セロトニントランスポーター（SERT）と
セロトニン 1A（5-HT_{1A}）受容体への
結合阻害活性を併せ持つ新規抗うつ薬の創製

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奈良先端科学技術大学院大学
物質創成科学研究科

吉永　英史

目次

略語表

【本文中の略語】

2-Pr: 2-propyl、2-プロピル
5-HT: serotonin、セロトニン
Ar: aryl、アリール
AUC: are under the curve、曲線下面積
Bn: benzyl、ベンジル
β-NADPH: β-nicotinamide adenine dinucleotide phosphate、β-ニコチンアミドアデニンジヌクレオチド
Boc: tert-butoxy carbonyl、tert-ブトキシカルボニル
CHO: Chinese hamster ovary、チャイニーズハムスターの卵巣
CYP2D6: cytochrome P450 family 2 subfamily D member 6、シトクロム P450 2D6
DA: dopamine、ドパミン
DDI: drug-drug interaction、薬物相互作用
DML: designed multiple ligand、デザインドマルチプルリガンド
DTT: dithiothreitol、ジチオトレイトール
EM: extensive metabolizer、エクステンシブメタボライザー
ESI: electrospray ionization、エレクトロスプレイイオン化
Et: ethyl、エチル
GDP: guanosine diphosphate、グアノシン二リン酸
GTP: guanosine triphosphate、グアノシン三リン酸
HPLC: high performance liquid chromatography、高速液体クロマトグラフィー
HRMS: high resolution mass spectrometry、高分解能質量分析
I.A.: intrinsic activity、内因活性
IC_{50}: 50% inhibitory concentration、50%阻害濃度
Ki: inhibition constant、阻害定数
LC-MSMS: Liquid Chromatography - tandem Mass Spectrometry、液体クロマトグラフィー質量分析法
LeuT: leucine transporter、ロイシントランスポーター
Me: methyl、メチル
MeO: methoxy、メトキシ
Mp: melting point、融点
NMR: nulear magnetic resonance、核磁気共鳴
PM: poor metabolizer、プアメタボライザー
p.o.: per oral、経口
r.t.: room temperature、室温
SAR: structure-activity relationship、構造活性相関

SEM: standard error of mean、標準誤差
SERT: serotonin transporter、セロトニントランスポーター
S_NAr: aromatic neulceophilic substitution、芳香族求核置換
SSRI: selective serotonin reuptake inhibitors、選択的セロトニン再取り込み阻害薬
SRI: serotonin reuptake inhibition、セロトニン再取り込み阻害
Ts: tosyl、トシル

【合成試薬：実験項に関する略語】
2-PrOH: 2-propanol、2-プロパノール
AcCl: acetyl chloride、塩化アセチル
AcOH: acetic acid、酢酸
AgOTf: silver trifluoromethansulfonate、トリフルオロメタンスルホン酸銀
$AlCl_3$: aluminium chloride、塩化アルミニウム
BBr_3: boron tribromide、三臭化ホウ素
BF_3-Et_2O: boron trifluoride etherate、三フッ化ホウ素ジエチルエーテル錯体
BH_3-THF: borane tetrahydrofuran complex、ボランテトラヒドロフラン錯体
Boc_2O: di-tert-butyl dicarbonate、二炭酸ジ-tert-ブチル
$B(OMe)_3$: trimethyl borate、ホウ酸トリメチル
BsOH: benzensulfonic acid、ベンゼンスルホン酸
$CaCl_2$: calcium chloride、塩化カルシウム
CH_2Cl_2: dichloromethane、ジクロロメタン
$CHCl_3$: chloroform、クロロホルム
DAIB: (diacetoxyiodo)benzene、(ジアセトキシヨード)ベンゼン
DMA: dimethylacetamide、ジメチルアセトアミド
DMF: dimethylformamide、ジメチルホルムアミド
DMSO: dimethylsulfoxide、ジメチルスルホキシド
Et_3N: triethylamine、トリエチルアミン
Et_2O: diethylether、ジエチルエーテル
EtOAc: ethyl acetate、酢酸エチル
EtOH: ethanol、エタノール
F-TEDA-BF_4: 1-Chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate)、1-クロロメチル-4-フルオロ-1,4-ジアゾニアビシクロ[2.2.2]オクタンビス(テトラフルオロボレイト)
H_2: hydrogen gas、水素ガス
HCl: hydrochloride、塩化水素
$HC(OMe)_3$: trimethyl orthoformate、オルトギ酸トリメチル
H_3PO_4: phosphoric acid、リン酸
K_2CO_3: potassium carbonate、炭酸カリウム

KH_2PO_4: potassium dihydrogenphosphate、リン酸二水素カリウム
$KHSO_4$: potassium hydrogen sulfate、硫酸水素カリウム
KI: potassium iodide、ヨウ化カリウム
KOH: potassium hydroxide、水酸化カリウム
$LiAlH_4$ (LAH): lithium aluminiumhydride、水素化リチウムアルミニウム
LiBr: lithium bromide、臭化リチウム
mCPBA: m-chloroperoxybenzoic acid、m-クロロ過安息香酸
$MeB(OH)_2$: methaneboronic acid、メチルボロン酸
MeCN: acetonitrile、アセトニトリル
MeN_3HCl: trimethylamine hydrochloride、トリメチルアミン塩酸塩
MeS^+I^-: trimethylsulfonium iodide、トリメチルスルホニウムヨージド
MeOH: metanol、メタノール
$MgCl_2$: magnesium chloride、塩化マグネシウム
$MgSO_4$: magunesium sulfate、硫酸マグネシウム
MnO_2: manganese dioxide、二酸化マンガン
MS3Å: molecular sieve 3Å、モレキュラーシーブ 3Å
MsCl: methansulfonyl chloride、メタンスルホニルクロライド
$NaBH_4$: sodium borohydride、水素化ホウ素ナトリウム
NaClO: sodium hypochlorite、次亜塩素酸ナトリウム
$NaClO_2$: sodium chlorite、亜塩素酸ナトリウム
$NaHCO_3$: sodium hydrogen carbonate、炭酸水素ナトリウム
Na_2HPO_4: sodium hydrogen phosphate、リン酸水素二ナトリウム
$NaHSO_3$: sodium hydrogen sulfite、亜硫酸水素ナトリウム
NaOH: sodium hydroxide、水酸化ナトリウム
Na_2SO_4: sodium sulfate、硫酸ナトリウム
NBS: N-bromosuccinimide、N-ブロモスクシンイミド
n-BuLi: n-butyl lithium、n-ブチルリチウム
n-BuMgCl: n-butyl magnesium chloride、n-ブチルマグネシウムクロライド
NCS: N-chlorosuccinimide、N-クロロスクシンイミド
NH_4Cl: ammonium chloride、塩化アンモニウム
NIS: N-iodosuccinimide、N-ヨードスクシンイミド
NMM: 4-methylmorpholine、4-メチルモルホリン
PDC: pyridinium dichromate、二クロム酸ピリジニウム
Pd-C: paradium carbon、パラジウム炭素
$Pd(PPh_3)_4$: Tetrakis(triphenylphosphine)palladium(0)、テトラキス(トリフェニルホスフィン)パラジウム(0)
PPA: polyphosphoric acid、ポリリン酸
PPh_3: triphenylphosphine、トリフェニルホスフィン

p-TsOH: p-toluenesulfonic acid、p-トルエンスルホン酸
$SOCl_2$: thionyl chloride、塩化チオニル
TCCA: trichloroisocyanuric acid、トリクロロイソシアヌル酸
TEMPO: 2,2,6,6-tetramethylpiperidine 1-oxyl、2,2,6,6-テトラメチルピペリジン 1-オキシル
TFA: trifluoroacetic acid、トリフルオロ酢酸
TFAA: trifluoroacetic anhydride、無水トリフルオロ酢酸
Tf_2O: trifluoromethanesulfonic anhydride、トリフルオロメタンスルホン酸無水物
TfOH: trifluoromethansulfonic acid、トリフルオロメタンスルホン酸
THF: tetrahydrofuran、テトラヒドロフラン
TsCl: toluenesulfonyl chloride、トルエンスルホニルクロライド
Rh-C: rhodium carbon、ロジウム炭素

第 1 章　本研究の背景

1-1. 大うつ病と治療薬

大うつ病は、全世界人口の４％が罹患する世界で最も問題となっている疾患の一つである。Figure 1 に示すようにセロトニン神経の起始核である背側縫線核等の縫線核から脳内の様々な領域にセロトニン神経は投射される。セロトニンは、投射先の脳領域で分泌され、気分・情動・記憶といった様々な神経機能に影響を与えることから、うつ病の発症と密接に関わっていることが知られている。[1]

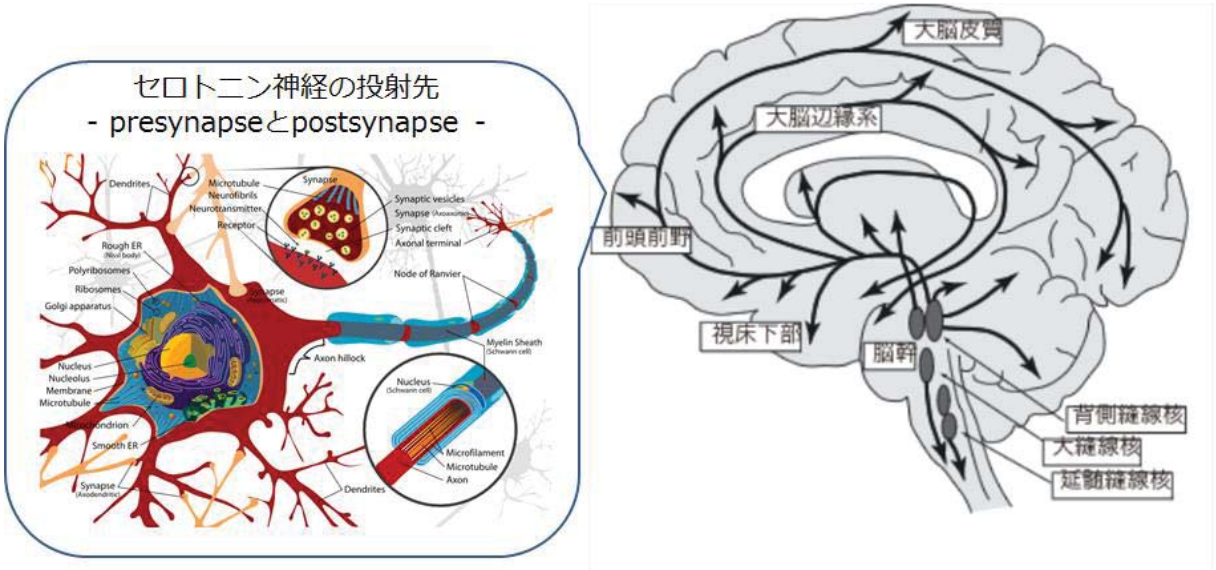
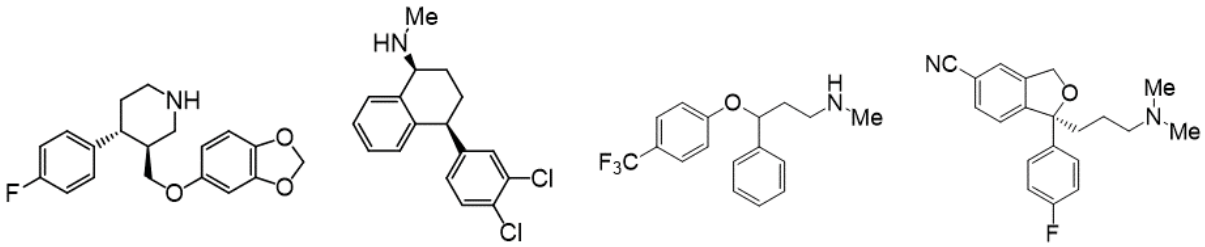


Figure 1. セロトニン神経細胞のある場所と軸索の投射領域 [1]

脳内でのセロトニン遊離量を調節するパロキセチンやフルオキセチンに代表されるセロトニン選択的再取り込阻害剤（SSRI）が、うつ病治療の第一選択薬として世界で広く使われている。しかしながら、SSRI は、投薬開始から治療効果の発現まで２～３週間かかるという治療オンセットの遅さや薬物治療を受けたうつ病患者のおよそ３分の１に治療効果が表れないという問題点があり、これらの問題点を克服した新規抗うつ薬が望まれている。[2-5] Figure 2 に代表的な SSRI とその化学構造を記す。



パロキセチン [6]　セルトラリン [7]　フルオキセチン [8]　エスシタロプラム [9]

Figure 2. 代表的な SSRI の化学構造

1-2. SSRI の作用メカニズムと問題点克服のためのストラテジー

脳内セロトニンの不足によるセロトニン神経伝達の低下がうつ病の原因の一つとするセロトニン仮説があり、SSRI はセロトニントランスポーター（SERT）を阻害することにより脳内セロトニン量を上げることで抗うつ作用を発揮すると考えられている。[10] 上述の通り、SSRI には投薬開始から治療効果の発現まで２～３週間かかる治療オンセットの遅さという問題点がある。この観点で、5-HT_{1A} 受容体阻害活性を持つピンドロール（Figure 3）をパロキセチン等の SSRI と併用すると治療オンセットが早まるという臨床研究結果が報告された。[12,13] SSRI の治療オンセットの遅さの原因として次の機構が考えられる。Figure 4 に、セロトニン神経のプレシナプスから遊離したセロトニンがポストシナプスに作用しシグナルが伝達される様子を、健常人の場合、うつ病患者の場合、そして、SSRI 治療による変化を模式図として示す。投薬開始時期には SSRI 投与によりシナプス間隙で一過的にセロトニンが上昇するが、その上昇したセロトニンが、プレシナプスに発現する 5-HT_{1A} 自己受容体に作用し、ネガティブフィードバック機構が働きセロトニン分泌が抑制される。従って、アゴニストであるセロトニンが 5-HT_{1A} 自己受容体に長時間作用することによって、細胞表面における発現レベルの低下による 5-HT_{1A} 自己受容体の感受性の低下、すなわち、脱感作が起こり、初めて脳内セロトニン量の上昇が起こるため、SSRI の連続投与が必要となる。[14,15] そのことが、SSRI の治療オンセットという問題の原因であると考えられている。

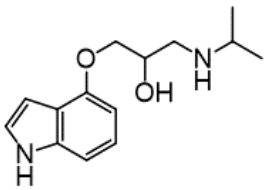


Figure 3. ピンドロールの化学構造 [11]

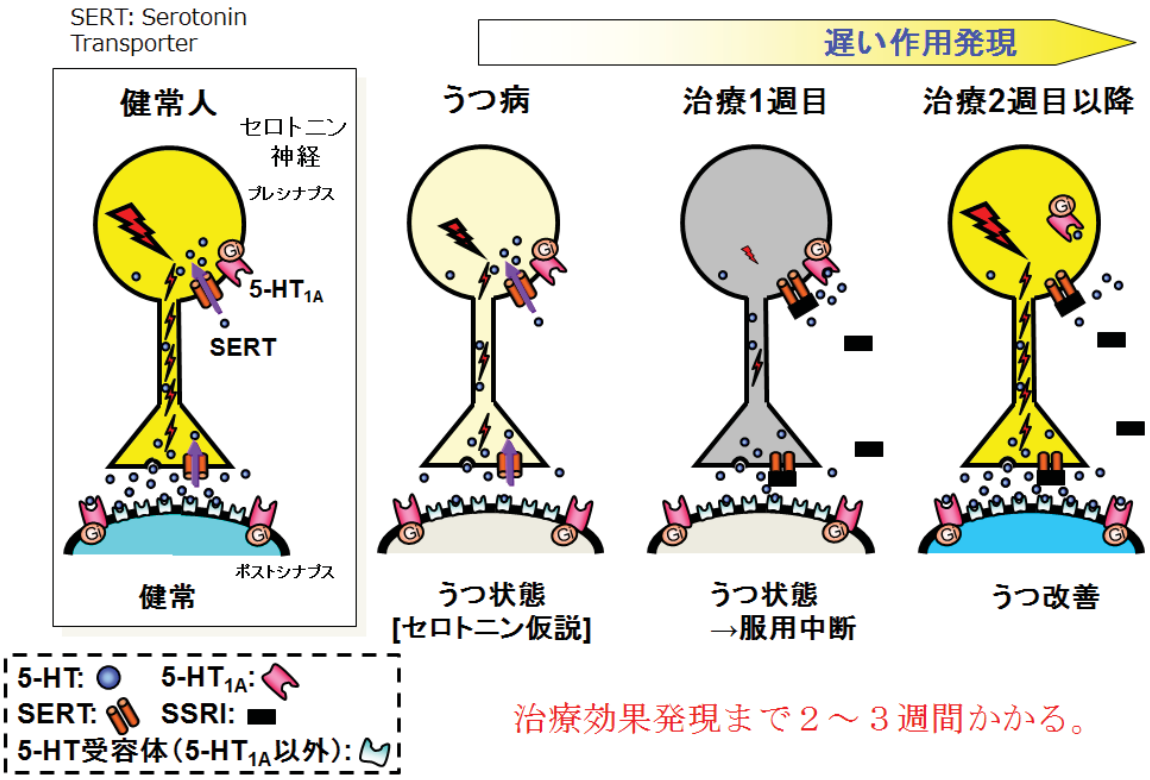


Figure 4. SSRI の作用機序

ピンドロールと SSRI を併用した臨床研究において、ピンドロールは 5-HT_{1A} 自己受容体を拮抗するため、セロトニン神経のネガティブフィードバック機構が阻害され SSRI の治療オンセットが早まったと考えられる。[16-18] すなわち、一つの分子でセロトニン取り込阻害（SRI）活性と 5-HT_{1A} 自己受容体阻害活性を併せ持つ化合物は、Figure 5 に示すように SRI 活性に基づくシナプス間隙でのセロトニン上昇作用を持ち、かつ、セロトニンの 5-HT_{1A} 自己受容体へのネガティブフィードバック機構を阻害できるため、SSRI の治療オンセットの遅さという問題点を改善する薬剤となりうる。そこで、本研究では、セロトニン取り込阻害（SRI）活性と 5-HT_{1A} 自己受容体阻害活性を併せ持つ薬剤の創製を目的とした。

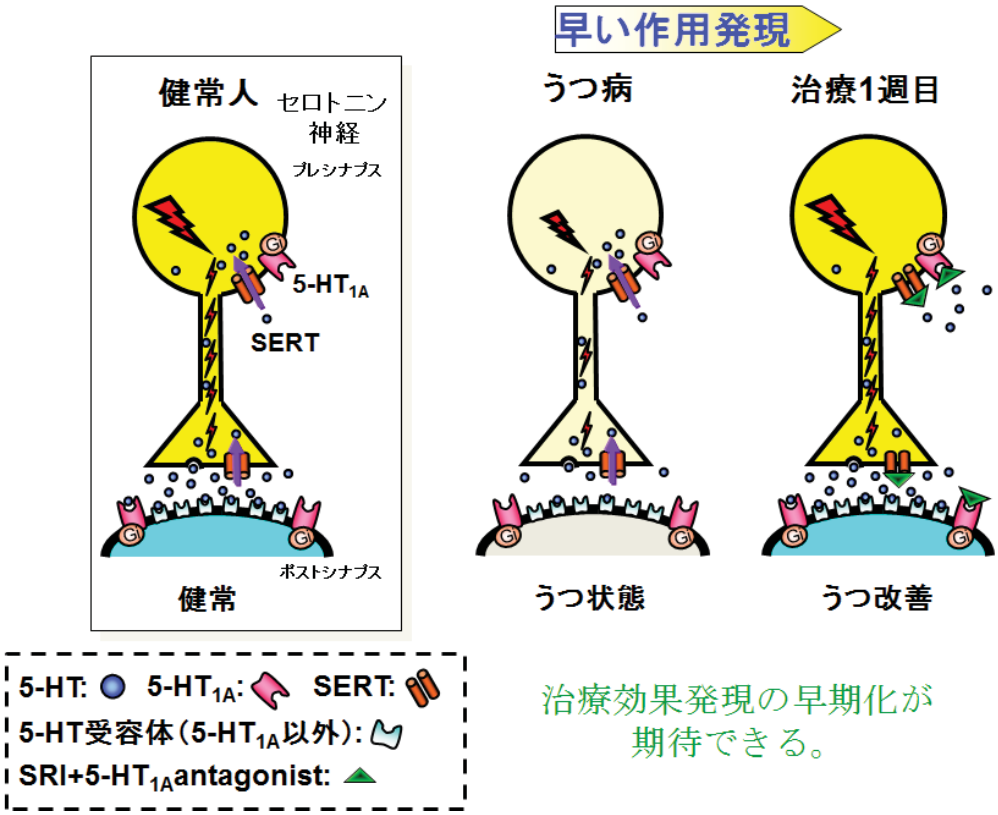


Figure 5. SRI+5-HT$_{1A}$ antagonist の作用機序

セロトニン取り込阻害（SRI）活性と 5-HT$_{1A}$ 自己受容体阻害活性を併せ持つ薬剤の取得を目指した創薬において、探索研究を効率化するべく次のような創薬ストラテジーを選択した。すなわち、セロトニントランスポーター（SERT）と 5-HT$_{1A}$ 受容体に対する結合阻害活性を指標に、より強い結合阻害活性を有する化合物を取得した後、SERT と 5-HT$_{1A}$ 受容体に対する機能評価を実施するというストラテジーである。その理由としては、次のことがあげられる。第一に、SRI 活性と SERT の結合阻害活性が良く相関することが知られており（Figure 6）、[19,20] また、SRI 活性評価はスループット性が良くないことから、SERT の結合阻害活性を指標に化合物を選択していくことが強い SRI 活性を有する化合物取得のために効率的であると考えた。第二に、私が目指すセロトニン取り込阻害（SRI）活性と 5-HT$_{1A}$ 自己受容体阻害活性を併せ持つ薬剤は、SRI 活性によりシナプス間隙にセロトニン（5-HT$_{1A}$ 受容体の内因性リガンドでフルアゴニストにあたる）の遊離量が増えた環境下での 5-HT$_{1A}$ 受容体に対する阻害活性を示す化合物であり、そのような環境下では、5-HT$_{1A}$ 部分作動薬も 5-HT$_{1A}$ 受容体に対する阻害活性を示しうることが知られている（Figure 7-1, 7-2）。[21] そこで、5-HT$_{1A}$ 受容体に対しより強い結合阻害活性を示す化合物を選択し、機能評価の結果、5-HT$_{1A}$ アンタゴニストあるいは 5-HT$_{1A}$ 部分作動薬であれば in vivo にてその化合物の抗うつ様作用を評価していくといった評価ステップが効率的と考えた。

