m, 2H). MS *m/z* 543 $(M + H)^+$. HPLC purity (220 nm) 99.9%. Anal. $C_{29}H_{31}FO_7S$: C, 64.19; H, 5.76. Found: C, 64.40; H, 5.92.

[(3*S*)-6-({3′-Chloro-2′,6′-dimethyl-4′-[3-(methylsulfonyl)propoxy]biphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetic Acid (96). Step 1: A mixture of **86b** (0.616 g, 1.36 mmol), **99b** (0.517 g, 1.77 mmol), and K_3PO_4 (0.433 g, 2.04 mmol) in DMF (2 mL) was stirred under nitrogen atmosphere at 90 °C for 2.5 h. The mixture was diluted with water and extracted with AcOEt. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 30:70–80:20) to give methyl [(3*S*)-6-({3′-chloro-2′,6′-dimethyl-4′-[3-(methylsulfonyl)propoxy]biphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetate (0.681 g, 88%) as a colorless viscous oil. ^{1}H NMR ($CDCl_3$) δ 1.97 (s, 3H), 2.05 (s, 3H), 2.35–2.46 (m, 2H), 2.55 (dd, *J* = 16.5, 9.1 Hz, 1H), 2.75 (dd, *J* = 16.5, 5.7 Hz, 1H), 2.99 (s, 3H), 3.31–3.41 (m, 2H), 3.71 (s, 3H), 3.75–3.86 (m, 1H), 4.20 (t, *J* = 5.9 Hz, 2H), 4.26 (dd,

J = 9.2, 6.2 Hz, 1H), 4.75 (t, *J* = 9.2 Hz, 1H), 5.06 (s, 2H), 6.43–6.51 (m, 2H), 6.69 (s, 1H), 6.99–7.07 (m, 2H), 7.13 (s, 1H), 7.37–7.47 (m, 2H). MS *m/z* 573 $(M + H)^+$. Step 2: Compound **96** was prepared by a method similar to that described for **75**-step 2 in 63% yield as colorless crystals (Et_2O–AcOEt). mp 127–128 °C. $[\alpha]_D$ +5.6° (c 0.30, CH_3CN). ^1H NMR ($CDCl_3$) δ 1.97 (s, 3H), 2.05 (s, 3H), 2.35–2.47 (m, 2H), 2.62 (dd, *J* = 16.8, 9.2 Hz, 1H), 2.81 (dd, *J* = 16.8, 5.5 Hz, 1H), 2.99 (s, 3H), 3.32–3.40 (m, 2H), 3.75–3.87 (m, 1H), 4.20 (t, *J* = 5.7 Hz, 2H), 4.29 (dd, *J* = 9.1, 6.0 Hz, 1H), 4.76 (t, *J* = 9.1 Hz, 1H), 5.06 (s, 2H), 6.44–6.52 (m, 2H), 6.69 (s, 1H), 7.02–7.08 (m, 2H), 7.13 (s, 1H), 7.37–7.47 (m, 2H). MS *m/z* 559 $(M + H)^+$. HPLC purity (220 nm) 99.63%. Anal. Calcd for $C_{29}H_{31}ClO_7S$: C, 62.30; H, 5.59. Found: C, 62.03; H, 5.58.

[(3*S*)-6-({3′,5′-Dichloro-2′,6′-dimethyl-4′-[3-(methylsulfonyl)propoxy]biphenyl-3-yl} methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetic Acid (97). The title compound was prepared from **7g** and **74n** by that described for **75**. Step 1: Methyl [(3*S*)-6-({3′,5′-dichloro-2′,6′-dimethyl-4′-[3-(methylsulfonyl)propoxy]biphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetate in 89% yield as a yellow oil. ^1H NMR ($CDCl_3$) δ 2.02 (s, 6H), 2.35–2.47 (m, 2H), 2.50–2.61 (m, 1H), 2.70–2.79 (m, 1H), 3.00 (s, 3H), 3.43–3.52 (m, 2H), 3.72 (s, 3H), 3.75–3.86 (m, 1H), 4.16 (t, *J* = 5.7 Hz, 2H), 4.26 (dd, *J* = 9.1, 6.0 Hz, 1H), 4.75 (t, *J* = 9.1 Hz, 1H), 5.06 (s, 2H), 6.43–6.50 (m, 2H), 6.99–7.05 (m, 2H), 7.11 (s, 1H), 7.39–7.49 (m, 2H). MS *m/z* 607 $(M + H)^+$. Step 2: **97** in 86% yield as colorless crystals (heptane–AcOEt). mp 115–116 °C. $[\alpha]_D$ +4.7° (c 0.30, CH_3CN). ^1H NMR ($CDCl_3$) δ 2.02 (s, 6H), 2.36–2.47 (m, 2H), 2.56–2.67 (m, 1H), 2.76–2.85 (m, 1H), 3.00 (s, 3H), 3.43–3.52 (m, 2H), 3.75–3.87 (m, 1H), 4.16 (t, *J* = 5.7 Hz, 2H), 4.29 (dd, *J* = 9.1, 6.0 Hz, 1H), 4.76 (t, *J* = 9.1 Hz, 1H), 5.06 (s, 2H), 6.44–6.51 (m, 2H), 7.00–7.08 (m, 2H), 7.11 (s, 1H), 7.39–7.49 (m, 2H). MS *m/z* 593 $(M + H)^+$. HPLC purity (220 nm) 99.8%. Anal. Calcd for $C_{29}H_{30}Cl_2O_7S$: C, 58.69; H, 5.09. Found: C, 58.69; H, 4.99.

(3-Methyloxetan-3-yl)methyl 4-Methylbenzenesulfonate (99a). To a suspension of *p*-TsCl (14.3 g, 75.0 mmol) in pyridine (60 mL) was added slowly 3-methyl-3-oxetane-methanol (**98a**) (5.11 g, 50.0 mmol) at 0 °C, and the mixture was stirred under nitrogen atmosphere at 0 °C for 4 h. The mixture was added to ice water, and stirred for 1 h. The precipitate was collected by filtration, washed with cold water, and dried to give **99a** (8.97 g, 70%) as colorless crystals. mp 60–61 °C. ^1H NMR ($CDCl_3$) δ 1.31 (s, 3H), 2.47 (s, 3H), 4.11 (s, 2H), 4.32–4.39 (m, 4H), 7.37 (d, *J* = 8.4 Hz, 2H), 7.81 (d, *J* = 8.4 Hz, 2H). MS *m/z* 257 $(M + H)^+$. HPLC purity (220 nm) 98.6%. Anal. Calcd for $C_{12}H_{16}O_4S$: C, 56.23; H, 6.29. Found: C, 56.21; H, 6.22.

3-(Methylsulfonyl)propyl 4-Methylbenzenesulfonate (99b). Step 1: To a solution of 3-methylthio-1-propanol (**98b**) (5.30 g, 50.0 mmol), Et_3N (10.5 mL, 75.0 mmol), and *N*,*N*,*N*′,*N*′-tetramethyl-1,6-hexanediamine (0.861 g, 5.00 mmol) in toluene (50 mL) was added dropwise *p*-TsCl (14.3 g, 75.0 mmol) in toluene (50 mL) at 0 °C, and the mixture was stirred

under nitrogen atmosphere at 0 °C for 3 h. The mixture was quenched with water and extracted with AcOEt. The extract was washed with brine, dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 10:90–40:60) to give 3-(methylthio)propyl 4-methylbenzenesulfonate (12.2 g, 94%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.87–1.98 (m, 2H), 2.04 (s, 3H), 2.45 (s, 3H), 2.51 (t, *J* = 7.1 Hz, 2H), 4.14 (t, *J* = 6.1 Hz, 2H), 7.35 (d, *J* = 8.2 Hz, 2H), 7.80 (d, *J* = 8.2 Hz, 2H). MS *m/z* 261 $(M + H)^+$. Step 2: To a solution of the obtained sulfonate (12.2 g, 46.9 mmol) in MeOH (250 mL) was added dropwise a solution of Oxone® (57.7 g, 93.8 mmol) in water (250 mL) at 0 °C, and the mixture was stirred at 0 °C to room temperature for 20 h. After evaporation of the solvent, the residue was diluted with water, and extracted with AcOEt. The extract was washed with brine, dried over anhydrous Na_2SO_4, and concentrated. The resulting crystals were washed with heptane–AcOEt to give **99b** (13.1 g, 96%) as colorless crystals. mp 88–89 °C. ^{1}H NMR ($CDCl_3$) δ 2.17–2.28 (m, 2H), 2.46 (s, 3H), 2.92 (s, 3H), 3.07–3.15 (m, 2H), 4.18 (t, *J* = 5.9 Hz, 2H), 7.37 (d, *J* = 8.3 Hz, 2H), 7.79 (d, *J* = 8.3 Hz, 2H). MS *m/z* 293 $(M + H)^+$. Anal. Calcd for $C_{11}H_{16}O_5S_2$: C, 45.19; H, 5.52. Found: C, 44.96; H, 5.53.

3′-(Hydroxymethyl)-2,6-dimethylbiphenyl-4-ol (100). To a solution of 4′-hydroxy-2′,6′-dimethylbiphenyl-3-carbaldehyde (**44**) (6.95 g, 30.7 mmol) in MeOH (30 mL) and THF (60 mL) was added gradually $NaBH_4$ (1.29 g, 30.7 mmol) at 0 °C, and the mixture was stirred under nitrogen atmosphere at 0 °C to room temperature for 20 h. The mixture was concentrated, quenched with water and 1 M HCl aqueous solution, and extracted with AcOEt. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated to give crystals. Recrystallization from heptane–AcOEt gave **100** (6.56 g, 93%) as colorless crystals. mp 175 °C. ^{1}H NMR ($CDCl_3$) δ 1.67 (t, *J* = 5.8 Hz, 1H), 1.98 (s, 6H), 4.65 (s, 1H), 4.74 (d, *J* = 5.8 Hz, 2H), 6.59 (s, 2H), 7.06 (dt, *J* = 7.3, 1.5 Hz, 1H), 7.12 (s, 1H), 7.33 (dt, *J* = 7.5, 1.5 Hz, 1H), 7.41 (t, *J* = 7.5 Hz, 1H). MS *m/z* 211 $(M - 18 + H)^+$. Anal. Calcd for $C_{15}H_{16}O_2$: C, 78.92; H, 7.06. Found: C, 78.76; H, 7.04.