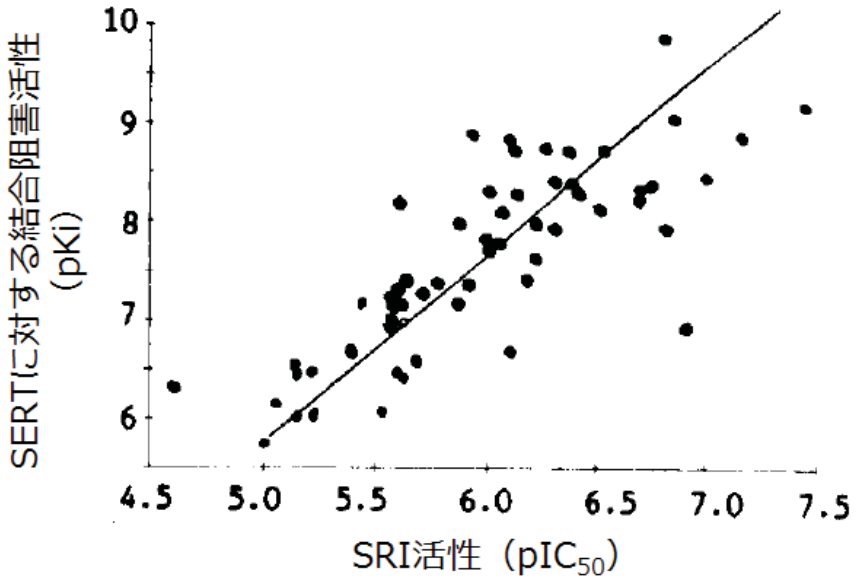


Figure 6. SERT 結合阻害活性と SRI 活性の相関 [19]

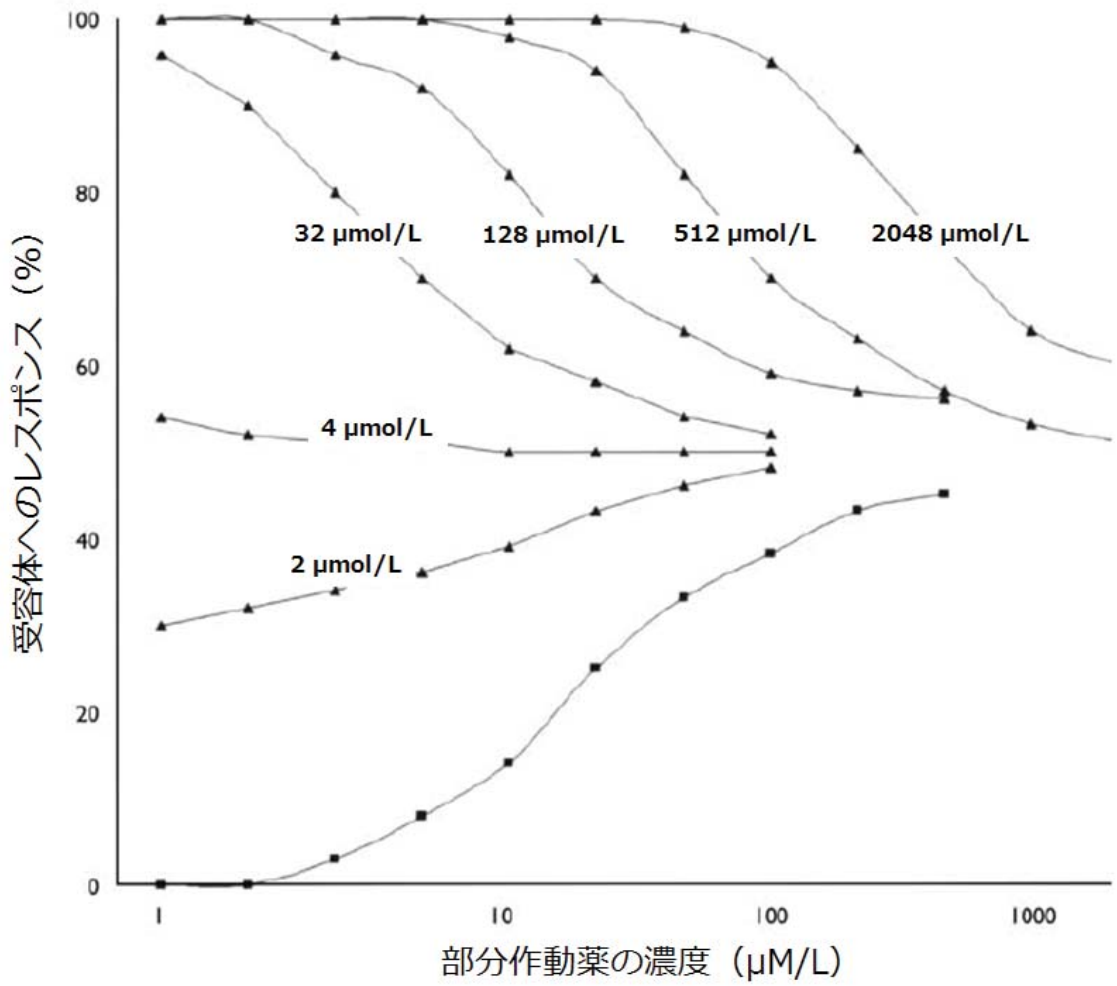


Figure 7-1. 理論上の受容体への部分作動薬の濃度-レスポンス曲線 [21]

■:アゴニストなし，▲:アゴニスト共存（数字は濃度）

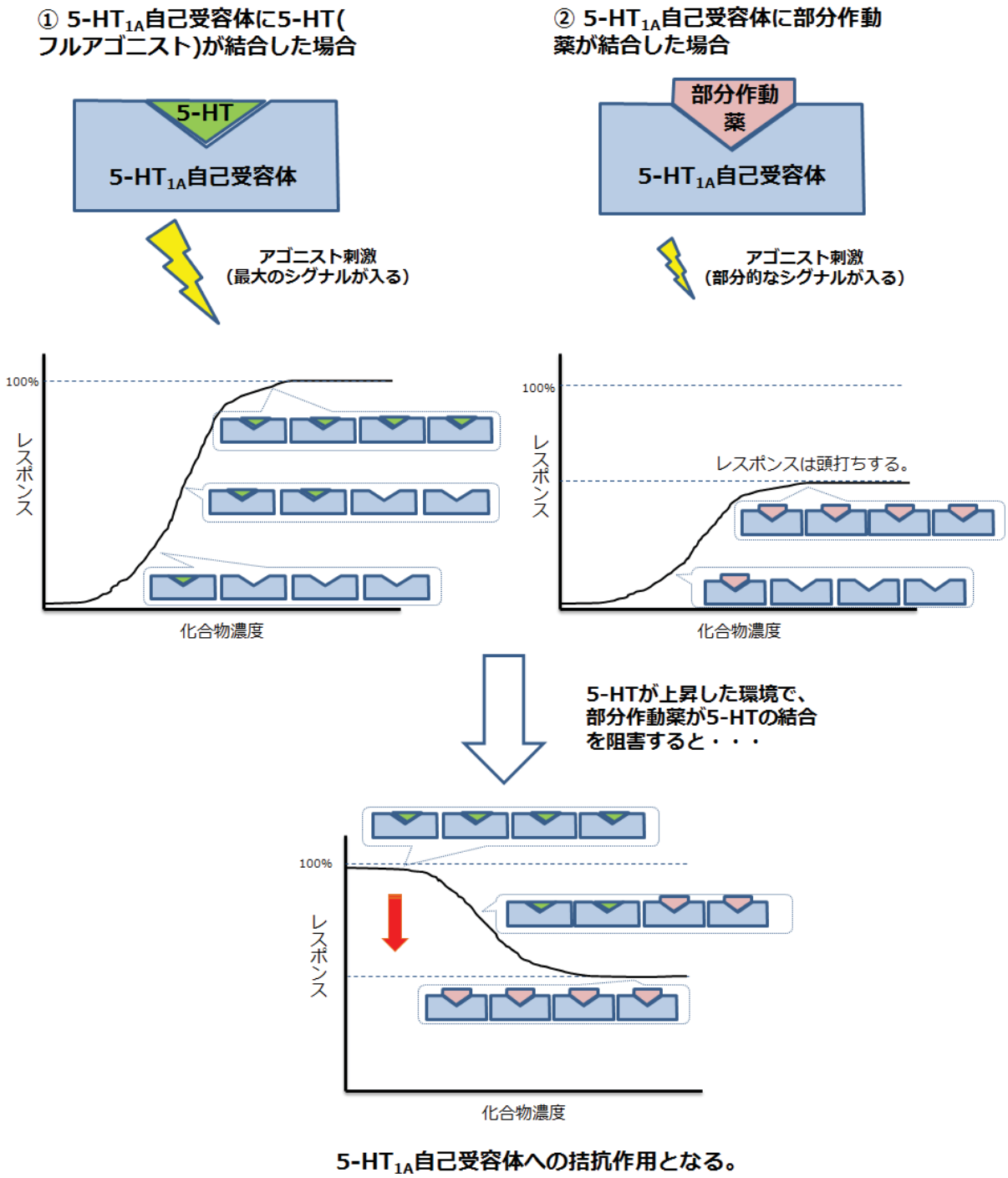


Figure 7-2. Figure 7-1 の模式図

さらに、SERT に対する結合阻害活性と 5-HT$_{1A}$ 受容体に対する結合阻害活性をバランスよく併せ持つ化合物が生体内で SERT と 5-HT$_{1A}$ 受容体に同程度の強さで作用すると考えられる。化合物が生体内でどのように分布するか、また、生体内の様々な組織に標的タンパクがどのように分布するかといった要因により、二つの標的タンパクに作用する化合物の治療効果を最大化するための最適な in vitro 活性のバランスが決まってくる。[22] しかしながら、SERT 結合阻害活性と 5-HT$_{1A}$ 結合阻害活性の最適なバランスについて、臨床上のエビデンスはない。そこで、SERT と 5-HT$_{1A}$ 受容体に対す

るバランスの良い結合阻害活性としては同程度の活性値と言える 10 倍以内の範囲で SERT と 5-HT_{1A} 受容体への阻害定数（Ki 値）を示す化合物と定義した。一方、パロキセチン，セルトラリン，フルオキセチン，エスシタロプラムといった多くの SSRI は、SERT に対し 0.1 nM から一桁 nM の範囲で非常に強い結合阻害活性を示す（パロキセチン: Ki = 0.10 nM，セルトラリン: Ki = 0.26 nM，フルオキセチン: Ki = 1.1 nM，エスシタロプラム: Ki = 1.1 nM）。[23] 従って、本研究では、SERT と 5-HT_{1A} 受容体に対し強くかつバランスの良い結合阻害活性を示す化合物として、SERT および 5-HT_{1A} に対し 10 nM 以下の Ki 値を示し、かつ、その Ki 値が 10 倍の範囲内である化合物の取得を目指すこととした。

1-3. 二つの作用を併せ持つ化合物創製のためのストラテジー

一つの分子で二つの作用を併せ持つ化合物のことを Designed Multiple Ligand（DML）と呼ぶ。DML を得るためのストラテジーとして、『designing IN』,『balancing』,『designing OUT』という三つのアプローチが知られている（Figure 8）。[24] 『designing IN』とは高い選択性で標的タンパク A あるいは標的タンパク B に作用する化合物を起点とし、もう一方の標的タンパクへの作用を付加させる化合物デザインアプローチである。続いて、『balancing』とは標的タンパク A への強い作用と弱いながらも標的タンパク B への作用も併せ持つ化合物を起点とし、例えば標的タンパク B への作用を増強し、標的タンパク A と標的タンパク B への作用をバランスよく併せ持つ化合物をデザインするアプローチである。最後に、『designing OUT』とは標的タンパク A と標的タンパク B への強い作用を併せ持つが副作用につながるような標的タンパク C への作用も併せ持つ化合物を起点とし、不要な標的タンパク C への作用を除去するような化合物デザインを行うアプローチである。DML を得るためには、起点となる化合物のプロファイル・特性を理解した上で、上記３つのアプローチの内どれが最適な方法かを考え選択することが重要である。

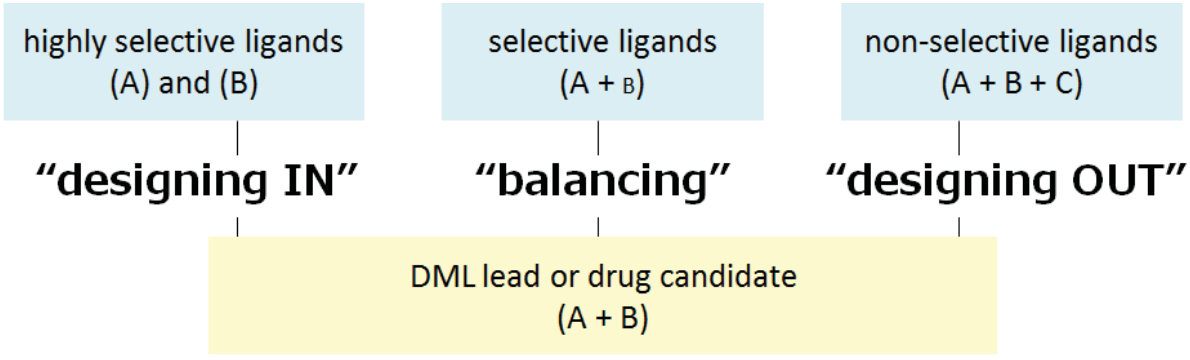


Figure 8. DML (Designed Multiple Ligand) を得るためのアプローチ [24]

1-4. 本研究論文の構成

本研究論文は、第 1 章から第 5 章までの構成とする。

本章では、研究の背景となる事項について述べた。

第 2 章では、化合物 **1** を起点とした『balancing』により **SMP-304** を見出すまでの探索合成戦略とその結果について述べる。また、第 3 章では、**SMP-304** の問題点とその課題を如何に解決し、**DSP-1053** を見出したかについて記す（Figure 9）。

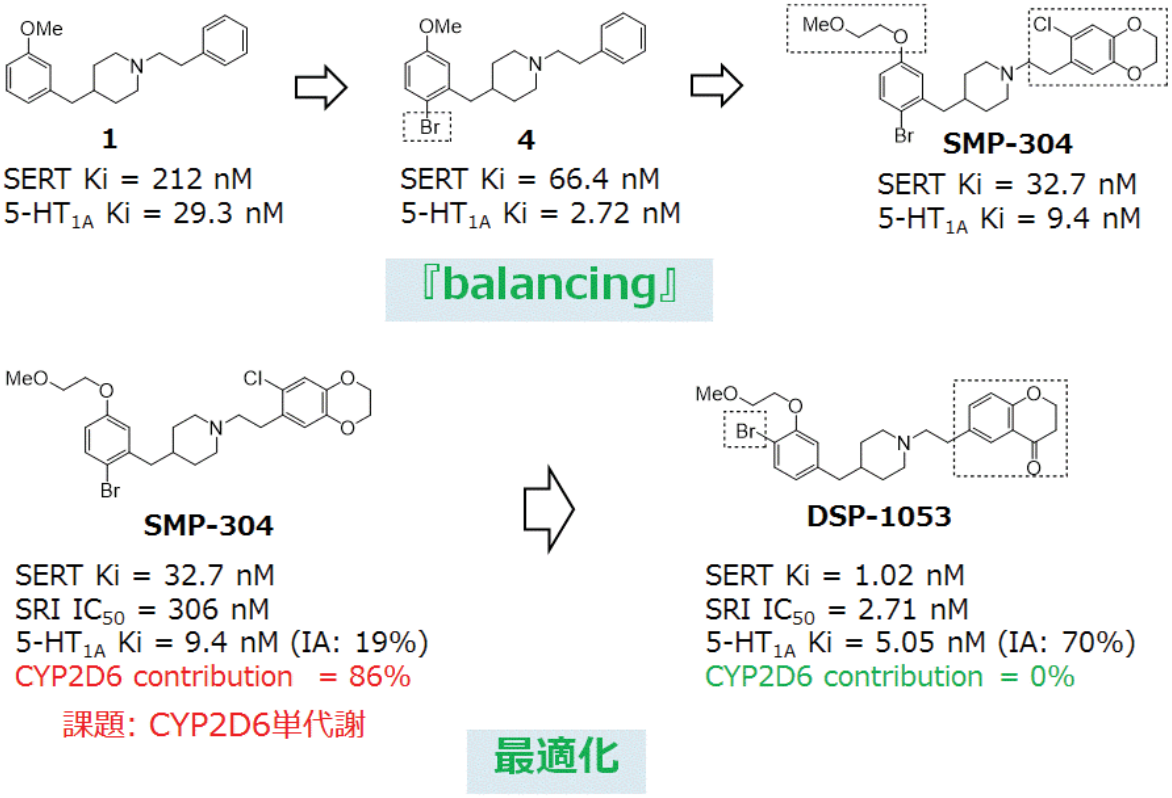
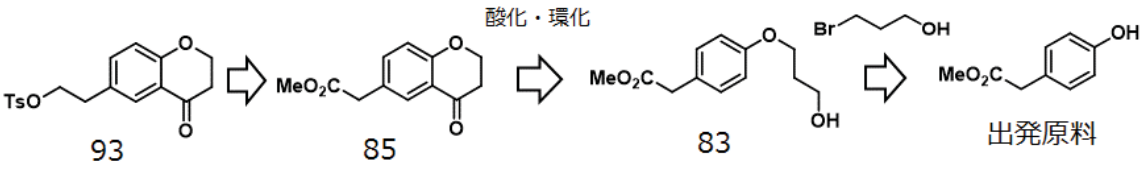
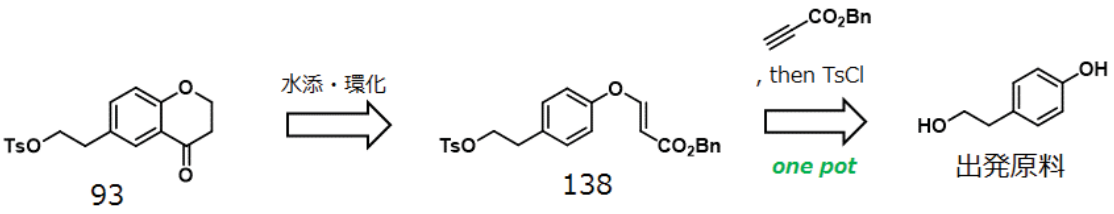


Figure 9. **SMP-304** および **DSP-1053** の創製

第 4 章では、**DSP-1053** の重要中間体である **93** の実践的合成法をその研究段階や検討期間に合わせ、合成的課題を解決し、第一世代合成法と第二世代合成法を段階的に構築し、**DSP-1053** の遅滞なき研究開発に貢献したので、その課題解決のための戦略と結果について述べる（Figure 10）。



第一世代合成法　総収率：49%，全反応工程数：8段階



第二世代合成法　総収率：71%，全反応工程数：5段階

Figure 10. 重要中間体 **93** の第一世代合成法と第二世代合成法

最後に、第 5 章では、本研究の結論をまとめる。

1-5. 引用文献

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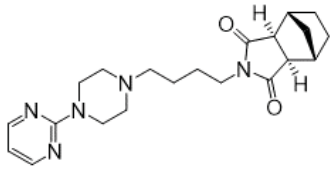
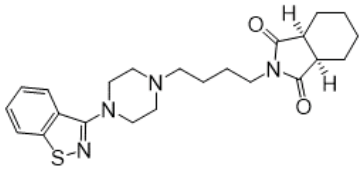
第 2 章　**SMP-304** の創製

2-1. 創薬研究の起点化合物 **1** について

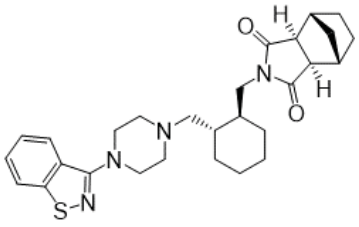
大日本住友製薬（株）では、古くからセロトニンやドパミンといった神経伝達物質の受容体やトランスポーターに作用する薬剤の研究開発が行われている（代表化合物：タンドスピロン、ペロスピロン、ルラシドン、ブロナンセリン等（Figure 11））。



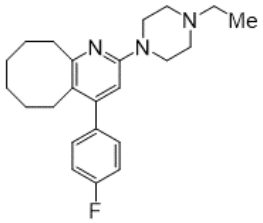
タンドスピロン [1]
（5-HT_{1A} 部分作動薬）



ペロスピロン [2]
（5-HT_{2A} / D_2 アンタゴニスト）



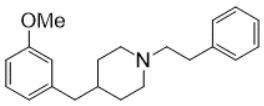
ルラシドン [3]
（5-HT_{2A} / D_2 / 5-HT_7 アンタゴニスト
＋5-HT_{1A} 部分作動薬）



ブロナンセリン [4]
（5-HT_{2A} / D_2 アンタゴニスト）

Figure 11. 大日本住友製薬（株）が創製したセロトニン受容体に作用する薬剤

これらの化合物の構造上の特徴は、塩基性アミンを有するピペラジン環といった環状アミン構造を部分構造として有しており、特に、Figure 11 に示すように、タンドスピロン、ペロスピロン、ルラシドンについては、その環状アミン構造を中心に一方にピリミジン環やベンゾイソチアゾール環といった芳香族環を有し、炭素リンカーを介してもう一方に環状構造を有している。大日本住友製薬（株）では、これらのプロジェクトを通じ、このような環状アミン構造を中心に左右両方にリンカーを介して環状構造を有する化合物を多く化合物ライブラリーとして保有している。その化合物ライブラリーの中で、Figure 12 に示すように、ピペリジン環といった環状アミン構造を中心に、ピペリジン環の 4 位にベンジル基を有し、ピペリジン環の 1 位に炭素リンカーを介しベンゼン環を有する化合物 **1** が、5-HT_{1A} 受容体に対し強い結合阻害活性を有し、弱いながらも SERT に対しても結合阻害活性を有することが分かった。そこで、化合物 **1** を起点とし、DML のうち『balancing』による SERT と 5-HT_{1A} 受容体に対して強い結合阻害活性を併せ持つ化合物の取得を目指した探索合成研究を開始することとした。



1

SERT Ki = 212 nM
5-HT_{1A} Ki = 29.3 nM

Figure 12. 化合物 **1** のプロファイル

2-2. 『balancing』による SERT と 5-HT_{1A} への強い結合阻害活性を併せ持つ化合物の創出

化合物 **1** を起点とした『balancing』により SERT と 5-HT_{1A} 受容体に対して強い結合阻害活性を併せ持つ化合物の取得を目指すにあたり、化合物 **1** の SERT に対する結合阻害活性の向上を試みた。化合物 **1** と同様のベンジルピペリジン誘導体のピペリジン環 4 位に置換したベンジル基のベンゼン環の 6 位に F 基や Br 基といったハロゲン原子を有する化合物において、強い SERT 結合阻害活性を示す化合物と 5-HT_{1A} 結合阻害活性を示す化合物が Bristol-Myers Squib 社から報告された。[5,6] そこで、化合物 **1** のピペリジン環 4 位に置換したベンジル基のベンゼン環の 6 位に各ハロゲン原子の導入を行った（Figure 13）。

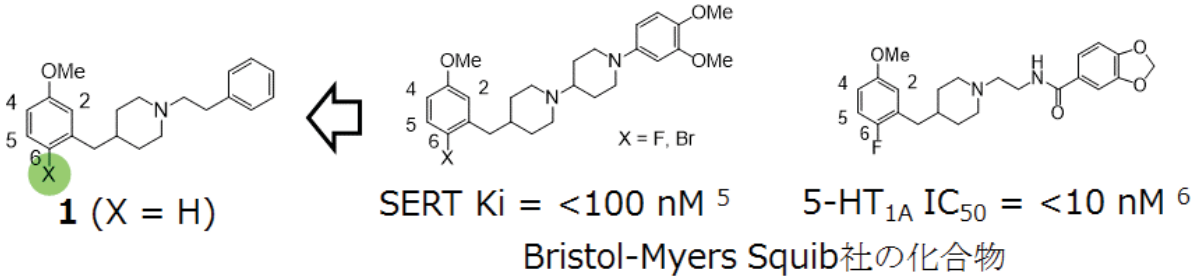
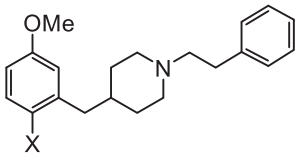


Figure 13. SERT に対する結合阻害活性向上へのストラテジー

その結果、ハロゲン原子が大きくなるにつれ SERT 結合阻害活性は向上し、Br 基を有する化合物 **4** が最も強い SERT 結合阻害活性を示した。また、ハロゲン原子の導入により、SERT 結合阻害活性とともに 5-HT_{1A} 結合阻害活性も向上した（Table 1）。

Table 1. ハロゲン原子の導入検討

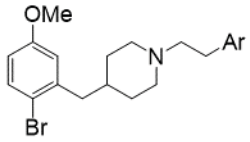


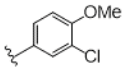
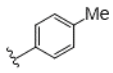
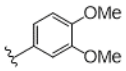
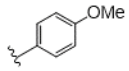
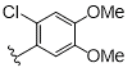
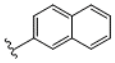
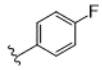
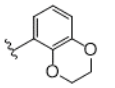
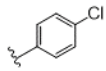
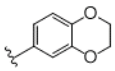
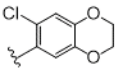
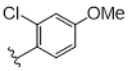
Compound	X	h-SERT[a]	h-5-HT$_{1A}$[a]
1	H	212 ± 46	29.3 ± 4.1
2	F	274 ± 70	6.04 ± 0.0
3	Cl	102 ± 15	2.27 ± 0.38
4	Br	66.4 ± 11.3	2.72 ± 0.81

[a] h = human. Ki values (nM) are the means of two independent experiments.

さらなる SERT 結合阻害活性の向上を目指し、ピペリジン環 1 位にリンカーを介して置換したベンゼン環への置換基導入を行った（Table 2）。まず、構造活性相関取得のために、F 基，Cl 基，Me 基，MeO 基を 2 位，3 位，4 位に導入した（化合物 **5**, **6**, **7**, **8**, **9**, **10**, **11**, **12**）。その結果、3 位と 4 位への置換基導入により SERT 結合阻害活性がより向上し、中でも MeO 基の導入が最も効果的であることがわかった（化合物 6, 7, 8）。続いて、SERT 結合阻害活性向上に効果的であった Cl 基と MeO 基が 2 つ以上置換した化合物の検討を行った。そして、3 位と 4 位に Cl 基あるいは MeO 基が置換した化合物 **14**, **15** において、さらなる SERT 結合阻害活性の向上が見られた。一方、化合物 **4** のベンゼン環を二環性のナフタレン環に変換したところ、SERT 結合阻害活性が向上した（化合物 **17**, **18**）。そこで、化合物 **15** の 3,4-ジメトキシフェニル基を参考に二環性のベンゾジオキサン環を右側パーツとして導入したところ強い SERT 結合阻害活性を示す化合物 **20** が得られた。さらに、Cl 基を導入した化合物 **21** が、最も強い SERT 結合阻害活性を示し、その 10 倍の範囲内でバランスよく 5-HT$_{1A}$ 結合阻害活性を示した。

Table 2. 右側 Ar 基の変換検討



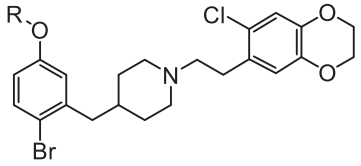
Compound	Ar	h-SERT[a]	h-5-HT$_{1A}$[a]	Compound	Ar	h-SERT[a]	h-5-HT$_{1A}$[a]
4		66.4 ± 11.3	2.72 ± 0.81	**14**		18.9 ± 0.2	1.04 ± 0.18
5		65.3 ± 7.7	1.40 ± 0.66	**15**		26.9 ± 1.0	1.56 ± 0.22
6		37.5 ± 4.1	0.81 ± 0.19	**16**		28.0 ± 2.9	1.96 ± 0.45
7		37.6 ± 3.0	3.03 ± 0.60	**17**		38.9 ± 6.8	4.81 ± 0.49
8		37.5 ± 4.1	0.82 ± 0.17	**18**		25.6 ± 5.2	0.89 ± 0.14
9		51.2 ± 6.5	1.04 ± 0.34	**19**		25.6 ± 0.3	2.12 ± 0.09
10		46.2 ± 1.5	1.34 ± 0.59	**20**		14.0 ± 1.4	0.36 ± 0.05
11		62.4 ± 3.1	4.08 ± 0.75	**21**		12.4 ± 1.5	6.46 ± 0.57
12		42.6 ± 1.2	2.93 ± 0.78				
13		30.5 ± 2.3	1.12 ± 0.48				

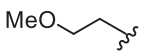
[a] h = human. Ki values (nM) are the means of two independent experiments.

強い SERT 結合阻害活性と 5-HT$_{1A}$ 結合阻害活性をバランスよく併せ持つ化合物 **21** を見出したが、本化合物は、強い CYP2D6 阻害活性を有することがわかった。シトクローム P450（CYP）は、生体内で様々な基質を酸化する薬物の主要な代謝酵素として知られている。一方、血中に複数種類の薬物が存在することにより、互いの代謝速度に変化をもたらし、薬剤の作用に対して影響を与えることを薬物相互作用（DDI）と呼ぶ。[7] CYP のサブタイプの一つである CYP2D6 の強い阻害活性を有する薬剤は、この DDI を引き起こす可能性があることが知られている。実際に、うつ病患者は複数の薬剤を処方されるケースが多いことから可能な限り DDI のリスクの少ない抗うつ剤が望まれている。[8] このような背景から、化合物 **21** の CYP2D6 阻害活性の改善検討を行った。化合物 **21** は強い SERT/5-HT$_{1A}$ 結合阻害活性を有することから可能な限りその構造は残すべきと考え、これまでに検討していなかった左側フェニル基に置換したメトキシ基を他のアルコキシ基への変換検討を行った（Table 3）。その結果、アルキル基が大きくなるにつれて CYP2D6 阻害活性は弱くなり、メトキシエチル基が置換

した化合物 **24**（**SMP-304**）は最も弱い CYP2D6 阻害活性を示した。本化合物は、Ki 値が 10 nM 以下ではないものの比較的強い SERT 結合阻害活性と 5-HT_{1A} 結合阻害活性をバランスよく併せ持ち CYP2D6 阻害活性が改善された化合物であることから、SERT や 5-HT_{1A} 受容体に対する機能評価及びコンセプト検証のための in vivo 薬効評価を行う化合物として選抜した。

Table 3. メトキシ基の変換検討



Compound	R	h-SERT[a]	h-5-HT_{1A}[a]	CYP2D6[b]
21	Me	12.4 ± 1.5	6.46 ± 0.57	<0.4
22	Et	18.7 ± 2.1	23.4 ± 6.1	1.4
23	2-Pr	13.9 ± 0.3	15.9 ± 3.6	1.5
24 (SMP-304)		32.7 ± 3.4	9.4 ± 0.2	3.0

[a] h = human. Ki values (nM) are the means of at least two independent experiments.
[b] IC_{50} value (uM).

2-3. **SMP-304** のラット強制水泳試験による抗うつ様作用評価

SMP-304 の SERT と 5-HT_{1A} 受容体に対する機能評価を行った。SRI 活性が IC_{50} = 306 nM、5-HT_{1A} 受容体に対する内因活性が 19%であり、目的とするセロトニン取り込阻害（SRI）活性と 5-HT_{1A} 自己受容体阻害活性を併せ持ちうる化合物であることが確認されたので、代表的な SSRI の一つであるパロキセチンとのラット強制水泳試験における抗うつ様作用のオンセットの早さについて比較試験を行った。ラット強制水泳試験とは、ラットを水中に長時間浮かべると次第に泳ぐのをやめ無動時間が増加する。この状態をうつ状態に陥ったと判断し、抗うつ作用の期待できる薬剤の投与後の無動時間の変化を測定することで抗うつ様作用を評価する系である（Figure 14）。Figure 14 は、**SMP-304** およびパロキセチン投与による無動時間の変化を示している。縦軸は無動時間を横軸は薬剤の投与量を示し、図 A および図 B の投与量 0 mg/kg すなわち溶媒群における無動時間と比較し有意な減少が見られた場合に抗うつ様作用が発現したと判断した。**SMP-304** は 1 mg/kg および 3 mg/kg の経口連続投与で二日目から抗うつ様作用を発現する（Figure 15, 図 A）のに対し、パロキセチンは経口連続投与で 10 mg/kg でも抗うつ様作用を発現しなかった（Figure 15, 図 B）。以上の結果から、**SMP-304** はラット強制水泳試験において代表的な SSRI の一つであるパロキセチンよりも早い抗うつ様作用のオンセットを示した。**SMP-304** は、目的とするセロトニン取り込阻害（SRI）活性と 5-HT_{1A} 自己受容体阻害活性を併せ持ち SSRI の課題の一

つである作用オンセットの遅さを克服しうる化合物であることがわかった。

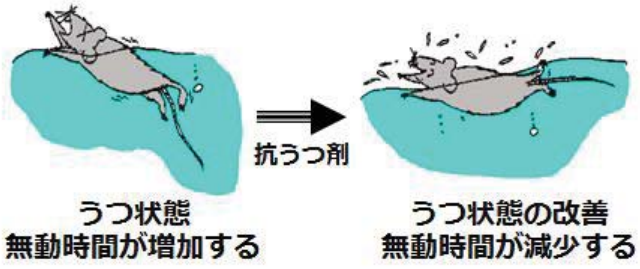
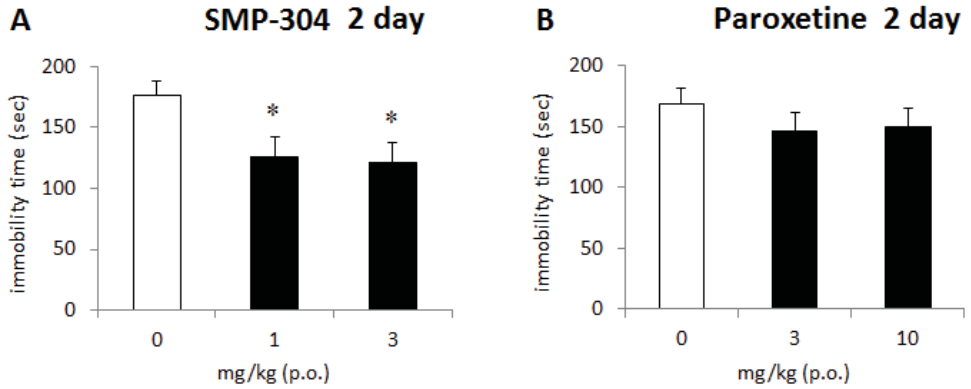


Figure 14. ラット強制水泳試験の概要



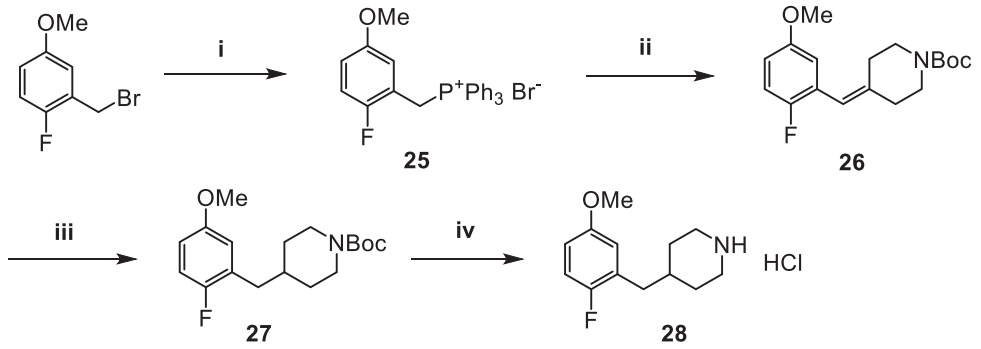
Each bar represents the mean ± S.E.M. n = 12 (forced swimming test) per group. * P < 0.05, significantly different from the vehicle-treated group (Dunnett's multiple comparison test).

Figure 15. ラット強制水泳試験の結果
(A) **SMP-304** 二日間投与でのラット強制水泳試験時の無動時間
(B) パロキセチン二日間投与でのラット強制水泳試験時の無動時間

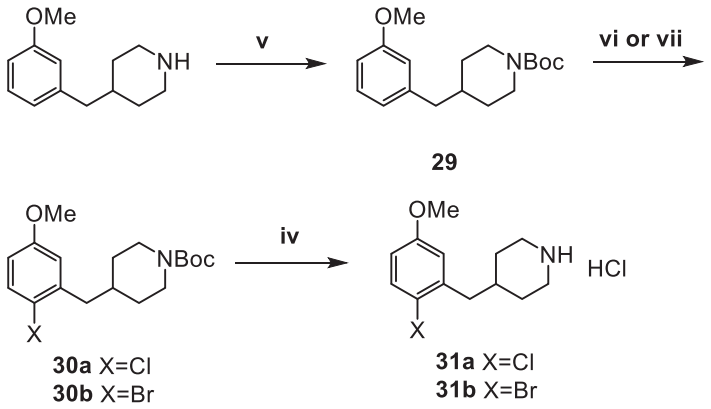
2-4. 化合物の合成

上記化合物は、左側パーツにあたるベンジルピペリジン中間体と右側パーツにあたるトシラートやブロマイド中間体をそれぞれ合成し、アルキル化反応により最終化合物とした。左側パーツのベンゼン環 6 位に F 基の置換したアミン中間体を合成するために、2-(bromomethyl)-1-fluoro-4-methoxybenzene とトリフェニルホスフィンを作用させホスホニウム塩 **25** とした後、Wittig 反応によりオレフィン **26** とした。続いて、パラジウム炭素を用いた水素添加反応により **27** とした後、塩化水素－1,4-ジオキサンによる脱 Boc 反応を行い、アミン中間体 **28** を得た（Scheme 1）。左側パーツのベンゼン環 6 位に Cl 基あるいは Br 基の置換したアミン中間体は、Scheme 2 に示したルートにより合成した。購入可能な 4-(3-methoxybenzyl)piperidine を Boc 化した後、NCS あるいは NBS によるハロゲン化により **30a, 30b** を合成した。これらを塩化水素－1,4-ジオキサンと作用させることによりアミン中間体 **31a, 31b** へと導いた。左側パーツのベンゼン環に置換したアルコキシ基の変換検討のためにエトキシ基，2-プロポキシ基，メトキシエトキシ基の置換した各アミン中間体を次の方法により合成した（Scheme 3）。アミン中間体 **31b** を BBr_3 に作用させ脱メチル化反応を行い、続いて、Boc 化反応に

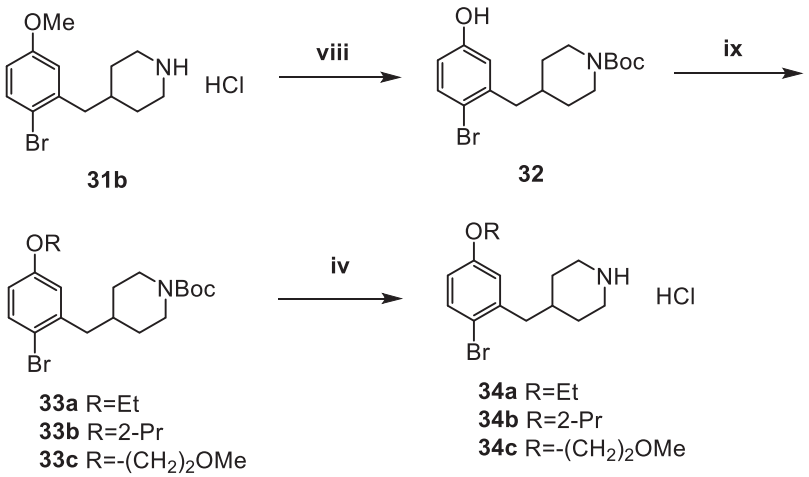
付すことでフェノール **32** を得た。**32** を各アルキル化剤と作用させ **33a – 33c** とした後、塩化水素－1,4-ジオキサンによる脱 Boc 化反応によりアミン中間体 **34a – 34c** を合成した。



Scheme 1. 試薬と反応条件: (i) PPh_3, toluene, reflux, (ii) *tert*-butyl 4-oxopiperidine-1-carboxylate, K_2CO_3, 2-PrOH, reflux, (iii) H_2, 10% Pd-C, MeOH, r.t., (iv) 4N HCl/1,4-dioxane, $CHCl_3$, r.t.