[4′-(2-Ethoxyethoxy)-2′,6′-dimethylbiphenyl-3-yl]methanol (74a). A mixture of **100** (4.57 g, 20.0 mmol), 2-chloroethyl ethyl ether (3.29 mL, 30.0 mmol), K_2CO_3 (3.32 g, 24.0 mmol), and KI (0.332 g, 2.00 mmol) in DMF (30 mL) was stirred under nitrogen atmosphere at 80 °C for 70 h. The mixture was diluted with water and extracted with AcOEt. The organic layer was washed sequentially with 1 M NaOH aqueous solution and brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by basic silica gel column chromatography (AcOEt:hexane = 20:80–60:40), and crystallized from heptane–AcOEt to give **74a** (4.43 g, 74%) as colorless crystals. mp 62–63 °C. ^{1}H NMR ($CDCl_3$) δ 1.25 (t, *J* = 7.1 Hz, 3H), 1.66 (t, *J* = 5.9 Hz, 1H), 2.00 (s, 6H), 3.62 (q, *J* = 7.1 Hz, 2H), 3.80 (t, *J* = 5.1 Hz, 2H), 4.14 (t, *J* = 5.1 Hz, 2H), 4.73 (d, *J* = 5.9 Hz, 2H), 6.69 (s, 2H), 7.06 (d, *J* = 7.3 Hz, 1H),

7.12 (s, 1H), 7.33 (d, J = 7.3 Hz, 1H), 7.40 (t, J = 7.3 Hz, 1H). MS *m/z* 301 $(M + H)^+$. Anal. Calcd for $C_{19}H_{24}O_3$: C, 75.97; H, 8.05. Found: C, 75.75; H, 8.10.

{4′-[2-(Ethylthio)ethoxy]-2′,6′-dimethylbiphenyl-3-yl}methanol (74e). The title compound was prepared from **100** and 2-chloroethyl ethyl sulfide by a method similar to that described for **74a** in 47% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.31 (t, J = 7.3 Hz, 3H), 1.67 (t, J = 5.8 Hz, 1H), 2.00 (s, 6H), 2.67 (q, J = 7.3 Hz, 2H), 2.92 (t, J = 7.0 Hz, 2H), 4.16 (t, J = 7.0 Hz, 2H), 4.73 (d, J = 5.8 Hz, 2H), 6.66 (s, 2H), 7.06 (dt, J = 7.3, 1.3 Hz, 1H), 7.12 (s, 1H), 7.30–7.36 (m, 1H), 7.41 (t, J = 7.3 Hz, 1H). MS *m/z* 299 $(M - 18 + H)^+$.

Compounds **101a**–**c** were prepared from **44** and appropriate alkylating agents (**99a**, 1-oxa-6-thiaspiro[2.5]octane, or **99b**) by a method similar to that described for **74a**.

2′,6′-Dimethyl-4′-[(3-methyloxetan-3-yl)methoxy]biphenyl-3-carbaldehyde (101a). 98% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.46 (s, 3H), 2.01 (s, 6H), 4.06 (s, 2H), 4.48 (d, J = 5.8 Hz, 2H), 4.65 (d, J = 5.8 Hz, 2H), 6.73 (s, 2H), 7.42 (dt, J = 7.6, 1.4 Hz, 1H), 7.59 (t, J = 7.6 Hz, 1H), 7.67 (t, J = 1.4 Hz, 1H), 7.87 (dt, J = 7.6, 1.4 Hz, 1H), 10.05 (s, 1H). MS *m/z* 333 $(M + Na)^+$.

4′-[(4-Hydroxytetrahydro-2*H*-thiopyran-4-yl)methoxy]-2′,6′-dimethylbiphenyl-3-carbaldehyde (101b). 89% yield as colorless crystals. ^{1}H NMR ($CDCl_3$) δ 1.70 (t, J = 5.8 Hz, 1H), 1.76–1.90 (m, 2H), 2.01 (s, 6H), 2.05–2.16 (m, 2H), 2.20 (s, 1H), 2.40–2.53 (m, 2H), 3.03–3.18 (m, 2H), 3.80 (s, 2H), 4.73 (d, J = 5.8 Hz, 2H), 6.67 (s, 2H), 7.02–7.09 (m, 1H), 7.12 (s, 1H), 7.31–7.37 (m, 1H), 7.41 (t, J = 7.4 Hz, 1H).

2′,6′-Dimethyl-4′-[3-(methylsulfonyl)propoxy]biphenyl-3-carbaldehyde (101c). 77% yield as colorless crystals. mp 91–94 °C. ^{1}H NMR ($CDCl_3$) δ 1.99 (s, 6H), 2.30–2.42 (m, 2H), 2.97 (s, 3H), 3.24–3.32 (m, 2H), 4.14 (t, J = 5.7 Hz, 2H), 6.67 (s, 2H), 7.41 (dt, J = 7.6, 1.5 Hz, 1H), 7.59 (t, J = 7.6 Hz, 1H), 7.66 (t, J = 1.5 Hz, 1H), 7.87 (dt, J = 7.6, 1.5 Hz, 1H), 10.05 (s, 1H). MS *m/z* 347 $(M + H)^+$. Anal. Calcd for $C_{19}H_{22}O_4S$: C, 65.87; H, 6.40. Found: C, 65.82; H, 6.47.

4′-{*tert*-Butyl(dimethyl)silyl}oxy}-2′,6′-dimethylbiphenyl-3-carbaldehyde (101d). To a solution of **44** (9.0 g, 39.8 mmol) and imidazole (2.98 g, 43.8 mmol) in DMF (100 mL) was added TBSCl (6.6 g, 43.8 mmol) at room temperature, and the mixture was stirred at room temperature for 4 h. The mixture was diluted with AcOEt, washed sequentially with water and brine, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 9:91–20:80) to give **101d** (10.5 g, 77%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 0.25 (s, 6H), 1.02 (s, 9H), 1.97 (s, 6H), 6.62 (s, 2H), 7.44 (dt, J = 7.5, 1.5 Hz, 1H), 7.59 (t, J = 7.5 Hz, 1H), 7.68 (t, J = 1.5 Hz, 1H), 7.86 (dt, J = 7.5, 1.5 Hz, 1H), 10.06 (s, 1H). MS *m/z* 341 $(M + H)^+$.

Compounds **74b**, **d**, **f**, and **g** were prepared from **101a**–**d** by a method similar to that described for **100**.

{2′,6′-Dimethyl-4′-[(3-methyloxetan-3-yl)methoxy]biphenyl-3-yl}methanol (74b). 92% yield as colorless crystals. mp 82 °C. ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 3H), 1.68 (t, *J* = 5.9 Hz, 1H), 2.01 (s, 6H), 4.05 (s, 2H), 4.47 (d, *J* = 5.9 Hz, 2H), 4.65 (d, *J* = 5.9 Hz, 2H), 4.74 (d, *J* = 5.9 Hz, 2H), 6.71 (s, 2H), 7.07 (d, *J* = 7.5 Hz, 1H), 7.13 (s, 1H), 7.32–7.37 (m, 1H), 7.41 (t, *J* = 7.5 Hz, 1H). MS *m/z* 313 $(M + H)^+$. HPLC purity (220 nm) 98.0%. Anal. Calcd for $C_{20}H_{24}O_3$: C, 76.89; H, 7.74. Found: C, 76.71; H, 7.87.

4-({[3′-(Hydroxymethyl)-2,6-dimethylbiphenyl-4-yl]oxy}methyl)tetrahydro-2*H*-thiopyran-4-ol (74d). 94% yield as colorless crystals. ^{1}H NMR ($CDCl_3$) δ 1.70 (t, *J* = 5.8 Hz, 1H), 1.76–1.90 (m, 2H), 2.01 (s, 6H), 2.05–2.16 (m, 2H), 2.20 (s, 1H), 2.40–2.53 (m, 2H), 3.03–3.18 (m, 2H), 3.80 (s, 2H), 4.73 (d, *J* = 5.8 Hz, 2H), 6.67 (s, 2H), 7.02–7.09 (m, 1H), 7.12 (s, 1H), 7.31–7.37 (m, 1H), 7.41 (t, *J* = 7.4 Hz, 1H).

{2′,6′-Dimethyl-4′-[3-(methylsulfonyl)propoxy]biphenyl-3-yl}methanol (74f). 97% yield as colorless crystals. mp 96–98 °C. ^{1}H NMR ($CDCl_3$) δ 1.68 (t, *J* = 5.9 Hz, 1H), 2.00 (s, 6H), 2.30–2.40 (m, 2H), 2.97 (s, 3H), 3.24–3.31 (m, 2H), 4.13 (t, *J* = 5.7 Hz, 2H), 4.73 (d, *J* = 5.9 Hz, 2H), 6.64 (s, 2H), 7.03–7.08 (m, 1H), 7.12 (s, 1H), 7.31–7.37 (m, 1H), 7.41 (t, *J* = 7.5 Hz, 1H). MS *m/z* 331 $(M - 18 + H)^+$. Anal. Calcd for $C_{19}H_{24}O_4S$: C, 65.49; H, 6.94. Found: C, 65.25; H, 7.19.

(4′-{*tert*-Butyl(dimethyl)silyl}oxy)-2′,6′-dimethylbiphenyl-3-yl)methnol (74g). 94% yield as colorless crystals. ^{1}H NMR ($CDCl_3$) δ 0.23 (s, 6H), 1.00 (s, 9H), 1.96 (s, 6H), 4.73 (s, 2H), 6.58 (s, 2H), 7.07 (d, *J* = 7.5 Hz, 1H), 7.13 (s, 1H), 7.32 (t, *J* = 7.5 Hz, 1H), 7.40 (t, *J* = 7.5 Hz, 1H).