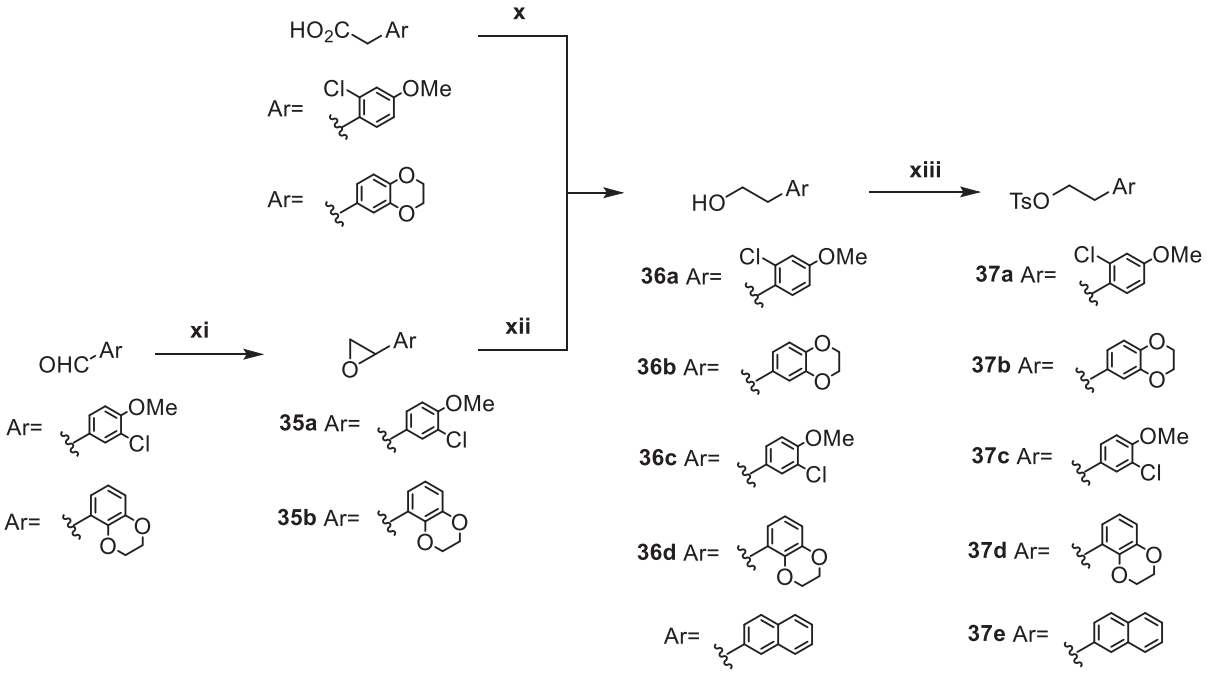


Scheme 2. 試薬と反応条件: (v) Boc_2O, THF, r.t., (vi) NCS, DMF, r.t., (vii) NBS, DMF, r.t., (iv) 4N HCl/1,4-dioxane, $CHCl_3$, r.t.

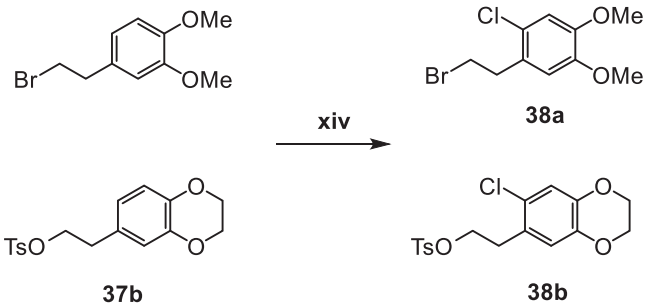


Scheme 3. 試薬と反応条件: (viii) BBr_3, CH_2Cl_2, r.t., then Boc_2O, THF, 20% aq.K_2CO_3, r.t., (ix) R-X (X = Br or I), K_2CO_3, DMF, (iv) 4N HCl/1,4-dioxane, $CHCl_3$, r.t.

右側パーツの最適化のために各トシラートあるいはブロマイド中間体を Scheme 4 に示す方法にて合成した。2-(2-chloro-4-methoxyphenyl)acetic acid あるいは 2-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)acetic acid とボラン-THF 錯体を反応させ、アルコール **36a, 36b** へと導いた。また、3-chloro-4-methoxybenzaldehyde あるいは 2,3-dihydrobenzo[b][1,4]dioxine-5-carbaldehyde を Corey-Chaykovsky 反応[9] によりエポキシ **35a, 35b** とした後、BF_3-Et_2O 錯体存在下、$NaBH_4$ と作用させることでエポキシの開環反応を行い、アルコール **36c, 36d** とした。**36a – 36d** あるいは 2-naphthalene ethanol をトリエチルアミンと触媒量のトリメチルアミン塩酸塩の存在下、トシルクロライドと作用させることで速やかにトシラート中間体 **37a – 37e** を得た。[10] また、4-(2-bromoethyl)-1,2-dimethoxy-benzene あるいはトシラート **37b** を直接クロル化することで簡便にブロモ中間体 **38a** あるいはトシラート中間体 **38b** を合成した（Scheme 5）。



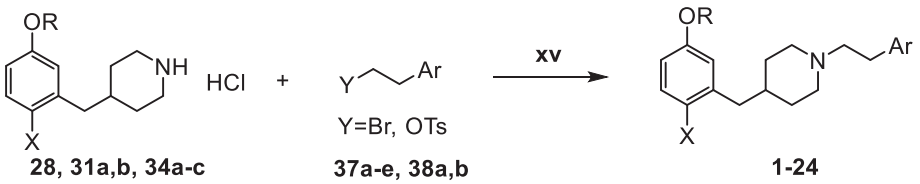
Scheme 4. 試薬と反応条件: (x) BH_3-THF, THF, r.t. (xi) MeS^+I^-, KOH, DMSO, 40°C, (xii) $NaBH_4$, BF_3-OEt_2, THF, r.t., (xiii) TsCl, MeN_3HCl, Et_3N, CH_2Cl_2, 0°C



Scheme 5. 試薬と反応条件: (xiv) NCS, DMF, r.t.

最後に、各アミン中間体とブロモあるいはトシラート中間体を炭酸カリウム存在下、アルキル化反応を行うことで、目的とする化合物 **1 – 24** をそれぞれ合成した（Scheme

6)。



Scheme 6. 試薬と反応条件: (xv) K_2CO_3, MeCN, reflux

2-5. 考察ならびに小括

SSRI と 5-HT_{1A} アンタゴニスト作用を有するピンドロールとの併用により抗うつ作用のオンセットが早まるといった臨床研究結果などから、セロトニン取り込阻害（SRI）活性と 5-HT_{1A} 自己受容体阻害活性を併せ持つ薬剤の創製を目的とする探索研究を開始した。大日本住友製薬（株）の過去のプロジェクト内で合成された化合物 **1** が強い 5-HT_{1A} 結合阻害活性と弱いながらも SERT 結合阻害活性を併せ持つことに着目し、化合物 **1** を起点とした比較的構造の近い誘導体の SAR 情報を活用した『balancing』により、SERT と 5-HT_{1A} に対しバランス良く強い結合阻害活性を併せ持ち、CYP2D6 阻害作用の比較的弱い **SMP-304** を見出した。機能評価の結果から、**SMP-304** は、SRI 活性を示し、5-HT_{1A} の弱い部分作動薬であり 5-HT_{1A} 自己受容体への阻害活性を有しうることから、ラット強制水泳試験にて抗うつ様作用の評価を行ったところ、既存 SSRI であるパロキセチンよりも早いオンセットを示し、私が目的とする SSRI の課題の一つである抗うつ作用のオンセットの遅さを克服しうる化合物であることがわかった。

SMP-304 が実際に生体内で 5-HT_{1A} 自己受容体阻害活性を有することを証明するためにはより詳細な検討が必要であるが、本研究結果より、SRI 活性に 5-HT_{1A} 自己受容体への阻害活性を併せ持つ化合物は SSRI よりも抗うつ作用のオンセットが早い新規抗うつ薬となり得ることが示唆された。また、二つの標的タンパクに強い作用を併せ持つ化合物（DML）の取得は容易なことではないが、一方の標的タンパクに強い作用を有し、もう一方の標的タンパクに弱いながらも作用を有する化合物を起点とした『balancing』は、DML 取得のための有用なアプローチの一つであることが実証された。**SMP-304** は期待通りの抗うつ作用の早いオンセットを示したものの、その後の検討で CYP2D6 の代謝寄与率が 86%と高値で CYP2D6 の単代謝であることが判明した。この **SMP-304** の課題に対する検討とその結果については、第 3 章で述べたい。

2-6. 実験の部

2-6-1. Synthesis

Melting points were determined on Stanford Research Systems OptiMelt MPA 100 without correction. NMR spectra were recorded at ambient temperature on a JEOL JMN-LA300 spectrometer. Chemical shifts are expressed in δ values (ppm) relative to a tetramethylsilane as an internal standard, and signals are expressed as s (singlet), d (doublet), t (triplet), m (multiplet) or br (broad). High-resolution mass spectra (HRMS) were recorded on a Thermo

Fisher Scientific LTQ orbitrap Discovery MS equipment. Elemental analysis was performed on a CE Instrument EA1110 and a Yokokawa analytical system IC7000. In general, reagents and solvents were used as obtained from commercial suppliers without further purification. Reaction progress was determined by thin layer chromatography (TLC) analysis on a Merck silica gel 60 F254 precoated glass plate. Visualization was done with UV light (254 nm) or iodine. Column chromatography was carried out using a Yamazen W-prep system and performed using prepacked silica-gel columns. All reactions were carried out under a nitrogen atmosphere unless otherwise mentioned.

2-6-1-1. 4-(3-Methoxybenzyl)-1-(2-phenylethyl)piperidine (**1**)

To a mixture of 4-(3-methoxy-benzyl)-piperidine (100 mg, 0.487 mmol) and potassium carbonate (101 mg, 0.731 mmol) in MeCN (2.4 mL) was added (2-bromoethyl)benzene (86.5 uL, 0.633 mmol). After reflux for 24 h, EtOAc (7.2 mL) was added to the reaction mixture, and the whole was filtered. The filtrate was evaporated in vacuo and the residue was purified by silica gel chromatography using 25% EtOAc/hexane and 10% MeOH/$CHCl_3$ as eluent to give 100 mg (66%) of the title compound as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.26-1.41 (2H, m), 1.46-1.59 (1H, m), 1.63-1.72 (2H, m), 1.95 (2H, td, J = 11.9, 2.3 Hz), 2.50-2.60 (4H, m), 2.76-2.84 (2H, m), 2.95-3.03 (2H, m), 3.80 (3H, s), 6.69-6.77 (3H, m), 7.15-7.23 (4H, m), 7.24-7.31 (2H, m); HRMS (ESI) m/z calcd for $C_{21}H_{28}NO$ $[M+H]^+$ 310.2165; found 310.2164.

2-6-1-2. 4-(2-Fluoro-5-methoxybenzyl)-1-(2-phenylethyl)piperidine (**2**)

To a mixture of the benzyl piperidine intermediate **28** (200 mg, 0.770 mmol) and potassium carbonate (266 mg, 1.93 mmol) in MeCN (3.0 mL) was added (2-bromoethyl)benzene (137 uL, 1.00 mmol). After reflux for 24 h, EtOAc (9.0 mL) was added to the reaction mixture and the whole was filtered. The filtrate was evaporated in vacuo and the residue was purified by silica gel chromatography using 0%-1% MeOH/$CHCl_3$ as eluent to give 228 mg (90%) of the title compound as a pale yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.28-1.46 (2H, m), 1.50-1.74 (3H, m), 1.96 (2H, br t, J = 10.7 Hz), 2.51-2.60 (4H, m), 2.75-2.84 (2H, m), 2.99 (2H, br d, J = 11.4 Hz), 3.77 (3H, s), 6.64-6.71 (2H, m), 6.88-6.97 (1H, m), 7.16-7.23 (3H, m), 7.24-7.32 (2H, m); HRMS (ESI) m/z calcd for $C_{21}H_{26}FNO$ $[M+H]^+$ 328.2071; found 328.2074.

2-6-1-3. 4-(2-Chloro-5-methoxybenzyl)-1-(2-phenylethyl)piperidine (**3**)

The title compound was prepared in a manner similar to that for the preparation of **2** using the benzyl piperidine intermediate **31a** and (2-bromoethyl)benzene. (94%) white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.32-1.49 (2H, m), 1.55-1.65 (2H, m), 1.68-1.74 (1H, m), 1.89-2.04 (2H, m), 2.49-2.61 (2H, m), 2.64 (2H, d, J = 6.4 Hz), 2.76-2.86 (2H, m), 2.95-3.04 (2H, m), 3.78 (3H, s), 6.65-6.74 (2H, m), 7.16-7.24 (4H, m), 7.24-7.31 (2H, m); HRMS (ESI) m/z calcd for

$C_{21}H_{27}ClNO$ $[M+H]^+$ 344.1776; found 344.1773.

2-6-1-4. 4-(2-Bromo-5-methoxybenzyl)-1-(2-phenylethyl)piperidine (**4**)

The title compound was prepared in a manner similar to that for the preparation of **2** using the benzyl piperidine intermediate **31b** and (2-bromoethyl)benzene. (72%) white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.31-1.49 (2H, m), 1.61-1.74 (3H, m), 1.90-2.03 (2H, m), 2.52-2.61 (2H, m), 2.64 (2H, d, J = 6.6 Hz), 2.76-2.85 (2H, m), 2.93-3.04 (2H, m), 3.78 (3H, s), 6.63 (1H, dd, J = 8.8, 2.9 Hz), 6.72 (1H, d, J = 3.1 Hz), 7.14-7.23 (3H, m), 7.24-7.31 (2H, m), 7.41 (1H, d, J = 8.6 Hz); HRMS (ESI) m/z calcd for $C_{21}H_{27}BrNO$ $[M+H]^+$ 388.1271; found 388.1270.

2-6-1-5. 4-(2-Bromo-5-methoxybenzyl)-1-[2-(4-methylphenyl)ethyl]piperidine (**5**)

The title compound was prepared in a manner similar to that for the preparation of **2** using the benzyl piperidine intermediate **31b** and 4-methylphenethyl bromide. (98%) white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.33-1.50 (2H, m), 1.62-1.73 (3H, m), 1.89-2.03 (2H, m), 2.31 (3H, s), 2.50-2.59 (2H, m), 2.64 (2H, d, J = 6.4 Hz), 2.72-2.82 (2H, m), 2.94-3.05 (2H, m), 3.78 (3H, s), 6.63 (1H, dd, J = 8.7, 3.0 Hz), 6.71 (1H, d, J = 3.1 Hz), 7.09 (4H, s), 7.41 (1H, d, J = 8.8 Hz); HRMS (ESI) m/z calcd for $C_{22}H_{29}BrNO$ $[M+H]^+$ 402.1427; found 402.1426.

2-6-1-6. 4-(2-Bromo-5-methoxybenzyl)-1-[2-(4-methoxyphenyl)ethyl]piperidine (**6**)

The title compound was prepared in a manner similar to that for the preparation of **2** using the benzyl piperidine intermediate **31b** and 4-methoxyphenethyl bromide. (98%) pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.37-1.59 (2H, m), 1.62-1.76 (3H, m), 1.93-2.13 (2H, m), 2.52-2.71 (4H, m), 2.74-2.88 (2H, m), 2.98-3.13 (2H, m), 3.78 (6H, s), 6.64 (1H, dd, J = 8.7, 3.0 Hz), 6.71 (1H, d, J = 2.9 Hz), 6.82 (2H, d, J = 8.8 Hz), 7.12 (2H, d, J = 8.6 Hz), 7.41 (1H, d, J = 8.8 Hz); HRMS (ESI) m/z calcd for $C_{22}H_{29}BrNO_2$ $[M+H]^+$ 418.1376; found 418.1377.

2-6-1-7. 4-(2-Bromo-5-methoxybenzyl)-1-[2-(2-methoxyphenyl)ethyl]piperidine (**7**)

The title compound was prepared in a manner similar to that for the preparation of **2** using the benzyl piperidine intermediate **31b** and 2-methoxyphenethyl bromide. (86%) pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.36-1.54 (2H, m), 1.58-1.74 (3H, m), 1.93-2.07 (2H, m), 2.50-2.60 (2H, m), 2.65 (2H, d, J = 6.4 Hz), 2.79-2.89 (2H, m), 2.98-3.09 (2H, m), 3.78 (3H, s), 3.80 (3H, s), 6.63 (1H, dd, J = 8.7, 3.0 Hz), 6.72 (1H, d, J = 3.1 Hz), 6.80-6.91 (2H, m), 7.11-7.22 (2H, m), 7.41 (1H, d, J = 8.8 Hz); HRMS (ESI) m/z calcd for $C_{22}H_{29}BrNO_2$ $[M+H]^+$ 418.1376; found 418.1371.

2-6-1-8. 4-(2-Bromo-5-methoxybenzyl)-1-[2-(3-methoxyphenyl)ethyl]piperidine (**8**)

The title compound was prepared in a manner similar to that for the preparation of **2** using the benzyl piperidine intermediate **31b** and 3-methoxyphenethyl bromide. (89%) pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.31-1.51 (2H, m), 1.63-1.75 (3H, m), 1.89-2.03 (2H, m),

2.52-2.61 (2H, m), 2.64 (2H, d, J = 6.6 Hz), 2.74-2.83 (2H, m), 2.94-3.04 (2H, m), 3.78 (3H, s), 3.79 (3H, s), 6.63 (1H, dd, J = 8.8, 2.9 Hz), 6.70-6.82 (4H, m), 7.19 (1H, dd, J = 8.1, 8.1 Hz), 7.41 (1H, d, J = 8.6 Hz); HRMS (ESI) m/z calcd for $C_{22}H_{29}BrNO_2$ $[M+H]^+$ 418.1376; found 418.1376.

2-6-1-9. 4-(2-Bromo-5-methoxybenzyl)-1-[2-(4-fluorophenyl)ethyl]piperidine (**9**)

The title compound was prepared in a manner similar to that for the preparation of **2** using the benzyl piperidine intermediate **31b** and 4-fluorophenethyl bromide. (98%) white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.30-1.49 (2H, m), 1.63-1.74 (3H, m), 1.88-2.03 (2H, m), 2.48-2.57 (2H, m), 2.64 (2H, d, J = 6.4 Hz), 2.73-2.82 (2H, m), 2.93-3.02 (2H, m), 3.78 (3H, s), 6.63 (1H, dd, J = 8.7, 3.0 Hz), 6.71 (1H, d, J = 3.1 Hz), 6.95 (2H, dd, J = 8.8, 8.8 Hz), 7.14 (2H, dd, J = 8.6, 5.5 Hz), 7.41 (1H, d, J = 8.8 Hz); HRMS (ESI) m/z calcd for $C_{21}H_{26}BrFNO$ $[M+H]^+$ 406.1176; found 406.1188.

2-6-1-10. 4-(2-Bromo-5-methoxybenzyl)-1-[2-(4-chlorophenyl)ethyl]piperidine (**10**)

The title compound was prepared in a manner similar to that for the preparation of **2** using the benzyl piperidine intermediate **31b** and 4-chlorophenethyl bromide. (98%) white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.30-1.48 (2H, m), 1.61-1.74 (3H, m), 1.88-2.01 (2H, m), 2.49-2.56 (2H, m), 2.64 (2H, d, J = 6.4 Hz), 2.73-2.80 (2H, m), 2.92-3.00 (2H, m), 3.78 (3H, s), 6.63 (1H, dd, J = 8.7, 3.0 Hz), 6.71 (1H, d, J = 3.1 Hz), 7.12 (2H, d, J = 8.4 Hz), 7.24 (2H, d, J = 8.4 Hz), 7.41 (1H, d, J = 8.8 Hz); HRMS (ESI) m/z calcd for $C_{21}H_{26}BrClNO$ $[M+H]^+$ 422.0881; found 422.0881.

2-6-1-11. 4-(2-Bromo-5-methoxybenzyl)-1-[2-(2-chlorophenyl)ethyl]piperidine (**11**)

The title compound was prepared in a manner similar to that for the preparation of **2** using the benzyl piperidine intermediate **31b** and 2-chlorophenethyl bromide. (54%) white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.33-1.51 (2H, m), 1.63-1.75 (3H, m), 1.95-2.08 (2H, m), 2.52-2.61 (2H, m), 2.65 (2H, d, J = 6.4 Hz), 2.90-2.97 (2H, m), 2.98-3.06 (2H, m), 3.78 (3H, s), 6.63 (1H, dd, J = 8.6, 3.1 Hz), 6.72 (1H, d, J = 3.1 Hz), 7.09-7.21 (2H, m), 7.24 (1H, dd, J = 7.2, 2.3 Hz), 7.33 (1H, dd, J = 7.4, 1.7 Hz), 7.41 (1H, d, J = 8.6 Hz); HRMS (ESI) m/z calcd for $C_{21}H_{26}BrClNO$ $[M+H]^+$ 422.0881; found 422.0882.

2-6-1-12. 4-(2-Bromo-5-methoxybenzyl)-1-[2-(3-chlorophenyl)ethyl]piperidine (**12**)

The title compound was prepared in a manner similar to that for the preparation of **2** using the benzyl piperidine intermediate **31b** and 3-chlorophenethyl bromide. (86%) pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.31-1.47 (2H, m), 1.62-1.73 (3H, m), 1.90-2.01 (2H, m), 2.50-2.58 (2H, m), 2.64 (2H, d, J = 6.4 Hz), 2.74-2.81 (2H, m), 2.92-3.00 (2H, m), 3.78 (3H, s), 6.63 (1H, dd, J = 8.6, 3.1 Hz), 6.71 (1H, d, J = 2.9 Hz), 7.05-7.10 (1H, m), 7.14-7.23 (3H, m), 7.42 (1H, d, J = 8.8 Hz); HRMS (ESI) m/z calcd for $C_{21}H_{26}BrClNO$ $[M+H]^+$ 422.0881;

found 422.0882.

2-6-1-13. 4-(2-Bromo-5-methoxybenzyl)-1-[2-(2-chloro-4-methoxyphenyl)ethyl]-piperidine (**13**)

The title compound was prepared in a manner similar to that for the preparation of **2** using the benzyl piperidine intermediate **31b** and the tosylate intermediate **37a**. (99%) white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.36-1.53 (2H, m), 1.58-1.74 (3H, m), 1.96-2.08 (2H, m), 2.49-2.59 (2H, m), 2.64 (2H, d, J = 6.4 Hz), 2.83-2.93 (2H, m), 2.98-3.07 (2H, m), 3.77 (3H, s), 3.78 (3H, s), 6.63 (1H, dd, J = 8.7, 3.0 Hz), 6.70-6.77 (2H, m), 6.89 (1H, d, J = 2.6 Hz), 7.13 (1H, d, J = 8.4 Hz), 7.41 (1H, d, J = 8.6 Hz); HRMS (ESI) m/z calcd for $C_{22}H_{28}BrClNO_2$ $[M+H]^+$ 452.0986; found 452.0986.

2-6-1-14. 4-(2-Bromo-5-methoxybenzyl)-1-[2-(3-chloro-4-methoxyphenyl)ethyl]-piperidine (**14**)

The title compound was prepared in a manner similar to that for the preparation of **2** using the benzyl piperidine intermediate **31b** and the tosylate intermediate **37c**. (98%) pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.32-1.50 (2H, m), 1.55-1.74 (3H, m), 1.89-2.02 (2H, m), 2.48-2.57 (2H, m), 2.64 (2H, d, J = 6.6 Hz), 2.69-2.77 (2H, m), 2.93-3.01 (2H, m), 3.78 (3H, s), 3.87 (3H, s), 6.63 (1H, dd, J = 8.7, 3.0 Hz), 6.71 (1H, d, J = 3.1 Hz), 6.84 (1H, d, J = 8.4 Hz), 7.05 (1H, dd, J = 8.3, 2.1 Hz), 7.20 (1H, d, J = 2.0 Hz), 7.41 (1H, d, J = 8.8 Hz); HRMS (ESI) m/z calcd for $C_{22}H_{28}BrClNO_2$ $[M+H]^+$ 452.0986; found 452.0982.

2-6-1-15. 4-(2-Bromo-5-methoxybenzyl)-1-[2-(3,4-dimethoxyphenyl)ethyl]piperidine (**15**)

The title compound was prepared in a manner similar to that for the preparation of **2** using the benzyl piperidine intermediate **31b** and 3,4-dimethoxyphenethyl bromide. (98%) pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.45-1.76 (5H, m), 1.95-2.15 (2H, m), 2.58-2.70 (4H, m), 2.77-2.87 (2H, m), 3.01-3.15 (2H, m), 3.78 (3H, s), 3.85 (3H, s), 3.87 (3H, s), 6.64 (1H, dd, J = 8.6, 3.1 Hz), 6.70-6.81 (4H, m), 7.42 (1H, d, J = 8.8 Hz); HRMS (ESI) m/z calcd for $C_{23}H_{31}BrNO_3$ $[M+H]^+$ 448.1482; found 448.1478.

2-6-1-16. 4-(2-Bromo-5-methoxybenzyl)-1-[2-(2-chloro-4,5-dimethoxyphenyl)ethyl]-piperidine (**16**)

The title compound was prepared in a manner similar to that for the preparation of **2** using the benzyl piperidine intermediate **31b** and the bromide intermediate **38a**. (97%) pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.41-1.75 (5H, m), 1.99-2.15 (2H, m), 2.53-2.62 (2H, m), 2.65 (2H, d, J = 6.4 Hz), 2.85-2.96 (2H, m), 3.00-3.11 (2H, m), 3.78 (3H, s), 3.84 (3H, s), 3.85 (3H, s), 6.64 (1H, dd, J = 8.6, 3.1 Hz), 6.72 (1H, d, J = 3.1 Hz), 6.76 (1H, s), 6.83 (1H, s), 7.42 (1H, d, J = 8.8 Hz); HRMS (ESI) m/z calcd for $C_{23}H_{30}BrClNO_3$ $[M+H]^+$ 482.1092; found 482.1091.

2-6-1-17. 4-(2-Bromo-5-methoxybenzyl)-1-[2-(5,8-dihydronaphthalen-1-yl)ethyl]-piperidine (**17**)

The title compound was prepared in a manner similar to that for the preparation of **2** using the benzyl piperidine intermediate **31b** and 2-(1-naphthyl)ethyl bromide. (99%) pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.39-1.55 (2H, m), 1.67-1.77 (3H, m), 2.00-2.14 (2H, m), 2.64-2.76 (4H, m), 3.05-3.16 (2H, m), 3.26-3.36 (2H, m), 3.79 (3H, s), 6.64 (1H, dd, J = 8.8, 2.9 Hz), 6.73 (1H, d, J = 3.1 Hz), 7.32-7.54 (5H, m), 7.71 (1H, d, J = 7.5 Hz), 7.82-7.87 (1H, m), 8.06 (1H, d, J = 7.7 Hz); HRMS (ESI) m/z calcd for $C_{25}H_{29}BrNO$ $[M+H]^+$ 438.1427; found 438.1427.

2-6-1-18. 4-(2-Bromo-5-methoxybenzyl)-1-[2-(naphthalen-2-yl)ethyl]piperidine (**18**)

The title compound was prepared in a manner similar to that for the preparation of **2** using the benzyl piperidine intermediate **31b** and the tosylate intermediate **37e**. (86%) white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.34-1.52 (2H, m), 1.63-1.75 (3H, m), 1.95-2.06 (2H, m), 2.61-2.71 (4H, m), 2.93-3.08 (4H, m), 3.78 (3H, s), 6.64 (1H, dd, J = 8.8, 3.1 Hz), 6.72 (1H, d, J = 2.9 Hz), 7.34 (1H, dd, J = 8.3, 1.7 Hz), 7.38-7.48 (3H, m), 7.63 (1H, br s), 7.74-7.82 (3H, m); HRMS (ESI) m/z calcd for $C_{25}H_{29}BrNO$ $[M+H]^+$ 438.1427; found 438.1429.

2-6-1-19. 4-(2-Bromo-5-methoxybenzyl)-1-[2-(2,3-dihydro-1,4-benzodioxin-5-yl)ethyl]-piperidine (**19**)

The title compound was prepared in a manner similar to that for the preparation of **2** using the benzyl piperidine intermediate **31b** and the tosylate intermediate **37d**. (99%) pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.35-1.52 (2H, m), 1.63-1.74 (3H, m), 1.92-2.05 (2H, m), 2.50-2.60 (2H, m), 2.64 (2H, d, J = 6.4 Hz), 2.74-2.84 (2H, m), 2.96-3.07 (2H, m), 3.78 (3H, s), 4.24 (4H, s), 6.63 (1H, dd, J = 8.7, 3.0 Hz), 6.68-6.77 (4H, m), 7.41 (1H, d, J = 8.6 Hz); HRMS (ESI) m/z calcd for $C_{23}H_{29}BrNO_3$ $[M+H]^+$ 446.1325; found 446.1324.

2-6-1-20. 4-(2-Bromo-5-methoxybenzyl)-1-[2-(2,3-dihydro-1,4-benzodioxin-6-yl)ethyl]-piperidine (**20**)

The title compound was prepared in a manner similar to that for the preparation of **2** using the benzyl piperidine intermediate **31b** and the tosylate intermediate **37b**. (72%) colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.31-1.47 (2H, m), 1.61-1.72 (3H, m), 1.88-2.00 (2H, m), 2.47-2.56 (2H, m), 2.60-2.74 (4H, m), 2.92-3.01 (2H, m), 3.78 (3H, s), 4.23 (4H, s), 6.60-6.68 (2H, m), 6.70-6.72 (2H, m), 6.77 (1H, d, J = 8.3 Hz), 7.41 (1H, d, J = 8.6 Hz); HRMS (ESI) m/z calcd for $C_{23}H_{29}BrNO_3$ $[M+H]^+$ 446.1325; found 446.1330.

2-6-1-21. 4-(2-Bromo-5-methoxybenzyl)-1-[2-(7-chloro-2,3-dihydro-1,4-benzodioxin-6-yl)-ethyl]piperidine (**21**)

The title compound was prepared in a manner similar to that for the preparation of **2** using the

benzyl piperidine intermediate **31b** and the tosylate intermediate **38b**. (83%) white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.33-1.50 (2H, m), 1.63-1.73 (3H, m), 1.93-2.05 (2H, m), 2.46-2.55 (2H, m), 2.64 (2H, d, J = 6.6 Hz), 2.76-2.85 (2H, m), 2.94-3.04 (2H, m), 3.78 (3H, s), 4.22 (4H, s), 6.63 (1H, dd, J = 8.7, 3.0 Hz), 6.71 (1H, d, J = 3.1 Hz), 6.73 (1H, s), 6.85 (1H, s), 7.41 (1H, d, J = 8.6 Hz); HRMS (ESI) m/z calcd for $C_{23}H_{28}BrClNO_3$ $[M+H]^+$ 480.0936; found 480.0927.

2-6-1-22. 4-(2-Bromo-5-ethoxybenzyl)-1-[2-(7-chloro-2,3-dihydro-1,4-benzodioxin-6-yl)-ethyl]piperidine (**22**)

The title compound was prepared in a manner similar to that for the preparation of **2** using the benzyl piperidine intermediate **34a** and the tosylate intermediate **38b**. (84%) white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.32-1.50 (5H, m), 1.63-1.73 (3H, m), 1.92-2.06 (2H, m), 2.46-2.57 (2H, m), 2.63 (2H, d, J = 6.4 Hz), 2.76-2.85 (2H, m), 2.94-3.04 (2H, m), 3.99 (2H, q, J = 7.0 Hz), 4.22 (4H, s), 6.61 (1H, dd, J = 8.8, 2.9 Hz), 6.71 (1H, d, J = 2.9 Hz), 6.73 (1H, s), 6.85 (1H, s), 7.39 (1H, d, J = 8.8 Hz); HRMS (ESI) m/z calcd for $C_{24}H_{29}BrClNO_3$ $[M+H]^+$ 494.1092; found 494.1099.

2-6-1-23. 4-[2-Bromo-5-(propan-2-yloxy)benzyl]-1-[2-(7-chloro-2,3-dihydro-1,4-benzo-dioxin-6-yl)ethyl]piperidine (**23**)

The title compound was prepared in a manner similar to that for the preparation of **2** using the benzyl piperidine intermediate **34b** and the tosylate intermediate **38b**. (81%) colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.32 (6H, d, J = 6.1 Hz), 1.35-1.50 (2H, m), 1.63-1.73 (3H, m), 1.93-2.07 (2H, m), 2.47-2.56 (2H, m), 2.62 (2H, d, J = 6.4 Hz), 2.76-2.86 (2H, m), 2.94-3.05 (2H, m), 4.22 (4H, s), 4.42-4.56 (1H, m), 6.61 (1H, dd, J = 8.8, 2.9 Hz), 6.70 (1H, d, J = 2.9 Hz), 6.73 (1H, s), 6.85 (1H, s), 7.39 (1H, d, J = 8.8 Hz); HRMS (ESI) m/z calcd for $C_{25}H_{31}BrClNO_3$ $[M+H]^+$ 508.1248; found 508.1251.

2-6-1-24. 4-[2-Bromo-5-(2-methoxyethoxy)benzyl]-1-[2-(7-chloro-2,3-dihydro-1,4-benzo-dioxin-6-yl)ethyl]piperidine hydrochloride (**24, SMP-304**)

The free form of the title compound was prepared in a manner similar to that for the preparation of **2** using the benzyl piperidine intermediate **34c** and the tosylate intermediate **38b**. (81%) colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.30-1.50 (2H, m), 1.62-1.73 (3H, m), 1.92-2.07 (2H, m), 2.46-2.57 (2H, m), 2.63 (2H, d, J = 6.2 Hz), 2.76-2.86 (2H, m), 2.94-3.05 (2H, m), 3.45 (3H, s), 3.71-3.77 (2H, m), 4.05-4.11 (2H, m), 4.22 (4H, s), 6.65 (1H, dd, J = 8.8, 3.1 Hz), 6.73 (1H, s), 6.76 (1H, d, J = 2.9 Hz), 6.85 (1H, s), 7.40 (1H, d, J = 8.8 Hz); HRMS (ESI) m/z calcd for $C_{25}H_{31}BrClNO_4$ $[M+H]^+$ 524.1197; found 524.1199. The product was converted into the title compound quantitatively by treated with hydrochloric acid (1.0 eq.) in MeOH at room temperature for 1h. Mp: 155-158°C. Anal. Calcd for $C_{25}H_{31}BrClNO_4HCl$: C, 53.49; H, 5.75; N, 2.50; Cl, 12.63; Br, 14.23. Found: C, 53.69; H,

5.76; N, 2.57; Cl, 12.69; Br, 14.22.

2-6-1-25. (2-Fluoro-5-methoxybenzyl)(triphenyl)phosphonium bromide (**25**)

To a solution of 2-fluoro-5-methoxybenzyl bromide (12.5 g, 57.1 mmol) in toluene (150 mL) was added triphenylphosphine (16.5 g, 62.8 mmol). After reflux for 4 h, the resulting solid was collected and washed with toluene (20 mL x 3) to give 19.8 g (64%) of the title compound as a white solid. ^{1}H NMR (300 MHz, DMSO-D_6) δ: 3.48 (3H, s), 5.10 (2H, d, J = 15.4 Hz), 6.47-6.54 (1H, m), 6.87-6.95 (1H, m), 7.06 (1H, dd, J = 9.2, 9.2 Hz), 7.66-7.80 (12H, m), 7.87-7.97 (3H, m); HRMS (ESI) m/z calcd for $C_{26}H_{23}FOP$ $[M+H]^+$ 410.1465; found 401.1472.

2-6-1-26. *tert*-Butyl 4-(2-fluoro-5-methoxybenzylidene)piperidine-1-carboxylate (**26**)

To a mixture of the phosphonium salt **25** (15 g, 27.7 mmol) and potassium carbonate (5.74 g, 41.6 mmol) in 2-PrOH was added 1-(tert-Butoxycarbonyl)-4-oxopiperidine (6.06 g, 30.4 mmol). After reflux for 5 h, the reaction mixture was filtered, and the filtrate was evaporated in vacuo. Et_2O (100 mL) was then added to the residue and stirred for 30 min at room temperature. The resulting solid was filtered out, and the filtrate was concentrated. The residue was purified by silica gel chromatography using 0-13% EtOAc/hexane as eluent to give 7.32 g (82%) of the title compound as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.47 (9H, s), 2.30-2.39 (4H, m), 3.41 (2H, t, J = 5.7 Hz), 3.52 (2H, t, J = 5.9 Hz), 3.77 (3H, s), 6.23 (1H, s), 6.65-6.76 (2H, m), 6.96 (1H, dd, J = 9.1, 9.1 Hz).

2-6-1-27. *tert*-Butyl 4-(2-fluoro-5-methoxybenzyl)piperidine-1-carboxylate (**27**)

The olefin intermediate **26** (5.30 g, 16.5 mmol) was dissolved in MeOH (30 mL) and hydrogenated over 10% Pd on carbon (water ~50%, 1.00 g) at room temperature for 5 h. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo. The residue was purified by silica gel chromatography using 3-23% EtOAc/hexane as eluent to give 4.95 g (93%) of the title compound as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.08-1.24 (2H, m), 1.45 (9H, s), 1.58-1.66 (2H, m), 1.66-1.78 (1H, m), 2.54 (2H, dd, J = 7.1, 1.0 Hz), 2.57-2.71 (2H, m), 3.77 (3H, s), 3.99-4.15 (2H, m), 6.62-6.71 (2H, m), 6.93 (1H, dd, J = 9.1, 9.1 Hz).

2-6-1-28. 4-(2-Fluoro-5-methoxybenzyl)piperidine hydrochloride (**28**)

To a solution of intermediate **27** (4.00 g, 12.4 mmol) in $CHCl_3$ (15 mL) was added 4 N HCl/1,4-dioxane (30 mL). After stirring at room temperature for 21 h, the reaction mixture was evaporated in vacuo. Et_2O (50 mL) was then added to the residue and stirred at room temperature for 30 min. The resulting solid was collected to obtain 3.16 g (98%) of the title compound as a white solid. ^{1}H NMR (300 MHz, DMSO-D_6) δ: 1.27-1.46 (2H, m), 1.62-1.75 (2H, m), 1.75-1.89 (1H, m), 2.53 (2H, d, J = 6.1 Hz), 2.71-2.85 (2H, m), 3.16-3.26 (2H, m),

3.72 (3H, s), 6.76-6.86 (2H, m), 7.08 (1H, dd, J = 9.2, 9.2 Hz), 8.72 (2H, br s); HRMS (ESI) m/z calcd for $C_{13}H_{18}FNO$ $[M+H]^+$ 224.1445; found 224.1451.

2-6-1-29. *tert*-Butyl 4-(3-methoxybenzyl)piperidine-1-carboxylate (**29**)

To a solution of 4-(3-methoxybenzyl)piperidine (1.70 g, 8.28mmol) in THF (15 mL) was added a solution of di-tert-butyl dicarbonate (1.90 g, 8.69 mmol) in THF (5 mL). After stirring at room temperature for 3h, the reaction mixture was concentrated. The residue was purified by silica gel chromatography using 1-22% EtOAc/hexane as eluent to give 2.57 g (quant.) of the title compound as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.05-1.22 (2H, m), 1.45 (9H, s), 1.52-1.74 (3H, m), 2.51 (2H, d, J = 6.8 Hz), 2.63 (2H, t, J = 12.4 Hz), 3.80 (3H, s), 3.97-4.16 (2H, m), 6.66-6.71 (1H, m), 6.71-6.78 (2H, m), 7.20 (1H, dd, J = 7.8, 7.8 Hz).

2-6-1-30. *tert*-Butyl 4-(2-chloro-5-methoxybenzyl)piperidine-1-carboxylate (**30a**)

To a solution of intermediate **29** (300 mg, 0.982 mmol) in DMF (3 mL) was added N-chlorosuccinimide (138 mg, 1.03 mmol). After stirring at 60°C for 12h, H_2O (30 mL) was added to the reaction mixture and the whole was extracted with EtOAc (30 mL x 2). The combined organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by silica gel chromatography using 1-22% EtOAc/hexane as eluent to give 290 mg (87%) of the title compound as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.11-1.28 (2H, m), 1.45 (9H, s), 1.58-1.67 (2H, m), 1.69-1.85 (1H, m), 2.56-2.72 (4H, m), 3.78 (3H, s), 3.99-4.17 (2H, m), 6.67-6.72 (2H, m), 7.21-7.26 (1H, m).