[2,6-Dimethyl-4-(tetrahydro-2*H*-thiopyran-4-yloxy)phenyl]boronic Acid (102). Step 1: 4-(4-Bromo-3,5-dimethylphenoxy)tetrahydro-2*H*-thiopyran was prepared from **43** and tetrahydro-4*H*-thiopyran-4-ol by a method similar to that described for **88**-step 1 in 86% yield as a white solid. ^{1}H NMR ($CDCl_3$) δ 1.93–2.07 (m, 2H), 2.10–2.23 (m, 2H), 2.37 (s, 6H), 2.49–2.62 (m, 2H), 2.85–2.98 (m, 2H), 4.25–4.36 (m, 1H), 6.65 (s, 2H). Step 2: To a solution of the obtained solid (3.01 g, 10.0 mmol) in THF (50 mL) was added dropwise a solution of 1.6 M *n*-BuLi in hexane (6.57 mL, 10.5 mmol) under argon atmosphere at −78 ˚C. The mixture was stirred at −78 °C for 1.5 h and then B(*i*-PrO)$_3$ (6.92 mL, 30.0 mmol) was added at the same temperature. The mixture was gradually warmed to room temperature and stirred for 16 h. After the mixture was cooled to 0 °C, 2 M HCl aqueous solution (50 mL) was added. The resulting mixture was stirred at 0 °C for 2.5 h. The phases were separated, and the aqueous phase was extracted with AcOEt (pH of the aqueous phase was adjusted to neutral with saturated $NaHCO_3$ aqueous solution). The combined organic phase was dried over anhydrous $MgSO_4$, and concentrated to give a white solid. The resulting solid was washed with cold hexane and dried to afford **102** (1.89 g, 71%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 1.90–2.06 (m, 2H), 2.09–2.23 (m, 2H), 2.35 (s, 6H), 2.48–2.62 (m, 2H), 2.83–2.98 (m, 2H), 4.28–4.40 (m, 1H), 6.51 (s, 2H), 6.59 (s, 2H).

{4′-[(1,1-Dioxidotetrahydro-2*H*-thiopyran-4-yl)oxy]-2′,6′-dimethylbiphenyl-3-yl}methanol (74c). Step 1: A mixture of **102** (1.41 g, 5.30 mmol), methyl 3-bromobenzoate (1.14 g, 5.30 mmol), and $Pd(PPh_3)_4$ (0.306 g, 0.265 mmol) in 2 M Cs_2CO_3 (6.35 mL) and DME (20 mL) was stirred under argon atmosphere at 95 °C for 24 h. The mixture was diluted with water, and extracted with AcOEt. The combined organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 0:100–25:75) to give crystals. Recrystallization from hexane–AcOEt gave methyl 2′,6′-dimethyl-4′-(tetrahydro-2*H*-thiopyran-4-yloxy)biphenyl-3-carboxylate (1.63 g, 86%) as colorless crystals. mp 69–71 °C. ^{1}H NMR ($CDCl_3$) δ 1.92–2.13 (m, 8H), 2.15–2.29 (m, 2H), 2.52–2.66 (m, 2H), 2.89–3.03 (m, 2H), 3.91 (s, 3H), 4.33–4.44 (m, 1H), 6.66 (s, 2H), 7.34 (dt, *J* = 7.8, 1.5 Hz, 1H), 7.49 (t, *J* = 7.8 Hz, 1H), 7.84 (t, *J* = 1.5 Hz, 1H), 8.01 (dt, *J* = 7.8, 1.5 Hz, 1H). MS *m/z* 379 $(M + Na)^+$. HPLC purity (220 nm) 99.7%. Anal. Calcd for $C_{21}H_{24}O_3S$: C, 70.75; H, 6.79. Found: C, 70.73; H, 6.80. Step 2: Methyl 4′-[(1,1-dioxidotetrahydro-2*H*-thiopyran-4-yl)oxy]-2′,6′-dimethylbiphenyl-3-carboxylate was prepared from the obtained crystals by a method similar to that described for **83**-step 2 in 85% yield as colorless crystals. mp 180 °C. ^{1}H NMR ($CDCl_3$) δ 1.99 (s, 6H), 2.30–2.45 (m, 2H), 2.45–2.59 (m, 2H), 2.88–3.02 (m, 2H), 3.37–3.53 (m, 2H), 3.92 (s ,3H), 4.63–4.72 (m, 1H), 6.69 (s, 2H), 7.33 (dt, *J* = 7.6, 1.4 Hz, 1H), 7.50 (t, *J* = 7.6 Hz, 1H), 7.83 (t, *J* = 1.6 Hz, 1H), 8.02 (dt, *J* = 7.6, 1.4 Hz, 1H). MS *m/z* 389 $(M + H)^+$. HPLC purity (220 nm) 98.6%. Step 3: To a solution of the obtained crystals (0.128 g, 0.33 mmol) in THF (2 mL) was added gradually $LiAlH_4$ (80%, 15.7 mg, 0.33 mmol) at 0 °C. The mixture was stirred at 0 °C for 1.5 h, followed by gradually addition of $Na_2SO_4 \cdot 10\ H_2O$ at the same temperature. After stirring at room temperature for 1 h, the mixture was filtered through a pad of Celite. The filtrate was concentrated to afford **74c** (0.111 g, 93%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 1.76 (t, *J* = 5.6 Hz, 1H), 2.00 (s, 6H), 2.29–2.44 (m, 2H), 2.44–2.58 (m, 2H), 2.87–3.02 (m, 2H), 3.37–3.53 (m, 2H), 4.63–4.70 (m, 1H), 4.74 (d, *J* = 5.6 Hz, 2H), 6.68 (s, 2H), 7.05 (dt, *J* = 7.4, 1.5 Hz, 1H), 7.12 (s, 1H), 7.31–7.38 (m, 1H), 7.42 (t, *J* = 7.4 Hz, 1H). MS *m/z* 343 $(M - 18 + H)^+$. HPLC purity (220 nm) 97.1%.

3,5-Diethylphenol (104). A mixture of 4-ethylphenol (**103**) (25.7 g, 210 mmol) and $AlCl_3$ (62.5 g, 469 mmol) was stirred under nitrogen atmosphere at 115 °C for 4 h. The reaction mixture was cooled to 60 °C, poured onto crushed ice, and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 0:100–25:75) to give **104** (12.3 g, 78%) as a reddish brown oil. ^{1}H NMR ($CDCl_3$) δ 1.21 (t, *J* = 7.7 Hz, 6H), 2.58 (q, *J* = 7.7 Hz, 4H), 4.66 (s, 1H), 6.49–6.52 (m, 2H), 6.60–6.63 (m, 1H). MS *m/z* 151 $(M + H)^+$.

4-Bromo-3,5-diethylphenol (105a). To a solution of **104** (3.00 g, 20.0 mmol) in MeOH (30 mL) was added *n*-Bu_4NBr_3 (9.64 g, 20.0 mmol) at room temperature, and the mixture was stirred for 1 h. After evaporation of the solvent, the residue was diluted with water, and

extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 0:100–25:75) to give **105a** (3.28 g, 72%). An analytical sample was obtained as colorless crystals (heptane). ^{1}H NMR ($CDCl_3$) δ 1.21 (t, J = 7.6 Hz, 6H), 2.73 (q, J = 7.6 Hz, 4H), 4.65 (s, 1H), 6.59 (s, 2H). Anal. Calcd for $C_{10}H_{13}BrO$: C, 52.42; H, 5.72. Found: C, 52.22; H, 5.66.

2-Hydroxy-3,4,6-trimethylbenzaldehyde (107). To a solution of 2,3,5-trimethylphenol (**106**) (13.6 g, 100 mmol) in CH_2Cl_2 (20 mL) was added dropwise $TiCl_4$ (41.7 g, 220 mmol) under nitrogen atmosphere at 0 °C over 0.5 h and the mixture was stirred at 0 °C for 1 h. To the mixture was added dropwise dichloromethyl methyl ether (11.5 g, 100 mmol), and the mixture was stirred at 0 °C for 6 h. The mixture was quenched with saturated NH_4Cl aqueous solution and extracted with CH_2Cl_2. The organic layer was washed sequentially with diluted HCl aqueous solution, $NaHCO_3$ aqueous solution and brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 5:95–50:50) to give **107** (6.58 g, 40%) as pale brown crystals. ^{1}H NMR ($CDCl_3$) δ 2.13 (s, 3H), 2.27 (s, 3H), 2.53 (s, 3H), 6.53 (s, 1H), 10.23 (s, 1H), 12.29 (s, 1H). MS *m/z* 165 $(M + H)^+$.

2,3,5,6-Tetramethylphenol (108). Compound **107** (6.58 g, 40.1 mmol) was hydrogenated on 10% Pd/C (1.0 g, containing 50% water) in MeOH (120 mL) under H_2 atmosphere (balloon pressure) at room temperature for 22 h. The catalyst was removed by filtration, and the filtrate was concentrated to give **108** (5.83 g, 97%). An analytical sample was obtained from MeOH as colorless plates. ^{1}H NMR ($CDCl_3$) δ 2.14 (s, 6H), 2.22 (s, 6H), 4.59 (s, 1H), 6.60 (s, 1H). MS *m/z* 151 $(M + H)^+$. Anal. Calcd for $C_{10}H_{14}O$: C, 79.96; H, 9.39. Found: C, 80.02; H, 9.42.

4-Bromo-2,3,5,6-tetramethylphenol (105b). To a suspension of **108** (5.10 g, 34.0 mmol) in AcOH (90 mL) was added dropwise a solution of Br_2 (1.98 mL, 38.6 mmol) in AcOH (30 mL) at room temperature, and the mixture was stirred at room temperature for 5 h. The mixture was concentrated, and the residue was diluted with AcOEt, washed sequentially with $Na_2S_2O_3$ aqueous solution and brine, dried over anhydrous $MgSO_4$, and concentrated to give **105b** (6.48 g, 83%). An analytical sample was obtained from petroleum ether as off-white crystals. ^{1}H NMR ($CDCl_3$) δ 2.23 (s, 6H), 2.40 (s, 6H), 4.59 (s, 1H).