2-6-1-31. 4-(2-Chloro-5-methoxybenzyl)piperidine hydrochloride (**31a**)

The title compound was prepared in a manner similar to that for the preparation of **28** using intermediate **30a**. (89%) white solid. ^{1}H NMR (300 MHz, DMSO-D_6) δ: 1.33-1.50 (2H, m), 1.64-1.75 (2H, m), 1.77-1.95 (1H, m), 2.62 (2H, d, J = 7.0 Hz), 2.73-2.86 (2H, m), 3.17-3.26 (2H, m), 3.75 (3H, s), 6.84 (1H, dd, J = 8.8, 3.0 Hz), 6.91 (1H, d, J = 3.0 Hz), 7.33 (1H, d, J = 8.8 Hz), 8.69 (2H, br s).

2-6-1-32. *tert*-butyl 4-(2-bromo-5-methoxybenzyl)piperidine-1-carboxylate (**30b**)

To a solution of intermediate 6 (150 mg, 0.491 mmol) in DMF (2.0 mL) was added N-bromosuccinimide (96.0 mg, 0.540 mmol) at 0°C. After stirring at room temperature for 12h, H_2O (20 mL) was added to the reaction mixture and the whole was extracted with EtOAc (20 mL x 2). The combined organic layers were dried over $MgSO_4$, filtered and concentrated. The residue was purified by silica gel chromatography using 1-22% EtOAc/hexane as eluent to give 169 mg (90%) of the title compound as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.12-1.29 (2H, m), 1.46 (9H, s), 1.52-1.68 (2H, m), 1.70-1.86 (1H, m), 2.56-2.71 (4H, m), 3.78 (3H, s), 3.98-4.16 (2H, m), 6.64 (1H, dd, J = 8.7, 3.1 Hz), 6.70 (1H, d, J = 3.1 Hz), 7.42 (1H, d, J = 8.7 Hz).

2-6-1-33. 4-(2-bromo-5-methoxybenzyl)piperidine hydrochloride (**31b**)

The title compound was prepared in a manner similar to that for the preparation of **28** using intermediate **30b**. (91%) white solid. ^{1}H NMR (300 MHz, DMSO-D_6) δ: 1.33-1.52 (2H, m), 1.63-1.75 (2H, m), 1.78-1.95 (1H, m), 2.62 (2H, d, J = 7.2 Hz), 2.72-2.86 (2H, m), 3.16-3.28 (2H, m), 3.74 (3H, s), 6.78 (1H, dd, J = 8.6, 3.0 Hz), 6.91 (1H, d, J = 3.0 Hz), 7.48 (1H, d, J = 8.6 Hz), 8.66 (2H, br s).

2-6-1-34. *tert*-butyl 4-(2-bromo-5-hydroxybenzyl)piperidine-1-carboxylate (**32**)

To a suspension of intermediate **31b** (1.50 g, 4.68 mmol) in CH_2Cl_2 (10 mL) was added dropwise 1N BBr_3 in CH_2Cl_2 solution (5.61 mL, 5.61 mmol) at 0°C for 10min. After stirring at room temperature for 12h, MeOH (10 mL) was added to the reaction mixture, and the whole was stirred at room temperature for 30min and concentrated. To a mixture of the residue, THF (10 mL) and 20% aqueous K_2CO_3 (50 g) was added di-tert-butyl dicarbonate (1.12 g, 5.15 mmol). After stirring at room temperature for 12h, the reaction mixture was extracted with EtOAc (50 mL x 2). The combined organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was crystallized using EtOAc (4 mL) and stirred at room temperature for 10min. Hexane (8 mL) was then added to the mixture, and the whole was stirred at room temperature for 20min. The resulting solid was filtered, rinsed with EtOAc/hexane = 1/2 (1 mL) and collected to give 1.38 g (80%) of the title compound as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.11-1.27 (2H, m), 1.46 (9H, s), 1.60-1.69 (2H, m), 1.70-1.87 (1H, m), 2.49-2.75 (4H, m), 3.99-4.15 (2H, m), 5.93 (1H, s), 6.58 (1H, dd, J = 8.6, 2.9 Hz), 6.68 (1H, d, J = 3.1 Hz), 7.35 (1H, d, J = 8.6 Hz).

2-6-1-35. *tert*-butyl 4-(2-bromo-5-ethoxybenzyl)piperidine-1-carboxylate (**33a**)

To a mixture of intermediate **32** (1.00 g, 2.70 mmol) and potassium carbonate (1.16 g, 8.37 mmol) in DMF (5 mL) was added iodoethane (0.648 mL, 8.10 mmol). After stirring at 70°C for 8 h, the reaction mixture was treated with H_2O (40 mL) and extracted with EtOAc (40 mL). To the organic layer was added toluene (40 mL), and the mixture was washed with H_2O (40 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by silica gel chromatography using 0~16% EtOAc/hexane as eluent to give 1.12 g (quant.) of the title compound as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.12-1.28 (2H, m), 1.40 (3H, t, J = 7.0 Hz), 1.45 (9H, s), 1.59-1.67 (2H, m), 1.69-1.86 (1H, m), 2.56-2.71 (4H, m), 3.94-4.15 (4H, m), 6.62 (1H, dd, J = 8.7, 3.0 Hz), 6.69 (1H, d, J = 3.1 Hz), 7.40 (1H, d, J = 8.8 Hz).

2-6-1-36. 4-(2-bromo-5-ethoxybenzyl)piperidine hydrochloride (**34a**)

The title compound was prepared in a manner similar to that for the preparation of **28** using intermediate **33a**. (94%) white solid. ^{1}H NMR (300 MHz, DMSO-D_6) δ: 1.31 (3H, t, J = 7.0 Hz), 1.34-1.51 (2H, m), 1.63-1.74 (2H, m), 1.78-1.94 (1H, m), 2.61 (2H, d, J = 7.0 Hz),

2.71-2.85 (2H, m), 3.17-3.26 (2H, m), 4.00 (2H, q, J = 7.0 Hz), 6.76 (1H, dd, J = 8.6, 2.9 Hz), 6.90 (1H, d, J = 3.1 Hz), 7.46 (1H, d, J = 8.8 Hz), 8.73 (2H, br s); HRMS (ESI) m/z calcd for $C_{14}H_{21}BrClNO$ $[M+H]^+$ 298.0801; found 298.0814.

2-6-1-37. *tert*-butyl 4-[2-bromo-5-(propan-2-yloxy)benzyl]piperidine-1-carboxylate (**33b**)

The title compound was prepared in a manner similar to that for the preparation of **33a** using intermediate **32**. (quant.) white solid. ^{1}H-NMR (300 MHz,$CDCl_3$) δ: 1.11-1.28 (2H, m), 1.32 (6H, d, J = 6.1 Hz), 1.46 (9H, s), 1.61-1.65 (2H, m), 1.69-1.87 (1H, m), 2.55-2.73 (4H, m), 3.95-4.19 (2H, m), 4.42-4.56 (1H, m), 6.62 (1H, dd, J = 8.6, 3.0 Hz), 6.68 (1H, d, J = 3.0 Hz), 7.39 (1H, d, J = 8.6 Hz).

2-6-1-38. 4-[2-bromo-5-(propan-2-yloxy)benzyl]piperidine hydrochloride (**34b**)

The title compound was prepared in a manner similar to that for the preparation of **28** using intermediate **33b**. (99%) white solid. ^{1}H-NMR (300 MHz, DMSO-D_6) δ: 1.24 (6H, d, J = 6.1 Hz), 1.30-1.48 (2H, m), 1.61-1.74 (2H, m), 1.75-1.92 (1H, m), 2.59 (2H, d, J = 7.0 Hz), 2.70-2.84 (2H, m), 3.14-3.25 (2H, m), 4.52-4.64 (1H, m), 6.74 (1H, dd, J = 8.8, 3.1 Hz), 6.87 (1H, d, J = 3.1 Hz), 7.43 (1H, d, J = 8.8 Hz), 8.50 (1H, br s); HRMS (ESI) m/z calcd for $C_{15}H_{23}BrClNO$ $[M+H]^+$ 312.0957; found 312.0971.

2-6-1-39. *tert*-butyl 4-[2-bromo-5-(2-methoxyethoxy)benzyl]piperidine-1-carboxylate (**33c**)

To a mixture of intermediate **32** (1.5 g, 4.05 mmol) and potassium carbonate (1.12 g, 8.10 mmol) in DMF (10 mL) was added 2-bromoethylmethyl ether (0.571 mL, 6.08 mmol). After stirring at 100°C for 3h, the reaction mixture was treated with H_2O (50 mL) and extracted with EtOAc (50 mL x 2). The combined organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by silica gel chromatography using 8~29% EtOAc/hexane as eluent to give 1.70 g (98%) of the title compound as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.11-1.28 (2H, m), 1.46 (9H, s), 1.57-1.68 (2H, m), 1.69-1.85 (1H, m), 2.55-2.71 (4H, m), 3.45 (3H, s), 3.74 (2H, t, J = 4.6 Hz), 4.00-4.16 (4H, m), 6.66 (1H, dd, J = 8.7, 3.0 Hz), 6.74 (1H, d, J = 3.0 Hz), 7.41 (1H, d, J = 8.7 Hz).

2-6-1-40. 4-[2-bromo-5-(2-methoxyethoxy)benzyl]piperidine hydrochloride (**34c**)

The title compound was prepared in a manner similar to that for the preparation of **28** using intermediate **33c**. (97%) white solid. ^{1}H NMR (300 MHz, DMSO-D_6) δ: 1.33-1.51 (2H, m), 1.64-1.75 (2H, m), 1.79-1.95 (1H, m), 2.62 (2H, d, J = 7.2 Hz), 2.72-2.86 (2H, m), 3.16-3.27 (2H, m), 3.30 (3H, s), 3.64 (2H, t, J = 4.6 Hz), 4.07 (2H, t, J = 4.6 Hz), 6.78 (1H, dd, J = 8.8, 3.1 Hz), 6.93 (1H, d, J = 3.1 Hz), 7.47 (1H, d, J = 8.8 Hz), 8.68 (2H, br s).

2-6-1-41. 2-(2-chloro-4-methoxyphenyl)ethanol (**36a**)

To a solution of (2-chloro-4-methoxyphenyl)acetic acid (1.50 g, 7.48 mmol) in THF (20 mL)

was added 0.9 M BH_3-THF complex in THF solution (10.8 mL, 9.72 mmol). After stirring at room temperature for 3 h, MeOH (10 mL) was added, and the reaction mixture was stirred at room temperature for 30 min and concentrated in vacuo. The residue was purified by silica gel chromatography using 26~47% EtOAc/hexane as eluent to give 1.44 g (quant.) of the title compound as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.36 (1H, t, J = 5.9 Hz), 2.95 (2H, t, J = 6.7 Hz), 3.79 (3H, s), 3.85 (2H, td, J = 6.4, 6.4 Hz), 6.77 (1H, dd, J = 8.4, 2.6 Hz), 6.93 (1H, d, J = 2.8 Hz), 7.17 (1H, d, J = 8.4 Hz).

2-6-1-42. 2-(2-chloro-4-methoxyphenyl)ethyl 4-methylbenzenesulfonate (**37a**)

To a mixture of intermediate **36a** (1.20 g, 6.43 mmol), triethylamine (1.08 mL, 7.72 mmol) and trimethylamine hydrochloride (61.5 mg, 0.643 mmol) in CH_2Cl_2 (12 mL) was added p-toluenesulfonyl chloride (1.35 g, 7.07 mmol) at 0°C. After stirring at 0°C for 1 h, the reaction mixture was treated with H_2O (50 mL) and extracted with $CHCl_3$ (30 mL x 2). The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by silica gel chromatography using 4~25% EtOAc/hexane as eluent to give 2.08 g (95%) of the title compound as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.43 (3H, s), 3.01 (2H, t, J = 6.9 Hz), 3.77 (3H, s), 4.20 (2H, t, J = 6.9 Hz), 6.71 (1H, dd, J = 8.4, 2.6 Hz), 6.82 (1H, d, J = 2.6 Hz), 7.08 (1H, d, J = 8.4 Hz), 7.27 (2H, d, J = 7.9 Hz), 7.68 (2H, d, J = 8.3 Hz).

2-6-1-43. 2-(2,3-dihydro-1,4-benzodioxin-6-yl)ethanol (**36b**)

The title compound was prepared in a manner similar to that for the preparation of **36a** using (2,3-dihydro-benzo[1,4]dioxin-6-yl)-acetic acid. (88%) 1.59 g. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.39 (1H, t, J = 5.9 Hz), 2.76 (2H, t, J = 6.5 Hz), 3.82 (2H, td, J = 6.1, 6.1 Hz), 4.25 (4H, s), 6.70 (1H, dd, J = 8.3, 2.0 Hz), 6.74 (1H, d, J = 2.0 Hz), 6.81 (1H, d, J = 8.3 Hz).

2-6-1-44. 2-(2,3-dihydro-1,4-benzodioxin-6-yl)ethyl 4-methylbenzenesulfonate (**37b**)

The title compound was prepared in a manner similar to that for the preparation of **37a** using intermediate **36b**. (quant.) 1.94 g. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.44 (3H, s), 2.84 (2H, t, J = 7.2 Hz), 4.15 (2H, t, J = 7.2 Hz), 4.23 (4H, s), 6.57 (1H, dd, J = 8.1, 2.2 Hz), 6.61 (1H, d, J = 2.0 Hz), 6.74 (1H, d, J = 8.1 Hz), 7.30 (2H, d, J = 8.4 Hz), 7.72 (2H, d, J = 8.3 Hz).

2-6-1-45. 2-(3-chloro-4-methoxyphenyl)oxirane (**35a**)

To a mixture of 3-chloro-4-methoxybenzaldehyde (2.00 g, 11.7 mmol) and trimethylsulfonium iodide (3.35 g, 16.4 mmol) in DMSO (12 mL) was added potassium hydroxide (0.920 g, 16.4 mmol). After stirring at 40°C for 7 h, the reaction mixture was diluted with EtOAc (50 mL) and washed with H_2O (100 mL). The aqueous layer was extracted with EtOAc (50 mL). To the combined organic layer was added toluene (100 mL), and the whole was washed with H_2O (100 mL), dried over Na_2SO_4, filtered and concentrated

in vacuo to give 2.25 g (quant.) of the title compound as a pale yellow oil. The obtained compound **35a** was used in the next reaction without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.77 (1H, dd, J = 5.3, 2.6 Hz), 3.13 (1H, dd, J = 5.3, 4.0 Hz), 3.80 (1H, dd, J = 4.0, 2.6 Hz), 3.90 (3H, s), 6.90 (1H, d, J = 8.4 Hz), 7.16 (1H, dd, J = 8.5, 2.1 Hz), 7.28 (1H, d, J = 2.2 Hz).

2-6-1-46. 2-(3-chloro-4-methoxyphenyl)ethanol (**36c**)

To a suspension of sodium borohydride (168 mg, 4.44 mmol) in THF (12 mL) was added BF_3-Et_2O complex (0.903 mL, 7.13 mmol). After stirring at room temperature for 15 min, a solution of intermediate **35a** (2.00 g, 10.8 mmol) in THF (12 mL) was added dropwise for 10 min to the reaction mixture at 0°C. After stirring at room temperature for 2 h, H_2O (50 mL) was added to the reaction mixture, and the whole was extracted with EtOAc (40 mL). The organic layer was washed with brine (20 mL), dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by silica gel chromatography using 30~51% EtOAc/hexane as eluent to give 1.65 g (81%) of the title compound as a colorless oil. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 1.37 (1H, t, J = 5.9 Hz), 2.79 (2H, t, J = 6.5 Hz), 3.83 (2H, td, J = 6.2, 6.2 Hz), 3.89 (3H, s), 6.88 (1H, d, J = 8.3 Hz), 7.09 (1H, dd, J = 8.4, 2.2 Hz), 7.25 (1H, d, J = 2.2 Hz).

2-6-1-47. 2-(3-chloro-4-methoxyphenyl)ethyl 4-methylbenzenesulfonate (**37c**)

The title compound was prepared in a manner similar to that for the preparation of **37a** using intermediate **36c**. (89%) colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.44 (3H, s), 2.86 (2H, t, J = 6.8 Hz), 3.88 (3H, s), 4.17 (2H, t, J = 6.8 Hz), 6.80 (1H, d, J = 8.4 Hz), 6.99 (1H, dd, J = 8.3, 2.2 Hz), 7.04 (1H, d, J = 2.0 Hz), 7.28 (2H, d, J = 8.4 Hz), 7.66 (2H, d, J = 8.4 Hz).

2-6-1-48. 5-(oxiran-2-yl)-2,3-dihydro-1,4-benzodioxine (**35b**)

The title compound was prepared in a manner similar to that for the preparation of **35a** using 2,3-dihydro-1,4-benzodioxine-5-carbaldehyde. (quant.) colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.75 (1H, dd, J = 5.7, 2.6 Hz), 3.14 (1H, dd, J = 5.7, 4.0 Hz), 4.14 (1H, dd, J = 4.0, 2.6 Hz), 4.26-4.30 (2H, m), 4.30-4.34 (2H, m), 6.69-6.73 (1H, m), 6.79-6.82 (2H, m).

2-6-1-49. 2-(2,3-dihydro-1,4-benzodioxin-5-yl)ethanol (**36d**)

The title compound was prepared in a manner similar to that for the preparation of **36c** using intermediate **35b**. (72%) colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.88 (2H, t, J = 6.5 Hz), 3.84 (2H, td, J = 6.2, 6.2 Hz), 4.23-4.31 (4H, m), 6.72-6.80 (3H, m).

2-6-1-50. 2-(2,3-dihydro-1,4-benzodioxin-5-yl)ethyl 4-methylbenzenesulfonate (**37d**)

The title compound was prepared in a manner similar to that for the preparation of as **37a** using intermediate **36d**. (89%) white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.43 (3H, s), 2.92 (2H, t, J = 7.0 Hz), 4.11-4.18 (4H, m), 4.21 (2H, t, J = 7.0 Hz), 6.64 (1H, dd, J = 6.6, 2.6 Hz),

6.68-6.78 (2H, m), 7.26 (2H, d, J = 8.1 Hz), 7.65 (2H, d, J = 8.3 Hz).

2-6-1-51. 2-(naphthalen-2-yl)ethyl 4-methylbenzenesulfonate (**37e**)

The title compound was prepared in a manner similar to that for the preparation of **37a** using 2-naphthalene ethanol. (91%) white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.34 (3H, s), 3.11 (2H, t, J = 6.9 Hz), 4.31 (2H, t, J = 6.9 Hz), 7.08-7.14 (2H, m), 7.21 (1H, dd, J = 8.4, 1.7 Hz), 7.41-7.50 (2H, m), 7.52 (1H, br s), 7.60 (2H, d, J = 8.5 Hz), 7.71 (2H, d, J = 8.5 Hz), 7.76-7.83 (1H, m).

2-6-1-52. 1-(2-bromoethyl)-2-chloro-4,5-dimethoxybenzene (**38a**)

To a solution of 3,4-dimethoxyphenethyl bromide (300 mg, 1.22 mmol) in DMF (6.0 mL) was added N-chlorosuccinimide (179 mg, 1.34 mmol). After stirring at room temperature for 20h, saturated aqueous $NaHCO_3$ (4 mL) and H_2O (20 mL) were added to the reaction mixture, and the whole was extracted with EtOAc (30 mL x 2). The combined organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by silica gel chromatography using 0-15% EtOAc/hexane as eluent to give 324 mg (95%) of the title compound as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.21 (2H, t, J = 7.6 Hz), 3.56 (2H, t, J = 7.5 Hz), 3.86 (3H, s), 3.88 (3H, s), 6.74 (1H, s), 6.86 (1H, s).

2-6-1-53. 2-(7-chloro-2,3-dihydro-1,4-benzodioxin-6-yl)ethyl 4-methylbenzene-sulfonate (**38b**)

To a solution of **37b** (500 mg, 1.50 mmol) in DMF (7.0 mL) was added N-chlorosuccinimide (179 mg, 1.34 mmol). After stirring at 50°C for 4h, H_2O (40 mL) was added to the reaction mixture, and the whole was extracted with EtOAc (40 mL x 2). The combined organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by silica gel chromatography using 10-31% EtOAc/hexane as eluent to give 481 mg (87%) of the title compound as a white solid. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.44 (3H, s), 2.94 (2H, t, J = 7.0 Hz), 4.18 (2H, t, J = 7.0 Hz), 4.22 (4H, s), 6.67 (1H, s), 6.79 (1H, s), 7.29 (2H, d, J = 8.3 Hz), 7.71 (2H, d, J = 8.3 Hz).