4-Bromo-2-fluoro-3,5-dimethylphenol (105c). A mixture of **43** (2.00 g, 9.95 mmol) and *N*-fluoropyridinium triflate (6.15 g, 24.9 mmol) in 1,2-dichloroethane (20 mL) was stirred at reflux for 7 h. The mixture was quenched with 1 M $Na_2S_2O_3$ aqueous solution and extracted with AcOEt. The extract was washed sequentially with water and brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 0:100–30:70) to afford **105c** (0.790 g, 36%) as colorless

crystals. ^{1}H NMR ($CDCl_3$) δ 2.29–2.36 (m, 6H), 5.04 (d, *J* = 4.0 Hz, 1H), 6.79 (d, *J* = 9.0 Hz, 1H).

Compound **109a–c** was prepared from **105a–c** and 3-formylphenylboronic acid by a method similar to that described for **74b**-step 1.

2′,6′-Diethyl-4′-hydroxybiphenyl-3-carbaldehyde (109a). 68% yield as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.00 (t, *J* = 7.5 Hz, 6H), 2.25 (q, *J* = 7.5 Hz, 4H), 4.92 (s, 1H), 6.65 (s, 2H), 7.44 (dt, *J* = 7.6, 1.5 Hz, 1H), 7.58 (t, *J* = 7.6 Hz, 1H), 7.68 (t, *J* = 1.5 Hz, 1H), 7.87 (dt, *J* = 7.6, 1.5 Hz, 1H), 10.05 (s, 1H). MS *m/z* 255 $(M + H)^+$.

4′-Hydroxy-2′,3′,5′,6′-tetramethylbiphenyl-3-carbaldehyde (109b). 79% yield as colorless crystals. mp 136–137 °C. ^{1}H NMR ($CDCl_3$) δ 1.90 (s, 6H), 2.22 (s, 6H), 4.73 (s, 1H), 7.39 (dt, *J* = 7.6, 1.5 Hz, 1H), 7.58 (t, *J* = 7.6 Hz, 1H), 7.63 (t, *J* = 1.5 Hz, 1H), 7.86 (dt, *J* = 7.6, 1.5 Hz, 1H), 10.05 (s, 1H). MS *m/z* 255 $(M + H)^+$. Anal. Calcd for $C_{17}H_{18}O_2$: C, 80.28; H, 7.13. Found: C, 80.36; H, 7.20.

3′-Fluoro-4′-hydroxy-2′,6′-dimethylbiphenyl-3-carbaldehyde (109c). 49% yield as colorless crystals (heptane–AcOEt). mp 116–117 °C. ^{1}H NMR ($CDCl_3$) δ 1.91–1.97 (m, 6H), 5.10 (d, *J* = 4.7 Hz, 1H), 6.78 (d, *J* = 8.9 Hz, 1H), 7.40 (dt, *J* = 7.6, 1.5 Hz, 1H), 7.61 (t, *J* = 7.6 Hz, 1H), 7.65 (t, *J* = 1.5 Hz, 1H), 7.88 (dt, *J* = 7.6, 1.5 Hz, 1H), 10.06 (s, 1H). MS *m/z* 245 $(M + H)^+$. Anal. Calcd for $C_{15}H_{13}FO_2$: C, 73.76; H, 5.36. Found: C, 73.64; H, 5.29.

3′-Chloro-4′-hydroxy-2′,6′-dimethylbiphenyl-3-carbaldehyde (109d). To a solution of **44** (11.3 g, 50.0 mmol) in DMF (50 mL) was added gradually NCS (6.68 g, 50.0 mmol) at 0 °C, and the mixture was stirred at room temperature for 13 h. The mixture was heated to 50 °C, and stirred for 3 h. To the mixture was added NCS (1.34 g, 10.0 mmol), and the mixture was stirred at 50 °C for 3 h. To the mixture was added NCS (0.668 g, 5.00 mmol), and the resulting mixture was stirred at 50 °C for 1 h. The mixture was poured into water, and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 5:95–40:60) to give crystals. Recrystallization from heptane–AcOEt gave **109d** (8.47 g, 65%) as colorless crystals. mp 85–86 °C. ^{1}H NMR ($CDCl_3$) δ 1.95 (s, 3H), 2.05 (s, 3H), 5.61 (s, 1H), 6.84 (s, 1H), 7.36–7.42 (m, 1H), 7.57–7.66 (m, 2H), 7.85–7.91 (m, 1H), 10.06 (s, 1H). MS *m/z* 261 $(M + H)^+$. Anal. Calcd for $C_{15}H_{13}ClO_2$: C, 69.10; H, 5.03. Found: C, 69.16; H, 4.97.

3′,5′-Dichloro-4′-hydroxy-2′,6′-dimethylbiphenyl-3-carbaldehyde (109e). To a solution of **44** (11.3 g, 50.0 mmol) in DMF (50 mL) was added gradually NCS (13.4 g, 100 mmol) at 0 °C, and the mixture was stirred at room temperature for 14 h. The mixture was heated to 50 °C, and the mixture was stirred for 2 h. The mixture was poured into water, and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The resulting crystals were triturated with heptane–AcOEt to give **109e**

(8.88 g, 60%) as colorless crystals. mp 116–117 °C. ^{1}H NMR ($CDCl_3$) δ 2.03 (s, 6H), 6.00 (s, 1H), 7.35–7.40 (m, 1H), 7.60–7.66 (m, 2H), 7.88–7.94 (m, 1H), 10.06 (s, 1H). MS *m/z* 294 $(M + H)^+$. Anal. Calcd for $C_{15}H_{12}Cl_2O_2$: C, 61.04; H, 4.10. Found: C, 60.91; H, 3.98.

Compounds **110b–c** were prepared from **109b–c** by a method similar to that described for **100**.

3′-(Hydroxymethyl)-2,3,5,6-tetramethylbiphenyl-4-ol (110b). 93% yield as colorless crystals (heptane−AcOEt). mp 152–153 °C. ^{1}H NMR ($CDCl_3$) δ 1.65 (t, *J* = 5.9 Hz, 1H), 1.91 (s, 6H), 2.21 (s, 6H), 4.68 (s, 1H), 4.73 (d, *J* = 5.9 Hz, 2H), 7.01–7.06 (m, 1H), 7.08–7.10 (m, 1H), 7.31–7.36 (m, 1H), 7.40 (t, *J* = 7.4 Hz, 1H). MS *m/z* 239 $(M - 18 + H)^+$. Anal. Calcd for $C_{17}H_{20}O_2$: C, 79.65; H, 7.86. Found: C, 79.32; H, 7.97.

3-Fluoro-3′-(hydroxymethyl)-2,6-dimethylbiphenyl-4-ol (110c). 65% yield as colorless crystals. mp 123–124 °C. ^{1}H NMR ($CDCl_3$) δ 1.68 (t, *J* = 6.0 Hz, 1H), 1.90–1.97 (m, 6H), 4.74 (d, *J* = 6.0 Hz, 2H), 5.04 (d, *J* = 4.7 Hz, 1H), 6.75 (d, *J* = 8.9 Hz, 1H), 7.00–7.07 (m, 1H), 7.11 (s, 1H), 7.32–7.46 (m, 2H). MS *m/z* 229 $(M - 18 + H)^+$.

Compounds **74j–l** were prepared from **110b–c** and appropriate tosylates (1,1-dioxidotetrahydro-2*H*-thiopyran-4-yl 4-methylbenzenesulfonate or **99b**) by a method similar to that described for **74a**.

{4′-[(1,1-Dioxidotetrahydro-2*H*-thiopyran-4-yl)oxy]-2′,3′,5′,6′-tetramethylbiphenyl-3-yl}methanol (74j). 88% yield as colorless crystals (heptane–AcOEt). mp 203–205 °C. ^{1}H NMR ($CDCl_3$) δ 1.67 (t, *J* = 5.9 Hz, 1H), 1.88 (s, 6H), 2.21 (s, 6H), 2.29–2.55 (m, 4H), 2.96–3.08 (m, 2H), 3.31–3.44 (m, 2H), 3.95–4.04 (m, 1H), 4.74 (d, *J* = 5.9 Hz, 2H), 7.02 (d, *J* = 7.4 Hz, 1H), 7.08 (s, 1H), 7.32–7.37 (m, 1H), 7.41 (t, *J* = 7.4 Hz, 1H). MS *m/z* 371 $(M - 18 + H)^+$. Anal. Calcd for $C_{22}H_{28}O_4S$: C, 68.01; H, 7.26. Found: C, 67.93; H, 7.32.

{2′,3′,5′,6′-Tetramethyl-4′-[3-(methylsulfonyl)propoxy]biphenyl-3-yl}methanol (74k). 85% yield as colorless crystals (heptane–AcOEt). mp 132–134 °C. ^{1}H NMR ($CDCl_3$) δ 1.66 (t, *J* = 5.9 Hz, 1H), 1.88 (s, 6H), 2.20 (s, 6H), 2.32–2.43 (m, 2H), 3.00 (s, 3H), 3.35–3.43 (m, 2H), 3.86 (t, *J* = 5.8 Hz, 2H), 4.73 (d, *J* = 5.9 Hz, 2H), 7.03 (dt, *J* = 7.3, 1.3 Hz, 1H), 7.09 (s, 1H), 7.31–7.36 (m, 1H), 7.41 (t, *J* = 7.3 Hz, 1H). MS *m/z* 359 $(M - 18 + H)^+$. Anal. Calcd for $C_{21}H_{28}O_4S$: C, 66.99; H, 7.50. Found: C, 66.67; H, 7.32.

{4′-[(1,1-Dioxidotetrahydro-2*H*-thiopyran-4-yl)oxy]-3′-fluoro-2′,6′-dimethylbiphenyl-3-yl}methanol (74l). A crude product (quantitative) as a colorless oil. MS *m/z* 361 $(M - 18 + H)^+$.

4′-{[*tert*-Butyl(dimethyl)silyl]oxy}-3′-chloro-2′,6′-dimethylbiphenyl-3-carbaldehyde (111a). The title compound was prepared from **109d** by a method similar to that described for **101d** in 88% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 0.27 (s, 6H), 1.06 (s, 9H), 1.92 (s, 3H), 2.04 (s, 3H), 6.68 (s, 1H), 7.37–7.42 (m, 1H), 7.56–7.66 (m, 2H), 7.85–7.90 (m, 1H), 10.05 (s, 1H). MS *m/z* 375 $(M + H)^+$.

Compounds **111b–d** were prepared from tosylate **99b** and phenols **109a**, **109c**, or **109e** by a method similar to that described for **74a**.

2′,6′-Diethyl-4′-[3-(methylsulfonyl)propoxy]biphenyl-3-carbaldehyde (111b). 80% yield as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.00 (t, *J* = 7.5 Hz, 6H), 2.27 (q, *J* = 7.5 Hz, 4H), 2.32–2.43 (m, 2H), 2.98 (s, 3H), 3.24–3.33 (m, 2H), 4.17 (t, *J* = 5.9 Hz, 2H), 6.69 (s, 2H), 7.40–7.46 (m, 1H), 7.58 (t, *J* = 7.6 Hz, 1H), 7.65–7.70 (m, 1H), 7.84–7.90 (m, 1H), 10.05 (s, 1H). MS *m/z* 375 $(M + H)^+$.

3′-Fluoro-2′,6′-dimethyl-4′-[3-(methylsulfonyl)propoxy]biphenyl-3-carbaldehyde (111c). 95% yield as colorless crystals (heptane–AcOEt). mp 117–118 °C. ^{1}H NMR ($CDCl_3$) δ 1.93 (d, *J* = 2.8 Hz, 3H), 1.97 (s, 3H), 2.34–2.45 (m, 2H), 2.99 (s, 3H), 3.28–3.36 (m, 2H), 4.22 (t, *J* = 5.7 Hz, 2H), 6.73 (d, *J* = 8.3 Hz, 1H), 7.39 (dt, *J* = 7.6, 1.4 Hz, 1H), 7.58–7.66 (m, 2H), 7.89 (dt, *J* = 7.6, 1.4 Hz, 1H), 10.06 (s, 1H). MS *m/z* 365 $(M + H)^+$. Anal. Calcd for $C_{19}H_{21}FO_4S$: C, 62.62; H, 5.81. Found: C, 62.66; H, 5.81.

3′,5′-Dichloro-2′,6′-dimethyl-4′-[3-(methylsulfonyl)propoxy]biphenyl-3-carbaldehyde (111d). 53% yield as colorless crystals (heptane–AcOEt). mp 135–136 °C. ^{1}H NMR ($CDCl_3$) δ 2.03 (s, 6H), 2.37–2.48 (m, 2H), 3.00 (s, 3H), 3.44–3.51 (m, 2H), 4.18 (t, *J* = 5.7 Hz, 2H), 7.34–7.39 (m, 1H), 7.61–7.68 (m, 2H), 7.89–7.94 (m, 1H), 10.06 (s, 1H). MS *m/z* 415 $(M + H)^+$. Anal. Calcd for $C_{19}H_{20}Cl_2O_4S$: C, 54.94; H, 4.85. Found: C, 54.93; H, 4.89.

Compounds **74h**, **i**, **m**, **n** were prepared from **111a–d** by a method similar to that described for **100**.

(4′-{[*tert*-Butyl(dimethyl)silyl]oxy}-3′-chloro-2′,6′-dimethylbiphenyl-3-yl)methanol (74h). 97% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 0.26 (s, 6H), 1.06 (s, 9H), 1.69 (br s, 1H), 1.93 (s, 3H), 2.05 (s, 3H), 4.74 (s, 2H), 6.66 (s, 1H), 7.01–7.07 (m, 1H), 7.09–7.13 (m, 1H), 7.32–7.45 (m, 2H). MS *m/z* 377 $(M + H)^+$.

{2′,6′-Diethyl-4′-[3-(methylsulfonyl)propoxy]biphenyl-3-yl}methanol (74i). 84% yield as colorless crystals (heptane–AcOEt). mp 115–116 °C. ^{1}H NMR ($CDCl_3$) δ 1.01 (t, *J* = 7.5 Hz, 6H), 1.66 (t, *J* = 5.9 Hz, 1H), 2.24–2.42 (m, 6H), 2.97 (s, 3H), 3.25–3.33 (m, 2H), 4.16 (t, *J* = 5.7 Hz, 2H), 4.73 (d, *J* = 5.9 Hz, 2H), 6.67 (s, 2H), 7.06–7.10 (m, 1H), 7.12–7.16 (m, 1H), 7.32–7.43 (m, 2H). MS *m/z* 359 $(M - 18 + H)^+$. Anal. Calcd for $C_{21}H_{28}O_4S$: C, 66.99; H, 7.50. Found: C, 66.92; H, 7.46.

{3′-Fluoro-2′,6′-dimethyl-4′-[3-(methylsulfonyl)propoxy]biphenyl-3-yl}methanol (74m). 94% yield as colorless crystals (heptane–AcOEt). mp 62–63 °C. ^{1}H NMR ($CDCl_3$) δ 1.70 (t, *J* = 5.9 Hz, 1H), 1.93 (d, *J* = 3.0 Hz, 3H), 1.97 (s, 3H), 2.32–2.45 (m, 2H), 2.98 (s, 3H), 3.27–3.37 (m, 2H), 4.20 (t, *J* = 5.8 Hz, 2H), 4.74 (d, *J* = 5.9 Hz, 2H), 6.70 (d, *J* = 8.3 Hz, 1H), 6.99–7.08 (m, 1H), 7.10 (s, 1H), 7.32–7.47 (m, 2H). MS *m/z* 349 $(M - 18 + H)^+$. Anal. Calcd for $C_{19}H_{23}FO_4S$: C, 62.27; H, 6.33. Found: C, 62.63; H, 6.65.

{3′,5′-Dichloro-2′,6′-dimethyl-4′-[3-(methylsulfonyl)propoxy]biphenyl-3-yl}methanol (74n). 98% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.76 (t, *J* = 5.7 Hz, 1H), 2.03 (s,

6H), 2.36–2.47 (m, 2H), 3.00 (s, 3H), 3.43–3.51 (m, 2H), 4.16 (t, J = 5.7 Hz, 2H), 4.75 (d, J = 5.7 Hz, 2H), 6.97–7.03 (m, 1H), 7.07–7.08 (m, 1H), 7.36–7.48 (m, 2H). MS m/z 417 $(M + H)^+$.

Caspase-3/7 Activity Assay. HepG2 cells were cultured at 37°C, 5% CO_2 in DMEM supplemented with 10% fetal bovine serum, 50 IU/ml penicillin and 50 μg/ml streptomycin. Cells were seeded at 2×10^4cells/well in a 96-well white plate (Costar), and cultured with test compounds in DMEM supplemented with 0.5% fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 50 IU/ml penicillin and 50 μg/ml streptomycin for 1 day. Caspase-3/7 activity was measured by using Caspase-GloTM 3/7 assay Kit (Promega) according to the manufacture's instruction. Caspase-3/7 activity was calculated (n = 3) to the following. Caspase-3/7 activity (%) = (RLU of compound - RLU of 1% DMSO) / (RLU of 30 μM Staurosporine - RLU of 1% DMSO)×100.

Pharmacokinetic Analysis in Rat Cassette Dosing. Test compounds were administered as a cassette dosing to fasted rats. After oral administration, blood samples were collected. The blood samples were centrifuged to obtain the plasma fraction. The plasma samples were deproteinized with MeCN containing an internal standard. After centrifugation, the supernatant was diluted and centrifuged again. The compound concentrations in the supernatant were measured by LC/MS/MS.

Homology Modeling and Ligand Docking. A homology model of GPR40 was constructed using the crystal structure of bovine rhodopsin (PDB code 1GZM),[74] which obtained from the RCSB Protein Data Bank, as a structural template. An alignment of the amino acid sequences between GPR40 and rhodopsin was created using Clustal X (version 2.0.11)[75] and manually revised. Procedures of homology modeling were performed in MOE (version 2008.10).[76] The CL2 loop on the extra cellular domain was excluded except Cys170 forming disulfide bond due to the difficulty of estimation. In the previous step, compound **7** was docked into the obtained receptor model using the program GOLD (version 4.1).[77] Then, the resultant docking modes with receptor models, replacing compound **18** with **85**, were subjected energy minimization with MOE after connecting each residual substituent. In the energy minimization process, the MMFF94s force field was used and the dielectric constant was set to 2*r, where r is the distance between two interacting atoms.

Oral Glucose Tolerance Test (OGTT). The care and use of the animals and the experimental protocols used in this research were approved by the Experimental Animal Care and Use Committee of Takeda Pharmaceutical Company Limited. Female Wistar fatty WF rats and Male GK rats were obtained from Takeda Rabics Limited (Hikari, Japan). They were fed the commercial diet CE-2 (Clea Japan Co.) and tap water ad libitum. Female WF (12 – 17 weeks old) and male GK (41 weeks old) rats were fasted overnight and orally given vehicle (0.5% methylcellulose) or compounds. All animals received an oral glucose load (1 g/kg) one or four hours after drug administration. Blood samples were collected from tail

vein before drug administration (pre), and just before glucose load (time 0), and 10, 30, 60 and 120 minutes after glucose load. Plasma glucose and plasma insulin levels were measured by Autoanalyzer 7080 (Hitachi, Japan) and radioimmunoassay (Millipore, USA), respectively. Statistical differences were analyzed by the Student's t-test or the Aspin-Welch test. In the dose-dependent study, statistical significances versus vehicle control were assessed by the one-tailed Williams test or the Shirley-Williams test.