2-6-2. Biological tests

2-6-2-1. Materials

All radioligands were purchased from Perkin Elmer Japan (Kanagawa, Japan). Cell membranes expressing human serotonin transporter and 5-HT_{1A} receptor were purchased from Perkin Elmer Japan. Chinese hamster ovary cells expressing human serotonin transporter used for [^{3}H]5-HT uptake assay were established in the Pharmacology Research Laboratories at Sumitomo Dainippon Pharma Co., Ltd.

2-6-2-2. Radioreceptor binding assay

In a total volume of 500 μL, 2.5 μL of test substance solution or dimethyl sulfoxide, 50 μL of [^{3}H]citalopram or [^{3}H]8-OH-DPAT solution, and 447.5 μL of cell membranes were mixed. Cell membranes expressing human serotonin transporter and 5-HT_{1A} receptor were diluted with the reaction buffer to a final concentration of 1 unit/447.5 μL beforehand. All samples were reacted at 25°C for 0.5 h (for 5-HT_{1A}) or 1 h (for serotonin transporter) in an incubator. The reaction was terminated by addition of 4 mL ice-cold reaction buffer, and the cell membranes were collected by vacuum filtration through GF/B glass filters. The glass filters were then washed with 4 mL of ice-cold reaction buffer and placed in scintillation vials with scintillation fluid. After more than 3 h, the radioactivity in each sample was measured with a liquid scintillation counter for 2 min, and the calculated dpm value was used for data analysis. In the serotonin transporter binding assay, GF/B glass filters were soaked in 0.05% polyethylenimine solution for more than 15 min before use. The inhibition constant (Ki) was calculated in Microsoft® Office Excel 2003 (Microsoft Corporation) using the Cheng–Prusoff equation [$Ki = IC_{50}/(1 + ([L]/Kd)]$, where L is the concentration of radioligand in the assay and Kd is the dissociation constant of the radioligand for the receptor.

2-6-2-3. [^{3}H]5-HT uptake assay

Phosphate-buffered saline containing 0.1 mmol/L $CaCl_2$ and 1 mmol/L $MgCl_2$ was used as reaction buffer. One microliter of dimethyl sulfoxide or test substance and 199 μL of the reaction buffer were mixed, and 50 μL of the mixed solution was added to human serotonin transporter-expressing CHO cells cultured in 96-well assay plates. The plates were pre-incubated at 37°C for 10 min. During that time, dimethyl sulfoxide or test substance was diluted with [^{3}H]5-HT solution in another 96-well plate. After cells pre-incubation, 50 μL of the prepared [^{3}H]5-HT solution containing dimethyl sulfoxide or test substance was added to the wells, and the mixture was incubated at 37°C for 20 min. After the incubation, the liquid layer was discarded, and the cells were rinsed twice with 200 μL reaction buffer before being lysed with 100 μL of the Solvable solution. Radioactivity in each lysate sample was measured as described in the previous section.

2-6-2-4. Guanosine 5'-(γ-thio) Triphosphate, [^{35}S]-(GTPγS) assay for 5-HT_{1A} receptor

To make up a total volume of 500 μL, 2.5 μL of test compound, containing GTPγS (2 mM, to measure nonspecific binding), DMSO (to measure basal [^{35}S]GTPγS binding) or serotonin (20 mM, to measure maximal [^{35}S]GTPγS binding); 50 μL of reaction buffer [HEPES-NaOH buffer (20 mM, pH 7.4) containing 100 mM NaCl, 10 mM $MgCl_2$, 0.1 mM DTT, and 1 μM GDP] containing 0.5 nM [^{35}S]GTPγS; and 447.5 μL of cell membranes expressing human 5-HT_{1A} receptors were mixed. The following manipulation was carried out as described in the above 5-HT transporter binding assay. Intrinsic activity was expressed as relative value of the activity of 100 μM serotonin, which was considered to be 100%.

2-6-2-4-1. Data analyses

The following formulae were used:

1) Basal [^{35}S]GTPγS binding

Basal [^{35}S]GTPγS binding (dpm) = Binding activity of the DMSO group (dpm) − Binding activity of the GTPγS group (dpm)

2) Maximal [^{35}S]GTPγS binding

Maximal [^{35}S]GTPγS binding (dpm) = Binding activity of the serotonin group (dpm) − Binding activity of the GTPγS group (dpm)

3) Specific binding of the test substance

Specific binding of the test substance (dpm) = Binding activity of test substance group (dpm) − Binding activity of GTPγS group (dpm)

4) Maximal specific binding

The maximal specific binding of the test substance was calculated using the Dx calculation (logistic curve fitting) with the "measurement value input" function in Stat Preclinica Client Version 1.0. The direct estimation method was used. The maximal specific binding was calculated using the logistic curve of the concentrations of the test substance and the specific binding values.

5) Intrinsic activity of the test substance

When the increment in maximal [^{35}S]GTPγS binding (Maximal [^{35}S]GTPγS binding − Basal [^{35}S]GTPγS binding) was considered as 100%, intrinsic activity of the test substance, which is the percentage of the increment in maximal specific binding of the test substance (Maximal specific binding of test substance − Basal [^{35}S]GTPγS binding), was calculated using the following formula:

Intrinsic activity of the test substance (%) = 100 ×{[Maximal specific binding of test substance (dpm) − Basal [^{35}S]GTPγS binding (dpm)]／[Maximal [^{35}S]GTPγS binding (dpm) − Basal [^{35}S]GTPγS binding (dpm)]}

2-6-2-5. CYP2D6 inhibition assay

2-6-2-5-1. Materials

Bufuralol was purchased from Sigma-Aldrich Corp., and Pooled Human Liver Microsomes were purchased from Xenotech, LLC.

2-6-2-5-1-1. Preparation of 0.5 M Potassium Phosphate Buffer (pH 7.4)

Monopotassium phosphate solution (150 mL, 0.5 M) and dipotassium phosphate solution (700 mL, 0.5 M) were mixed, giving a solution with pH 7.4.

2-6-2-5-1-2. Preparation of Magnesium Chloride Solution (165 mM)

Magnesium chloride hexahydrate (3.35 g) was dissolved in distilled water (100 ml) to a final concentration of 165 mM ($MgCl_2.6H_2O$).

2-6-2-5-1-3. Preparation of Human Liver Microsome Solution

Human Liver Microsome Solution was prepared by mixing Pooled Human Liver Microsomes (150 μL, 20 mg/mL), potassium phosphate buffer (12 mL, 0.5 M), magnesium chloride solution (1.2 mL, 165 mM), and distilled water (34.65 mL).

2-6-2-5-1-4. Preparation of β-NADPH Solution (13 mM)

β-NADPH was dissolved in distilled water to a final concentration of 11.75 mg/mL.

2-6-2-5-1-5. Preparation of Substrate Solution

Bufuralol was dissolved in DMSO to a final concentration of 2.0 mM and then diluted 200-fold with distilled water.

2-6-2-5-2. Experimental Procedures

Step 1: The test drug in DMSO solution (10 mM) was serially diluted 5-fold with DMSO to prepare 10, 2, 0.4 and 0.08 mM test drug solutions.

Step 2: Each test drug solution and DMSO were separately diluted 96-fold with the human liver microsome solution, and 80 uL of each dilution was dispensed into 96-well microplates.

Step 3: The substrate solution (10 uL) and β-NADPH solution (10 uL) were added to each well, and the plate was incubated at 37 °C for 10 min.

Step 4: The reaction was terminated by addition of 300 uL of methanol.

Step 5: The reaction mixture was filtered, and the filtrate was loaded onto an LC-MSMS system.

2-6-2-5-3. Quantification and Calculation

The amount of 1'-hydroxybufuralol produced was quantified by LC-MSMS and used as CYP2D6 metabolic activity. The remaining activity of each sample was determined by comparing the activity in DMSO to that in the presence of the test drug. IC_{50} value for CYP2D6 inhibition was determined from test drug concentration and the remaining activity. The IC_{50} value was calculated by linear interpolation between two points that span the remaining activity (50%). A larger IC_{50} value for CYP2D6 inhibition indicates weak CYP2D6 inhibition.

2-6-2-6. Rat forced swimming test

On the first day of the experiment, male Wistar rats weighing 121.3 – 178.1 g were placed in plastic cylinders (40 cm in height, 19 cm in diameter) containing water (25 ± 1°C) to a depth of 19 cm. After spending 15 min in the water, the rats were removed from the cylinders and wiped with paper towels (training session). **SMP-304** (1, 3 mg/kg), paroxetine (3, 10 mg/kg), or vehicle (0.5% methylcellulose) was orally administered to each rats 30 min after the beginning of the training session. The test session was carried out the following day with each

rat treated with one of the test-drugs or vehicle 2 h before the start of the test session. During the test session each animal behavior was videotaped for 6 min, and a rat was judged to be immobile whenever it remained floating in water without movement, except for slight movement to keep posture. An observer blind to test-drugs measured twice the immobility time for each animal. When the difference between the first and second measured immobility time was within 30 sec, the first recorded immobility time was used for data analysis. When the difference between the two measured immobility times was more than 30 sec, another measurement was conducted, and the obtained immobility time was used for data analysis. After all measurements were completed, animals' assignment to test drugs was disclosed.

2-7. 引用文献

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第 3 章　**DSP-1053** の創製

3-1. **SMP-304** の課題と課題解決のためのストラテジー

第 2 章で述べたとおり、セロトニントランスポーター（SERT）と 5-HT_{1A} 受容体への比較的強くかつバランスの良い結合阻害活性を示す **SMP-304** を見出した。**SMP-304** は、セロトニン取り込阻害（SRI）活性と 5-HT_{1A} 受容体の弱い部分作動性を示し、目的とする SRI 活性と 5-HT_{1A} 自己受容体阻害活性を併せ持ちうる化合物であり、期待通りに既存 SSRI よりも早い抗うつ様作用のオンセットを示した。しかしながら、本化合物は CYP2D6 の代謝寄与率が 86%と非常に高く、CYP2D6 の単代謝といった課題があることが分かった。薬物の主要な代謝酵素であるシトクローム P450（CYP）のサブタイプの一つである CYP2D6 には、遺伝多形があることが知られており、その発現量や代謝活性について個人差があることが知られている。従って、CYP2D6 の単代謝である薬剤は、個人間でその血中濃度のばらつきが大きくなることが予想され、血中濃度コントロールが非常に難しくなることから個人間での治療効果や副作用の発現頻度・程度が異なってくる。個人間で治療効果が弱くなることは大切な治療期間の損失を意味し、個人間で副作用の発現頻度・程度の差が大きくなることはその薬剤には非常に高い安全性が要求されることを意味する。CYP2D6 の発現量や代謝活性が高くその基質となる薬剤を速やかに代謝するヒトを extensive metabolizer（EM）と呼び、CYP2D6 の基質となる薬剤の代謝が遅いヒトを poor metabolizer（PM）と呼ぶ。EM と PM 間での CYP2D6 の基質となる薬剤の代謝速度の違いは、3.5 倍から 53 倍となることが知られており、その値は薬剤の代謝に対する CYP2D6 の寄与率に依存して大きくなる。薬剤の代謝に対する CYP2D6 の寄与率が 60%以上となる場合に、その薬剤の EM と PM 間での血中濃度のばらつきが大きくなることが報告されており、一定の基準となる（Figure 16）。[1]

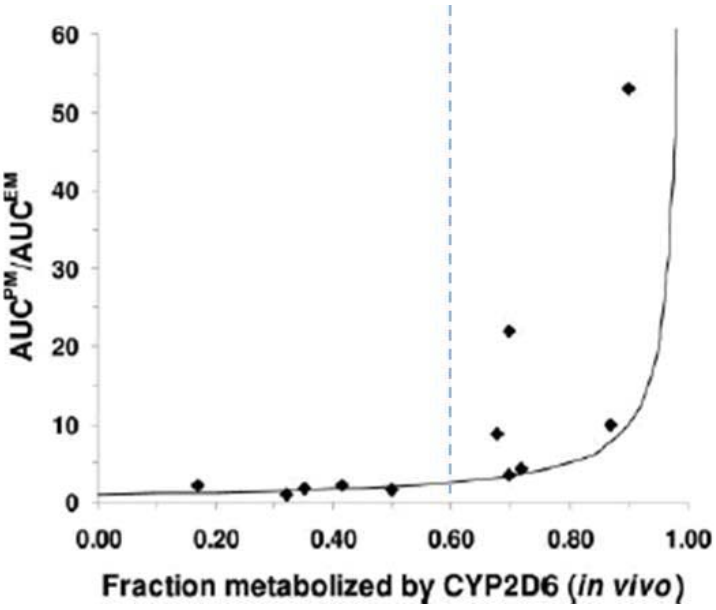


Figure 16. CYP2D6 の代謝寄与率と EM/PM 間での血中濃度比の相関 [1]
実線:理論上の相関曲線，◆:実薬でのデータ

多くの SSRI は、SERT に対し 0.1 nM から一桁 nM の範囲で非常に強い SERT に対する結合阻害活性を示す（パロキセチン: Ki = 0.10 nM, セルトラリン: Ki = 0.26 nM, フルオキセチン: Ki = 1.1 nM, エスシタロプラム: Ki = 1.1 nM）。[2] **SMP-304** は、Ki 値 32.7 nM と比較的強い SERT に対する結合阻害活性を示すが、パロキセチン, セルトラリン, フルオキセチン, エスシタロプラムといった既存 SSRI と比較するとその結合阻害活性は 10 倍以上弱い値となり、より強い SERT 結合阻害活性が望まれる。

以上から、**SMP-304** の SERT に対する結合阻害活性の向上と CYP2D6 の代謝寄与率の低減を目的とする探索研究を開始した。まず、SERT 結合阻害活性の向上のためのストラテジーについて述べる。第 2 章で述べた通り、化合物 **1** の 6 位へのハロゲン原子、特に、Br 基の導入により SERT 結合阻害活性が向上したが、その置換位置の最適化については未検討であったため、**SMP-304** の Br 基の置換位置の異なる類縁体を合成・評価することとした（Figure 17）。

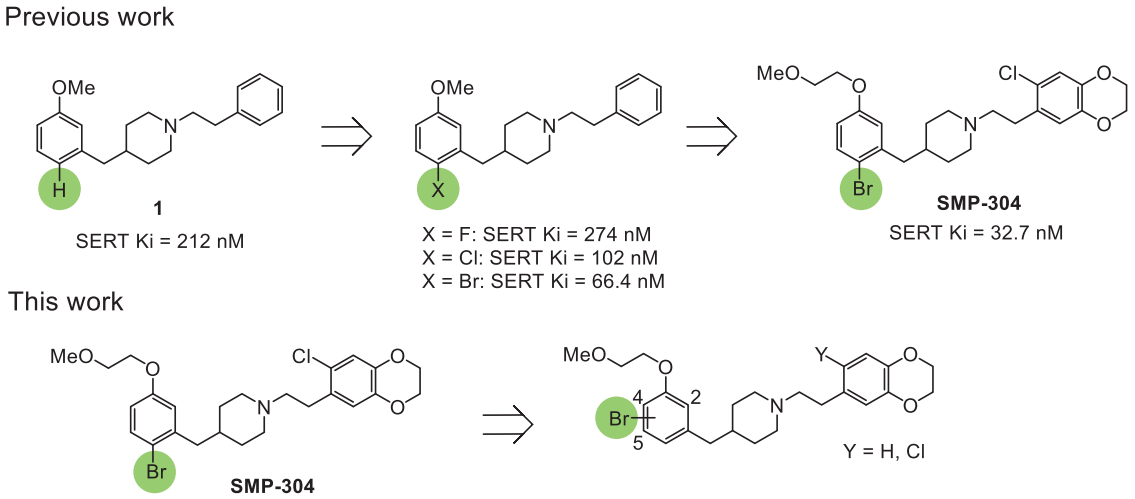


Figure 17. SERT 結合阻害活性向上のためのストラテジー

続いて、CYP2D6 の代謝寄与率の低減についてであるが、CYP2D6 の基質となる薬剤の構造的な特徴として塩基性アミンを有しその窒素原子の 5-7 Å の距離に脂溶性の部分構造を有するという点が上げられる。Figure 18 に CYP2D6 の基質となる薬剤の例とその代謝経路を示すが、上記の通り塩基性アミンを持ちその窒素原子からおよそ 5-7 Å 離れた位置に脂溶性の部分構造を有しその位置が代謝されている。[3] **SMP-304** は、右側パーツのベンゾジオキサン環が代謝されることが推定されたことから、CYP2D6 の代謝寄与率の低減を目的とし、ベンゾジオキサン環の変換検討を行うこととした（Figure 19）。

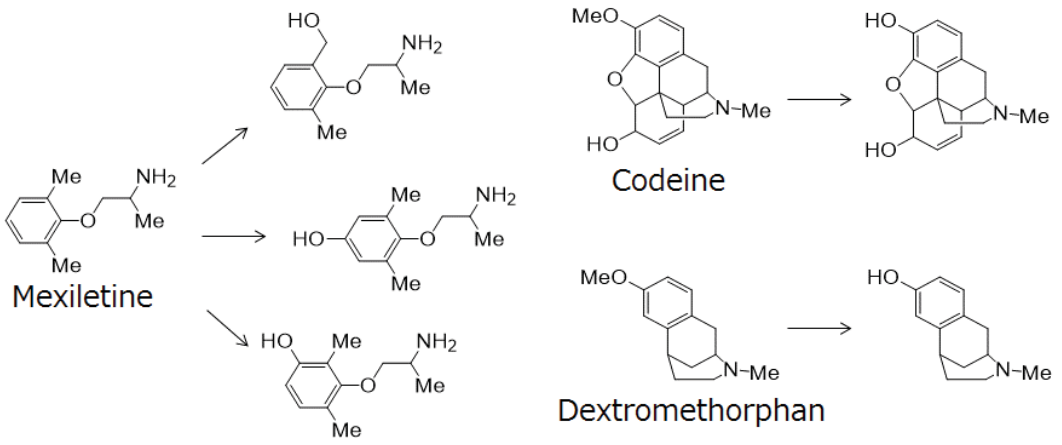


Figure 18. CYP2D6 の基質となる薬剤、並びに、CYP2D6 によるその代謝経路 [3]

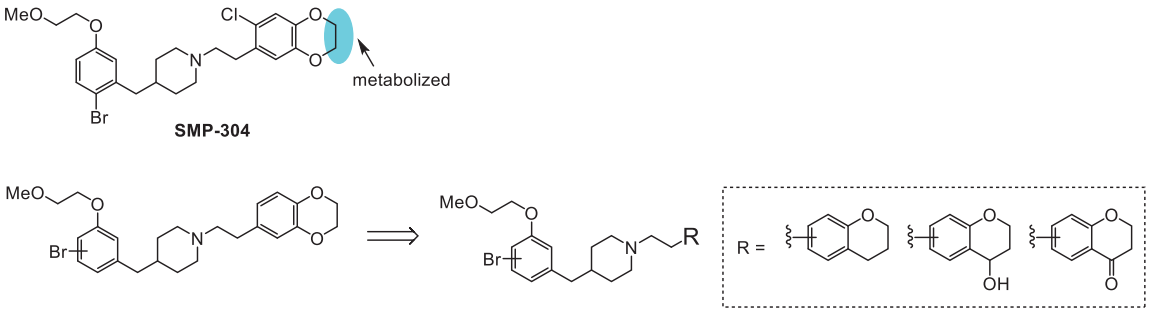
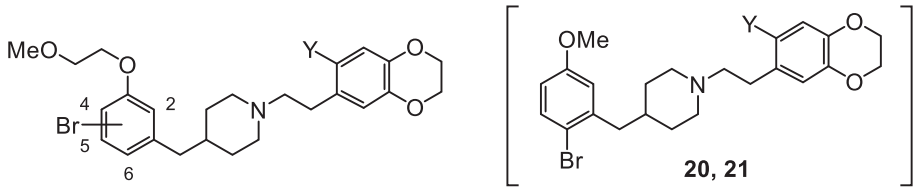


Figure 19. CYP2D6 の代謝寄与率低減のためのストラテジー

3-2. **SMP-304** の課題改善検討

SMP-304 の SERT に対する結合阻害活性の向上のため、Br 基の置換位置の変換検討を行った（Table 4）。その結果、2 位へ Br 基の置換位置を変換すると SERT, 5-HT_{1A} ともに結合阻害活性が減弱した。4 位あるいは 5 位への Br 基の置換位置の変換により、SERT 結合阻害活性は向上したが、5-HT_{1A} 結合阻害活性は減弱した。**SMP-304** の創製時に、得られた構造活性相関として、化合物 **21** から化合物 **20** への Cl 基の除去といった構造変換により、5-HT_{1A} 結合阻害活性が向上することが分かっていた。本構造活性相関情報を活用し、化合物 **40** および **41** の Cl 基の除去による化合物 **42** および **43** への変換を行った。その結果、SERT 結合阻害活性は維持したまま、5-HT_{1A} 結合阻害活性が向上した。なお、本構造変換による CYP2D6 代謝寄与率の改善効果は、軽微であった。

Table 4. ブロモ基の置換位置の変換検討



Compound	Br	Y	h-SERT[a]	h-5-HT$_{1A}$[a]	CYP2D6 contribution
24 (SMP-304)	6-Br	Cl	32.7 ± 3.4	9.4 ± 0.20	86%
39	2-Br	Cl	74.6 ± 8.2	468 ± 36	N.T.
40	4-Br	Cl	5.47 ± 0.70	251 ± 21	75.6%
41	5-Br	Cl	14.1 ± 1.7	546 ± 14	64.4%
42	4-Br	H	2.39 ± 0.27	16.0 ± 2.1	55.6%
43	5-Br	H	18.5 ± 0.05	57.8 ± 5.9	49.4%
20		H	14.0 ± 1.4	0.36 ± 0.05	N.T.
21		Cl	12.4 ± 1.5	6.46 ± 0.57	N.T.