A. Crystal Data.

Empirical Formula	$C_{29}H_{32}O_7S$ .1/2H_2O
Formula Weight	533.64
Crystal Color, Habit	colorless, platelet
Crystal Dimensions	0.30 x 0.20 x 0.05 mm
Crystal System	triclinic
Lattice Type	Primitive
No. of Reflections Used for Unit Cell Determination (2θ range)	25654 (7.3–136.5°)
Indexing Images	3 oscillations at 3.0 minutes
Camera Radius	127.40 mm
Lattice Parameters	a = 7.912(2) *Å* b = 9.698(3) *Å* c = 36.602(9) *Å* α = 91.59(2)° β = 92.35(2)° γ = 107.59(2)° V = 2672(4) $Å^3$
Space Group	P1(#1)
Z value	4
D_{calc}	1.326 g/cm^3
F_{000}	1132.00
μ(CuKα)	14.80 cm^{-1}

B. Intensity Measurements

Diffractometer	Rigaku RAXIS-RAPID Imaging Plate
Radiation	CuKα (λ = 1.54186 *Å*) graphite monochromated
Temperature	–173.0 °C
Voltage, Current	50 kV, 100 mA
Collimator Size	0.5 mm
Detector Aperture	460.0 mm x 256.0 mm

Data Images	45 exposures at 1.5 minutes per degree
Oscillation Range (ϕ=0.0°,χ=50.0°)	ω50.0 - 230.0° with 20.0° step
Oscillation Range (ϕ=90.0°,χ=50.0°)	ω50.0 - 230.0° with 20.0° step
Oscillation Range (ϕ=195.0°,χ=50.0°)	ω50.0 - 230.0° with 20.0° step
Oscillation Range (ϕ=270.0°,χ=50.0°)	ω50.0 - 230.0° with 20.0° step
Oscillation Range (ϕ=60.0°,χ=10.0°)	ω50.0 - 230.0° with 20.0° step
Camera Radius	127.40 mm
Pixel Size	0.100 mm
$2\theta_{max}$	136.5°
No. of Reflections Measured	Total: 27623
	Unique: 8873 (R_{int} = 0.040)
Corrections	Lorentz-polarization
	Absorption
	(trans. factors: 0.6381–0.9287)

C. Structure Solution and Refinement

Structure Solution	Direct Methods (SIR92)
Refinement	Full-matrix least-squares (SHELXL-97)
Function Minimized	$\Sigma\omega(Fo^2 - Fc^2)^2$
Least Squares Weights	$\omega = [\sigma^2(Fo^2)+(0.0587P)^2 + 0.0000P]^{-1}$
	where $P=(Fo^2 + 2Fc^2)/3$
No. of Reflections	12146
No. Variables	1348
Reflection/Parameter Ratio	9.01
Residuals: R; Rw	0.063 ; 0.166
Goodness of Fit Indicator	1.01
Max Shift/Error in Final Cycle	0.00
Maximum peak in Final Diff. Map	0.70 $e^-/Å^3$
Minimum peak in Final Diff. Map	–0.55 $e^-/Å^3$
Flack Parameter	-0.05(2)

参考文献およびノート

1. International Diabetes Federation. IDF Diabetes Atlas, 5th ed., **2012**. http://www.diabetesatlas.org.
2. Rendell, M. The role of sulphonylureas in the management of type 2 diabetes mellitus. *Drugs* **2004**, *64*, 1339–1358.
3. Burge, M. R.; Sood, V.; Sobhy, T. A.; Rassam, A. G.; Schade, D. S. Sulphonylurea-induced hypoglycaemia in type 2 diabetes mellitus: a review. *Diabetes, Obesity and Metabolism* **1999**, *1*, 199–206.
4. Maedler, K.; Carr, R. D.; Bosco, D.; Zuellig, R. A.; Berney, T.; Donath, M. Y. Sulfonylurea induced β-cell apoptosis in cultured human islets. *J. Clin. Endocrinol. Metab.* **2005**, *90*, 501–506.
5. Tzoulaki, I.; Molokhia, M.; Curcin, V.; Little, M. P.; Millett, C. J.; Ng, A.; Hughes, R. I.; Khunti, K.; Wilkins, M. R.; Majeed, A.; Elliott, P. Risk of cardiovascular disease and all cause mortality among patients with type 2 diabetes prescribed oral antidiabetes drugs: retrospective cohort study using UK general practice research database. *Br. Med. J.* **2009**, *339*, b4731.
6. Karagiannis, T.; Paschos, P.; Paletas, K.; Matthews, D. R.; Tsapas, A. Dipeptidyl peptidase-4 inhibitors for treatment of type 2 diabetes mellitus in the clinical setting: systematic review and meta-analysis. *Br. Med. J.* **2012**, *344*, e1369.
7. Meier, J. J. GLP‑1 receptor agonists for individualized treatment of type 2 diabetes mellitus. *Nature Rev. Endocrinol.* **2012**, *8*, 728–742.
8. Haber, E. P.; Ximenes, H. M. A.; Proco´Pio, J.; Carvalho, C. R. O.; Curi, R.; Carpinelli, A. R. Pleiotropic effects of fatty acids on pancreatic beta-cells. *J. Cell Physiol*. **2003**, *194*, 1–12.
9. Dobbins, R. L.; Chester, M. W.; Daniels, M. B.; McGarry, J. D.; Stein, D. T. Circulating fatty acids are essential for efficient glucose-stimulated insulin secretion after prolonged fasting in humans. *Diabetes* **1998**, *47*, 1613–1618.
10. Stein, D. T.; Esser, V.; Stevenson, B. E.; Lane, K. E.; Whiteside, J. H.; Daniels, M. B.; Chen, S.; McGarry, J. D. Essentiality of circulating fatty acids for glucose-stimulated insulin secretion in the fasted rat. *J. Clin. Invest*. **1996**, *97*, 2728–2735.
11. McGarry, J. D.; Dobbins, R. L. Fatty acids, lipotoxicity and insulin secretion. *Diabetologia* **1999**, *42*, 128–138.
12. Shimabukuro, M.; Zhou, Y.-T.; Levi, M.; Unger, R. H. Fatty acid-induced β-cell apoptosis: A link between obesity and diabetes. *Proc. Natl. Acad. Sci. USA* **1998**, *95*,

2498–2502.
13. Boden, G. Obesity, insulin resistance and free fatty acids. *Curr. Opin. Endocrinol. Diabetes Obes.* **2011**, *18*, 139−143.
14. International Union of Basic and Clinical Pharmacology (IUPHAR) のデータベースにおいて、GPR40 は FFA1 と規定されているが、他にも free fatty acid receptor 1 の略として FFAR1 などの名称も使用されている。
15. Itoh, Y.; Kawamata, Y.; Harada, M.; Kobayashi, M.; Fujii, R.; Fukusumi, S.; Ogi, K.; Hosoya, M.; Tanaka, Y.; Uejima, H.; Tanaka, H.; Maruyama, M.; Satoh, R.; Okubo, S.; Kizawa, H.; Komatsu, H.; Matsumura, F.; Noguchi, Y.; Shinohara, T.; Hinuma, S.; Fujisawa, Y.; Fujino, M. Free fatty acids regulate insulin secretion from pancreatic β cells through GPR40. *Nature* **2003**, *422*, 173–176.
16. GPCR は、細胞外から情報を受け取ると立体構造の変化が生じ、共役している三量体 G タンパク質 (α、β および γ サブユニット) の活性化を介して細胞内へとシグナルを伝達する。不活性状態の G タンパク質は α サブユニットに GDP が結合したヘテロ三量体構造をとっているが、GPCR によって活性化された G タンパク質は、α サブユニットから GDP が解離し、代わりに GTP が結合して活性型 Gα と Gβγ に解離する。GPR40 の場合、$G\alpha_q$ がホスホリパーゼ C に結合し、その後のシグナル伝達を引き起こす。
17. Sawzdargo, M.; George, S. R.; Nguyen, T.; Xu, S. J.; Kolakowski, L. F.; Odowd, B. F. A cluster of four novel human G protein-coupled receptor genes occurring in close proximity to CD22 gene on chromosome 19q13.1. *Biochem. Biophys. Res. Commun.* **1997**, *239*, 543–547.
18. Briscoe, C. P.; Tadayyon, M.; Andrews, J. L.; Benson, W. G.; Chambers, J. K.; Eilert, M. M.; Eillis, C.; Elshourbagy, N. A.; Goetz, A. S.; Minnick, D. T.; Murdock, P. R.; Sauls, H. R.; Shabon, U.; Spinage, L. D.; Strum, J. C.; Szekeres, P. G.; Tan, K. B.; Way, J. M.; Ignar, D. M.; Wilson, S.; Muir, A. I. The orphan G protein-coupled receptor GPR40 is activated by medium and long chain fatty acids. *J. Biol. Chem.* **2003**, *278*, 11303–11311.
19. Kotarsky, K.; Nilsson, N. E.; Flodgren, E.; Owman, C.; Olde, B. A human cell surface receptor activated by free fatty acids and thiazolidinedione drugs. *Biochem. Biophys. Res. Commun.* **2003**, *301*, 406-410.
20. Shapiro, H.; Shachar, S.; Sekler, I.; Hershfinkel, M.; Walker, M. D. Role of GPR40 in fatty acid action on the β cell line INS-1E. *Biochem. Biophys. Res. Commun.* **2005**, *335*, 97–104.
21. Fujiwara, K.; Maekawa, F.; Yada, T. Oleic acid interacts with GPR40 to induce Ca^{2+} signaling in rat islet β-cells: mediation by PLC and L-type Ca^{2+} channel and link to insulin secretion. *Am. J. Physiol. Endocrinol. Metab.* **2005**, *289*, E670–E677.
22. siRNA とは 21–23 残基から成る低分子 2 本鎖 RNA であり、メッセンジャーRNA

の破壊によって配列特異的に遺伝子の発現を抑制する、RNA 干渉と呼ばれる現象を引き起こす。本手法は遺伝子をノックダウンする方法として、生物学および医薬分野の基礎研究に応用されていると共に、臨床への応用も期待されている。

23. FLIPR はモレキュラーデバイス社の蛍光イメージングプレートリーダーのことであり、細胞内のカルシウムイオン (Ca^{2+}) 濃度の変動をハイスループットで測定することができる装置である。Ca^{2+}蛍光プローブ (Fluo-4 など) を、細胞内 Ca^{2+}恒常性を乱すことなく細胞に取り込ませ、リガンド刺激時のカルシウムシグナルの変動を蛍光シグナルとして検出、解析できる (*MEDCHEM NEWS* **2011**, *21 (2)*, 19)。
24. Sasaki, S.; Kitamura, S.; Negoro, N.; Suzuki, M.; Tsujihata, Y.; Suzuki, N.; Santou, T.; Kanzaki, N.; Harada, M.; Tanaka, Y.; Kobayashi, M.; Tada, N.; Funami, M.; Tanaka, T.; Yamamoto, Y.; Fukatsu, K.; Yasuma, T.; Momose, Y. Design, synthesis, and biological activity of potent and orally available G protein-coupled receptor 40 agonists. *J. Med. Chem.* **2011**, *54*, 1365–1378.
25. 薬物は酵素や受容体などの生体分子に結合し薬理作用を示すが、多くの場合、期待した薬理作用を導く生体分子だけでなく、予期せぬ分子 (オフターゲット) にも結合し、時に望まない作用をもたらす。有害反応は、医薬品の市場撤退や臨床研究時における候補化合物のドロップアウトの原因となるため、医薬品開発過程の早い段階でその確認をする作業は必要不可欠である (*ファルマシア* **2013**, *49 (1)*, 72)。
26. Negoro, N.; Sasaki, S.; Mikami, S.; Ito, M.; Suzuki, M.; Tsujihata, Y.; Ito, R.; Harada, A.; Takeuchi, K.; Suzuki, N.; Miyazaki, J.; Santou, T.; Odani, T.; Kanzaki, N.; Funami, M.; Tanaka, T.; Kogame, A.; Matsunaga, S.; Yasuma, T.; Momose, Y. Discovery of TAK-875: a potent, selective, and orally bioavailable GPR40 agonist. *ACS Med. Chem. Lett.* **2010**, *1*, 290–294.
27. Negoro, N.; Sasaki, S.; Ito, M.; Kitamura, S.; Tsujihata, Y.; Ito, R.; Suzuki, M.; Takeuchi, K.; Suzuki, N.; Miyazaki, J.; Santou, T.; Odani, T.; Kanzaki, N.; Funami, M.; Tanaka, T.; Yasuma, T.; Momose, Y. Identification of fused-ring alkanoic acids that act as G protein-coupled receptor 40/free fatty acid receptor 1 agonists with improved pharmacokinetic profile. *J. Med. Chem.* **2012**, *55*, 1538–1552.
28. Negoro, N.; Sasaki, S.; Ito, M.; Tsujihata, Y.; Ito, R.; Suzuki, M.; Takeuchi, K.; Suzuki, N.; Miyazaki, J.; Santou, T.; Odani, T.; Kanzaki, N.; Funami, M.; Morohashi, A.; Nonaka, Matsunaga, S.; M.; Yasuma, T.; Momose, Y. Optimization of (2,3-dihydro-1-benzofuran-3-yl)acetic acids: discovery of a non-free fatty acid-like, highly bioavailable G protein-coupled receptor 40/free fatty acid receptor 1 agonist as a glucose-dependent insulinotropic agent. *J. Med. Chem.* **2012**, *55*, 3960–3974.
29. Node, M.; Kumar, K.; Nishide, K.; Ohsugi, S.; Miyamoto, T. Odorless substitutes for foul-smelling thiols: syntheses and applications. *Tetrahedron Lett.* **2001**, *42*, 9207–9210.

30. Conconi, M. T.; Guiotto, A.; Manzini, P.; Chilin, A.; Bassani, V.; Parnigotto, P. P. 4-Hydroxymethyltetrahydro- and 4-hydroxymethyl-benzopsoralen: synthesis and biological activity. *Farmaco* **1995**, *50*, 125–130.
31. Fall, Y.; Santana, L.; Teijeira, M.; Uriarte, E. A convenient synthesis of benzofuran-3-acetic acids. *Heterocycles* **1995**, *41*, 647–650.
32. Kametani, T.; Nemoto, H.: Ishikawa, H.; Shiroyama, K.; Fukumoto, K. A formal regio- and stereoselective total synthesis of estrone. A convenient synthesis of *D*-homoestrone. *J. Am. Chem. Soc.* **1976**, *98*, 3378–890.
33. Nishide, K.; Node, M. Development of odorless sulfur reagents and their applications. *J. Syn. Org. Chem. Jpn.* **2004**, *62*, 895–910.
34. Gao, Y.; Zhang, Q.; Xu, J. A convenient and effective method for synthesizing β-amino-α,β-unsaturated esters and ketones. *Synth. Commun.* **2004**, *34*, 909–916.
35. Anghelide, N.; Draghici, C.; Raileanu, D. New dienamino esters and their cyclization to α–pyridones of nicotinic acid. *Tetrahedron* **1974**, *30*, 623–632.
36. Ramirez, F.; Paul, A. P. Studies on α–pyridones. II. Derivatives of pyrindine. *J. Am. Chem. Soc.* **1955**, *77*, 1035–1040.
37. Fukuyama, T.; Jow, C.-K.; Cheung, M. 2- and 4-Nitrobenzenesulfonamides: exceptionally versatile means for preparation of secondary amines and protection of amines. *Tetrahedron Lett*. **1995**, *36*, 6373–6374.
38. FFA は生体内で血清アルブミンに結合して存在することが知られている。GPR40 作動薬研究において、血清アルブミン存在下で活性が減弱する化合物が存在したことから、血清タンパクの影響を受けにくい化合物を見出すために、BSA 存在下における化合物の活性を評価した。
39. Yamagami, C.; Ogura, T.; Takao, N. Hydrophobicity parameters determined by reverse-phase liquid chromatography I. Relationship between capacity factors and octanol-water partition coefficients for monosubstituted pyrazines and the related pyridines. *J. Chromatogr.* **1990**, *514*, 123–136.
40. Waring, M. J. Lipophilicity in drug discovery. *Expert Opin. Drug Discov.* **2010**, *5*, 235–248.
41. ドラッグライクネスとは、Lipinski 博士によって提唱された Rule of 5 に代表される概念で、低分子の薬物が目指すべき性質のことを指す (*Adv. Drug Deliv. Rev.* **2001**, *46*, 3–26)。分子量や cLogP、水素結合アクセプター／ドナー (HBA/HBD)、回転可能結合数 (Rotatable bond)、2 次元極性表面積 (TPSA) などの経口吸収性に関与する指標に加えて、代謝酵素に対する反応性などの薬物相互作用の要素なども考慮される。
42. Li, J.; Edwards, P. C.; Burghammer, M.; Villa, C.; Schertler, G. F. X. Structure of bovine rhodopsin in a trigonal crystal form. *J. Mol. Biol.* **2004**, *343*, 1409–1438.
43. CL_{total} は、体内からの薬物排出総能力 (尿中排泄や肝代謝の和) を指す。クリアラ

ンスは「単位時間あたりに薬物が消失した循環血液の体積」として定義される。

44. $AUC_{po,0–8h}$ は、薬物濃度時間曲線下面積のことを表す。薬物を経口投与後 8 時間までの血中濃度を Y 軸、時間を X 軸にプロットした時の曲線の下側の面積を指す。体内の薬物暴露の指標となる。

45. Ikeda, H.; Shino, A.; Matsuo, T.; Iwatsuka, H.; Suzuoki, Z. A new genetically obese-hyperglycemic rat (Wistar fatty). *Diabetes* **1981**, *30*, 1045–50.
46. Garrido, D. M.; Corbett, D. F.; Dwornik, K. A.; Goetz, A. S.; Littleton, T. R.; McKeown, S. C.; Mills, W. Y.; Smalley, T. L.; Briscoe, C. P.; Peat, A. J. Synthesis and activity of small molecule GPR40 agonists. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1840–1845.
47. Zhou, C. Y.; Tang, C.; Chang, E.; Ge, M.; Lin, S. N.; Cline, E.; Tan, C. P.; Feng, Y.; Zhou, Y. P.; Eiermann, G. J.; Petrov, A.; Salituro, G.; Meinke, P.; Mosley, R.; Akiyama, T. E.; Einstein, M.; Kumar, S.; Berger, J.; Howard, A. D.; Thornberry, N.; Mills, S. G.; Yang, L. H. Discovery of 5-aryloxy-2,4-thiazolidinediones as potent GPR40 agonists. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1298–1301.
48. Song, F. B.; Lu, S. F.; Gunnet, J.; Xu, J. Z.; Wines, P.; Proost, J.; Liang, Y.; Baumann, C.; Lenhard, J.; Murray,W. V.; Demarest, K. T.; Kuo, G. H. Synthesis and biological evaluation of 3-aryl-3-(4-phenoxy)-propionic acid as a novel series of G protein-coupled receptor 40 agonists. *J. Med. Chem.* **2007**, *50*, 2807–2817.
49. Houze, J. B.; Zhu, L.; Sun, Y.; Akerman, M.; Qiu, W.; Zhang, A. J.; Sharma, R.; Schmitt, M.; Wang, Y.; Liu, J.; Liu, J.; Medina, J. C.; Reagan, J. D.; Luo, J.; Tonn, G.; Zhang, J.; Lu, J. Y-L.; Chen, M.; Lopez, E.; Nguyen, K.; Yang, L.; Tang, L.; Tian, H.; Shuttleworth, S. J.; Lin, D. C-H. AMG 837: A potent, orally bioavailable GPR40 agonist. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 1267−1270.
50. Christiansen, E.; Due-Hansen, M. E.; Urban, C.; Merten, N.; Pfleiderer, M.; Karlsen, K. K.; Rasmussen, S. S.; Steensgaard, M.; Hamacher, A.; Schmidt, J.; Drewke, C.; Petersen, R. K.; Kristiansen, K.; Ullrich, S.; Kostenis, E.; Kassack, M. U.; Ulven, T. Structure-Activity study of dihydrocinnamic acids and discovery of the potent FFA1 (GPR40) agonist TUG-469. *ACS Med. Chem. Lett.* **2010**, *1*, 345–349.
51. Christiansen, E.; Urban, C.; Merten, N.; Liebscher, K.; Karlsen, K. K.; Hamacher, A.; Spinrath, A.; Bond, A. D.; Drewke, C.; Ullrich, S.; Kassack, M. U.; Kostenis, E.; Ulven, T. Discovery of potent and selective agonists for the free fatty acid receptor 1 (FFA(1)/GPR40), a potential target for the treatment of type II diabetes. *J. Med. Chem.* **2008**, *51*, 7061–7064.
52. Christiansen, E.; Urban, C.; Grundmann, M.; Due-Hansen, M. E.; Hagesaether, E.; Schmidt, J.; Pardo, L.; Ullrich, S.; Kostenis, E.; Kassack, M.; Ulven, T. Identification of a potent and selective free fatty acid receptor 1 (FFA1/GPR40) agonist with favorable physicochemical and in vitro ADME properties. *J. Med. Chem.* **2011**, *54*, 6691–6703.
53. Song, Z.; Song, M.; Lee, D. Y. W.; Liu, Y.; Deaciuc, I. V.; McClain, C. J. Silymarin

prevents palmitate-induced lipotoxicity in HepG2 cells: involvement of maintenance of Akt kinase activation. *Basic Clin. Pharmacol. Toxicol.* **2007**, *101*, 262–268.

54. Mikami, S.; Kitamura, S.; Negoro, N.; Sasaki, S.; Suzuki, M.; Tsujihata, Y.; Ito, R.; Takeuchi, K.; Suzuki, N.; Santou, T.; Miyazaki, J.; Kanzaki, N.; Funami, M.; Yasuma, T.; Momose, Y. Discovery of phenylpropanoic acid derivatives containing polar functionalities as potent and orally bioavailable G protein-coupled receptor 40 agonists for the treatment of type 2 diabetes. *J. Med. Chem.*, **2012**, *55*, 3756–3776.
55. エナンチオマー間で薬理活性に差がある場合、活性の強い方をユートマー (eutomer)、弱い方をディストマー (distomer) という。
56. Yoshida, Y.; Shimonishi, K.; Sakakura, Y.; Okada, S.; Aso, N.; Tanabe, Y. Facile and practical methods for the sulfonylation of alcohols using Ts(Ms)Cl and $Me_2N(CH_2)_nNMe_2$ as a key base. *Synthesis* **1999**, 1633–1636.
57. Baddeley, G. The action of aluminum chloride on some phenol homologues. *J. Chem. Soc.* **1943**, 527–531.
58. X-ray crystallographic data for compound **85** has been deposited with the Cambridge Crystallographic Data Center as CCDC 837682. The crystallographic data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).
59. カスパーゼ (Caspase) とは、Cysteine-ASPartic-acid-proteASE を略したもので、細胞にアポトーシスを起こさせるシグナル伝達経路を構成する、一群のシステインプロテアーゼである。システインプロテアーゼは活性部位にシステイン残基をもつタンパク質分解酵素であり、カスパーゼは基質となるタンパク質のアスパラギン酸残基の後ろを切断する。発生の過程で、あるいは X 線や抗がん剤など DNA を損傷するストレス刺激や、細胞へのウイルス感染やがん化させる刺激など、さまざまな刺激に対する生体防御機構の 1 つとして、自らアポトーシスを起こして自殺する機構を持っている。カスパーゼファミリーは、複数のカスパーゼが順に活性化されていくカスパーゼカスケードと呼ばれる一連のシグナル伝達経路を形成しており、アポトーシス誘導刺激に反応してこのシグナル伝達が行われることで、細胞にアポトーシスが誘導される。Caspase-3/7 はそのカスケードの最終段階を担う酵素である。
60. Kepp, O.; Galluzzi, L.; Lipinski, M.; Yuan, J.; Kroemer, G. Cell death assays for drug discovery. *Nature Rev. Drug Discovery* **2011**, *10*, 221–237.
61. カセットドージングとは、複数の薬物を相互作用のない程度少量同時に動物に投与し、薬物動態プロファイルを調べる方法である。使用する動物を低減でき、評価時間を短縮できるなどの利点がある。一方、単独投与に比べて薬物相互作用による血中濃度上昇が懸念されるため、スクリーニング段階で大まかな評価に用いられる。
62. Brown, J. A.; Goldsworthy, S. M.; Barnes, A. A.; Eilert, M. M.; Tcheang, L.; Daniels, D.;

Muir, A. I.; Wigglesworth, M. J.; Kinghorn, I.; Fraser, N. J.; Pike, N. B.; Strum, J. C.; Steplewski, K. M.; Murdock, P. R.; Holder, J. C.; Marshall, F. H.; Szekeres, P. G.; Wilson, S.; Ignar, D. M.; Foord, M., S.; Wise, A.; Dowell, S. J. The orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. *J. Biol. Chem.* **2003**, *278*, 11312–11319.

63. Hirasawa, A.; Tsumaya, K.; Awaji, T.; Katsuma, S.; Adachi, T.; Yamada, M.; Sugimoto, Y.; Miyazaki S.; Tsujimoto, G. Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120. *Nat. Med.* **2005**, *11*, 90–94.
64. Imai, Y. N.; Inoue, Y.; Yamamoto, Y. Propensities of polar and aromatic amino acids in noncanonical interactions: nonbonded contacts analysis of protein−ligand complexes in crystal structures. *J. Med. Chem.*, **2007**, *50*, 1189–1196.
65. V_d (volume of distribution) は、化合物の組織移行の程度を血漿の体積に換算した数値であるが、血漿タンパクとの結合によって影響を受ける。$V_{d(ss)}$は定常状態分布容積を指す。
66. 日本人成人男性 (n = 8) に **85** (TAK-875: Fasiglifam) を25 mg単回投薬した際の半減期は、26.49 時間であった。
    Matsuno, K.; Hirayama, M.; Araki, T.; Dote, N.; Kondo, T.; Nakamura, K. Pharmacokinetics, safety and tolerability of single and multiple doses of TAK-875, a novel GPR40 agonist, in Japanese healthy male subjects. *Diabetes* **2010**, *59*, A193.
67. Naik, H.; Vakilynejad, M.; Wu, J.; Viswanathan, P.; Dote, N.; Higuchi, T.; Leifke, E. Safety, tolerability, pharmacokinetics, and pharmacodynamic properties of the GPR40 agonist TAK-875: results from a double blind, placebo controlled single oral dose-rising study in healthy volunteers. *J. Clin. Pharmacol.* **2012**, *52*, 1007–1016.
68. Tsujihata, Y.; Ito, R.; Suzuki, M.; Harada, A.; Negoro, N.; Yasuma, T.; Momose, Y.; Takeuchi, K.; TAK-875, an orally available GPR40/FFA1 agonist enhances glucose-dependent insulin secretion and improves both postprandial and fasting hyperglycemia in type 2 diabetic rats. *J. Pharmacol. Exp. Ther.* **2011**, *339*, 228–237.
69. 本試験系において、Glibenclamide (10 mg/kg) および Nateglinide (50 mg/kg) を投薬した際には、コントロール群と比較して、それぞれ 30 mg/dl 程度のグルコース濃度の低下を認め、低血糖に至ることを確認している。
70. Burant, C. F.; Viswanathan, P.; Marcinak, J.; Cao, C.; Vakilynejad, M.; Xie, B.; Leifke, E. TAK-875, a G protein-coupled receptor 40 agonist, for the treatment of type 2 diabetes mellitus: a phase 2, randomized, double-blind, placebo-controlled study. *Lancet* **2012**, *379*, 1403–1411.
71. Araki, T.; Hirayama, M.; Hiroi, S.; Kaku, K. GPR40-induced insulin secretion by the novel agonist TAK-875: first clinical findings in patients with type 2 diabetes. *Diabetes Obes. Metab.* **2012**, *14*, 271–278.
72. Leifke, E.; Naik, H.; Wu, J.; Viswanathan, P.; DeManno, D.; Kipnes, M.; Vakilynejad,

M. A multiple-ascending-dose study to evaluate safety, pharmacokinetics, and pharmacodynamics of a novel GPR40 agonist, TAK-875, in subjects with type 2 diabetes. *Clin. Pharmacol. Ther.* **2012**, *92*, 29–39.

73. Kaku, K.; Araki, T.; Yoshinaka, R. Randomized, double-blind, dose-ranging study of TAK-875, a novel GPR40 agonist, in Japanese patients with inadequately controlled type 2 diabetes. *Diabetes Care* **2013**, *36*, 245–250.
74. Li, J.; Edwards, P. C.; Burghammer, M.; Villa, C.; Schertleret, G. F. X. Structure of bovine rhodopsin in a trigonal crystal form. *J. Mol. Biol.* **2004**, *343*, 1409–1438.
75. Larkin, M. A.; Blackshields, G.; Brown, N. P.; Chenna, R.; McGettigan, P. A.; McWilliam, H.; Valentin, F.; Wallace, I.M.; Wilm, A.; Lopez, R.; Thompson, J. D.; Gibson, T. J.; Higgins, D. G. Clustal W and Clustal X version 2.0. *Bioinformatics*, **2007**, *23*, 2947–2948.
76. MOE, version 2008.10: Chemical Computing Group Inc., 1010 Sherbrooke St. W, Suite 910 Montreal, Quebec, Canada H3A 2R7. www.chemcomp.com.
77. GOLD, version 4.1: CCDC, Business & Administration, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, U.K. http://www.ccdc.cam.ac.uk.