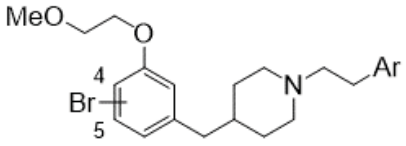
[a] h = human. Ki values (nM) are the means of two or three independent experiments.; N.T. = not tested

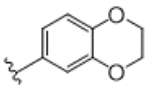
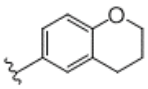
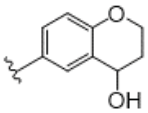
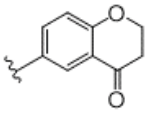
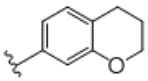
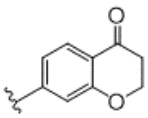
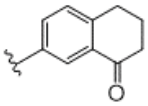
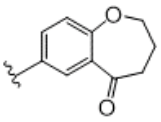
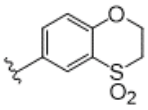
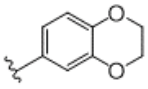
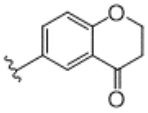
続いて、CYP2D6 の代謝寄与率の低減のために、化合物 **42** および **43** の右側パーツの変換検討を行った（Table 5）。合成上の効率性を重視し、同じ中間体から右側パーツの合成中間体となるトシラートが合成可能であることから、化合物 **42** のベンゾジオキサン環のエーテル結合をメチレンや水酸基、および、ケントン基へと変換した化合物 **44**, **45**, **46**, **47** および **48** を合成・評価した。その結果、エーテル結合をメチレンへと変換した化合物 **44** および **47** においても、また、エーテル結合と比較し脂溶性低減につながる水酸基やケトン基が導入された化合物 **45**, **46** および **48** においても、CYP2D6 の代謝寄与率は大幅に低減された。中でも、6 位置換クロマン-4-オン環を有する化合物 **46** は SERT および 5-HT$_{1A}$ に対して非常に強い結合阻害活性を示した。ベンゾジオキサン環から 6 位置換クロマン-4-オン環への変換が CYP2D6 の代謝寄与率の低減と SERT/5-HT$_{1A}$ 結合阻害活性の向上に効果的であったことから（化合物 **42** vs 化合物 **46**）、同様にケトン基を有する二環性構造 3,4-ジヒドロナフタレン-1-オンや 3,4-ジヒドロベンゾオキセピン-5-オンの導入検討、および、比較検討のためクロマン-4-オンのケトン基をスルホン基へと変換した 2,3-ジヒドロベンゾオキサチイン-4,4-ジオキシドの導入検討を行った（化合物 **49**, **50**, **51**）。その結果、ケトン基を有する 3,4-ジヒドロナフタレン-1-オンおよび 3,4-ジヒドロベンゾオキセピン-5-オンを導入した化合物 **49** および **50** において CYP2D6 の代謝寄与率の改善が見られたのに対し、スルホン基を有する 2,3-ジヒドロベンゾオキサチイン-4,4-ジオキシドを導入した化合物 **51** においては CYP2D6 の代謝寄与率の改善は見られなかった。また、左側ベンゼン環の

5 位に Br 基が置換した化合物 **43** においても右側ベンゾジオキサン環の 6 位置換クロマン-4-オン環への変換検討を行ったところ、化合物 **42** から化合物 **46** への変換と同様 CYP2D6 の代謝寄与率が大幅に低減した。

以上、まとめると **SMP-304** の代謝部位であるベンゾジオキサン環の変換は、CYP2D6 の代謝寄与率低減に効果的であることがわかった。その構造変換の適応範囲は、比較的広いものの、中にはエーテル結合からスルホン基への変換のように CYP2D6 の代謝寄与率低減に効果を示さないものもあることがわかった。中でも 6 位置換クロマン-4-オン環を有する化合物 **46** は、CYP2D6 の代謝寄与率が低減され非常に強い SERT および 5-HT_{1A} に対する結合阻害活性を示すことがわかった。

Table 5. 右側ベンゾジオキサン環の変換検討



Compound	Br	Ar	h-SERT[a]	h-5-HT$_{1A}$[a]	CYP2D6 contribution
42	4-Br		2.39 ± 0.27	16.0 ± 2.1	55.6%
44	4-Br		1.74 ± 0.07	43.1 ± 4.0	0%
45	4-Br		8.44 ± 0.28	32.8 ± 8.4	36.4%
46	4-Br		1.02 ± 0.06	5.05 ± 1.1	0%
47	4-Br		5.40 ± 0.19	19.0 ± 6.2	6.8%
48	4-Br		1.41 ± 0.11	42.8 ± 3.9	3.6%
49	4-Br		0.67 ± 0.07	12.4 ± 2.0	11.8%
50	4-Br		1.17 ± 0.13	71.9 ± 10.1	4.2%
51	4-Br		2.59 ± 0.03	11.3 ± 2.7	55.8%
43	5-Br		18.5 ± 0.05	57.8 ± 5.9	49.4%
52	5-Br		5.32 ± 0.68	44.3 ± 1.9	14%

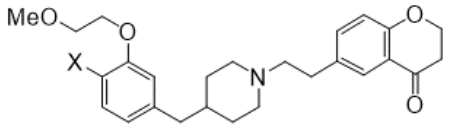
[a] h = human. Ki values (nM) are the means of two or three independent experiments.

3-3. 化合物 **46** の Br 基の変換による SERT に対する結合阻害活性の影響

化合物 **1** から化合物 **4** へのピペリジン環の 4 位に置換したベンジル基のベンゼン環 6 位への Br 基の導入により SERT 結合阻害活性が向上した点や **SMP-304** の Br 基の置換位置を 4 位へと変換した化合物 **40** において SERT 結合阻害活性の向上が見られた点から化合物 **46** の非常に強い SERT 結合阻害活性には Br 基の存在が重要であることが推察される。そこで、化合物 **46** の Br 基の他のハロゲン原子や低級アルキル基への変換を行った（Table 6）。その結果、2-Pr 基への変換を除いたすべての変換において 5-HT_{1A} 結合阻害活性にはあまり影響がなかったのに対し、SERT 結合阻害活性には予想していた通り大きな影響が見られた。まず、他のハロゲン原子への変換検討の結果、SERT 結合阻害活性は、I > Br > Cl > F の順となった。この順番は、化合物が酸素原子や窒素原子と σ ホールを介したハロゲン原子との相互作用として定義されるハロゲン結合によって標的タンパクと相互作用する場合に予測される活性強度の順番と一致する。[4,5] Figure 20 に記すようにハロゲン結合の強さは正電荷を帯びた σ ホールの大きさによって決まり、それはハロゲン原子の大きさにともなって増大する（I > Br > Cl > F）。[6] 一方、ハロゲン原子のサイズが大きくなるにつれ SERT 結合阻害活性が向上したが、これは疎水性相互作用や van der Waals 相互作用の増強によると見ることもできる。しかしながら、低級アルキル基を導入した場合に、その置換基の大きさや脂溶性の増大にともなって SERT 結合阻害活性は増強しなかった（化合物 **57, 58, 59**）。以上、構造活性相関という間接的なデータではあるものの化合物 **46** は SERT とハロゲン結合を介し相互作用していることが示唆された。

Zhou らによって、バクテリアの SERT 同族体であるロイシントランスポーター（LeuT）とセルトラリンや*(R)*-フルオキセチン／*(S)*-フルオキセチンといった SSRI との共結晶構造が報告された。[7] セルトラリンや*(R)*-フルオキセチン／*(S)*-フルオキセチンといった SSRI はいずれもハロゲン原子を有しており、Figure 21 に示すようにこれらのハロゲン置換ベンゼンがハロゲンバインディングポケットという LeuT の特異的なポケットにはまっていることが示されている。本既報において、セルトラリンや*(R)*-フルオキセチン／*(S)*-フルオキセチンとハロゲンバインディングポケットとの相互作用は van der Waals 相互作用によると考察されているが、化合物 **46** とその類縁体において Table 6 に示すようにハロゲン原子を介したより特異的な SERT との相互作用を示唆する構造活性相関が得られている。化合物 **46** やハロゲン原子が置換した類縁体と SERT との共結晶構造が取得されれば、SERT とのハロゲン結合といったハロゲン原子特異的な相互作用の考察が可能になると期待される。

Table 6. ブロモ基の変換検討



Compound	X	h-SERT[a]	h-5-HT$_{1A}$[a]	CYP2D6 contribution
53	H	202 ± 25	2.41 ± 0.11	N.T.
54	F	111 ± 3.5	2.25 ± 0.14	N.T.
55	Cl	3.37 ± 0.11	2.81 ± 0.03	53.5%
46	Br	1.02 ± 0.06	5.05 ± 1.07	0%
56	I	0.41 ± 0.06	8.76 ± 0.95	N.T.
57	Me	5.25 ± 1.25	7.72 ± 1.42	48.5%
58	Et	9.85 ± 1.36	19.3 ± 0.50	18.4%
59	2-Pr	246 ± 27	69.9 ± 4.0	N.T.

[a] h = human. Ki values (nM) are the means of two or three independent experiments.; N.T. = not tested

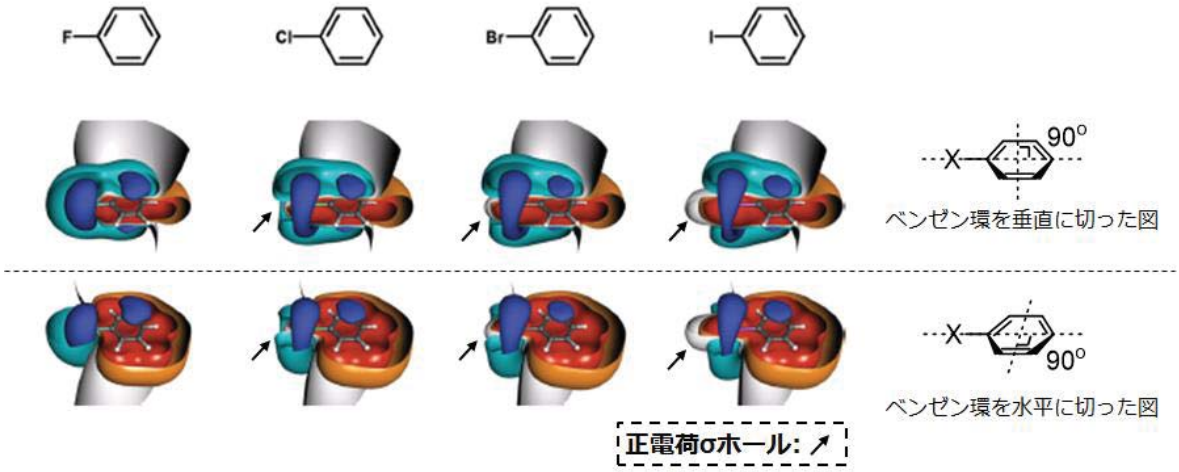


Figure 20. ハロゲン化ベンゼンの静電ポテンシャル [6]
青色: 負電荷，赤色: 正電荷

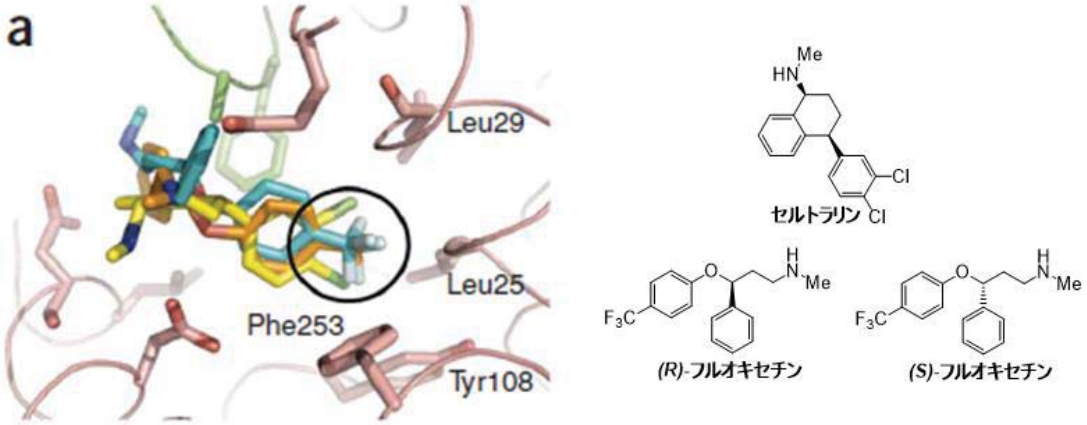
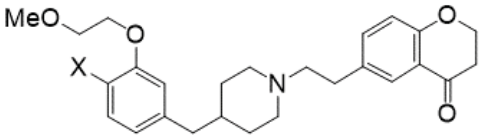


Figure 21. LeuT とセルトラリン・(*R*)-,(*S*)-フルオキセチンの共結晶 [7]
赤色: LeuT とハロゲンバインディングポケット
黄色: セルトラリン
オレンジ色: (*R*)-フルオキセチン
水色: (*S*)-フルオキセチン

3-4. SERT および 5-HT$_{1A}$ に対する機能評価

これまでの検討で、SERT および 5-HT$_{1A}$ に対し、その Ki 値が 10 倍の範囲内という良好なバランスで、かつ、一桁 nM という非常に強い結合阻害活性を示し、CYP2D6 の代謝寄与率が 60%以下であった化合物 **46**, **55** および **57** のセロトニン取り込阻害（SRI）活性および 5-HT$_{1A}$ の内因活性の評価を行った（Table 7）。その結果、これら３つの化合物は SERT 結合阻害活性の順に従い SRI 活性を示した。これは、SRI 活性を有する化合物を効率的に取得するために SERT 結合阻害活性を基準に化合物を選択するというストラテジーがうまく機能していることを示した。中でも、化合物 **46** は一桁 nM の IC$_{50}$ 値を示し最も強い SRI 活性を有していた。続いて、5-HT$_{1A}$ の内因活性の評価から、すべての化合物が 5-HT$_{1A}$ 部分作動薬であることが示された。

Table 7. 化合物 **46**, **55**, **57** の SRI 活性と 5-HT$_{1A}$ アゴニスト活性の評価



Compound	X	h-SERT binding[a] (Ki, nM)	h-SRI[b] (IC_{50}, nM)	h-5-HT$_{1A}$ binding[a] (Ki, nM)	h-5-HT$_{1A}$ I.A.[c] (%)	CYP2D6 contribution (%)
46	Br	1.02 ± 0.06	2.71 ± 0.41	5.05 ± 1.1	70.0	0
55	Cl	3.37 ± 0.11	16.4 ± 4.96	2.81 ± 0.03	60.8	53.5
57	Me	5.25 ± 1.25	45.3 ± 12.0	7.72 ± 1.42	49.5	48.5

h = human.
[a] Ki values (nM) are the means of two or three independent experiments.
[b] IC_{50} values (nM) are the means of at least three independent experiments. [c] I.A.: intrinsic activity

3-5. ラット前頭前皮質におけるセロトニン遊離量上昇作用の評価

化合物 **46, 55** および **57** は SRI 活性を示し、5-HT_{1A} 部分作動薬であることがわかったことから、これらのラット前頭前皮質におけるセロトニン遊離量上昇作用の評価を行うため、ラットマイクロダイアリシスを実施した。ラットマイクロダイアリシスとは、半透膜を使用したプローブをラットの脳内に挿入し、連続的に膜内部に液体を潅流し、膜を通過した液体を回収することで、プローブ半透膜外の成分すなわち脳内の物質を採取することができる。プローブには連続的に液体が流れているので、目的とするラットの脳内の物質を時間軸に沿ってその濃度変化を測定することができる評価系である（Figure 22）。[8]

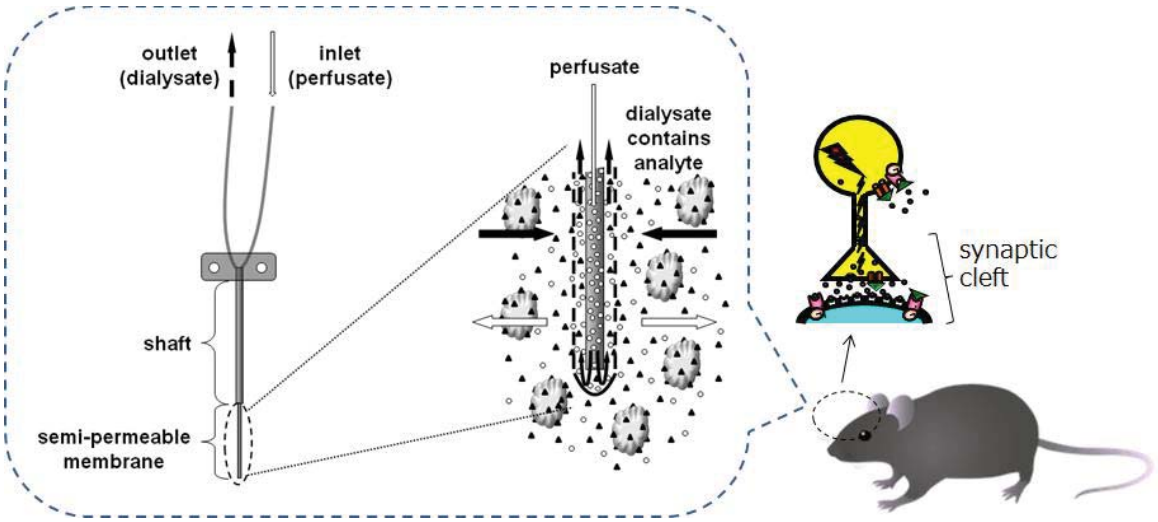
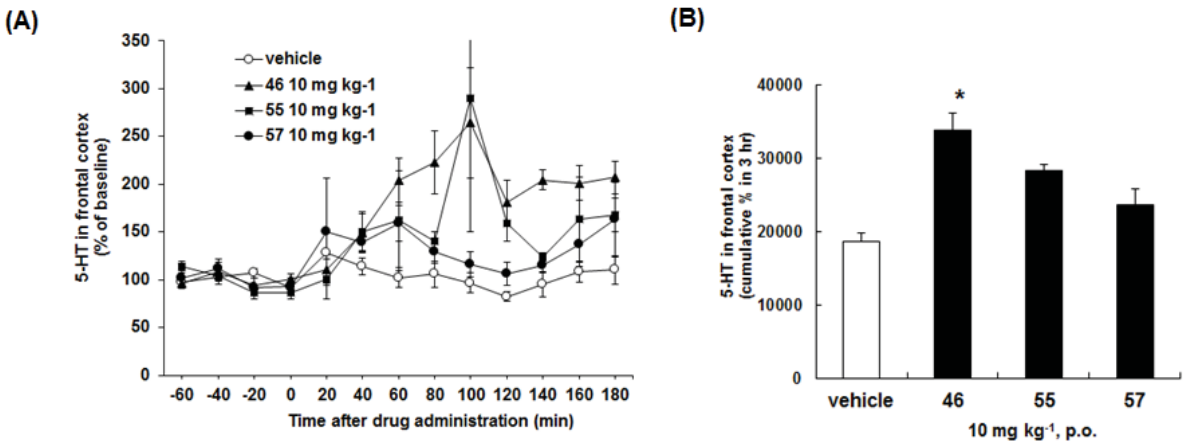


Figure 22. ラットマイクロダイアリシスの概要 [8]

化合物 **46, 55** および **57** を 10 mg/kg 経口投与した際の溶媒投与群に対するセロトニン遊離量の上昇率の経時的変化を測定した（Figure 23）。その結果、いずれの化合物もラット前頭前皮質におけるセロトニン遊離量上昇作用を示した。その作用の強さは、SERT に対する結合阻害活性や SRI 活性と相関し、**46** が最も強いセロトニン遊離量上昇作用を示した。以上の結果から、化合物 **46**（**DSP-1053**）を有望な候補化合物としてさらなるラット in vivo 評価を行うこととした。

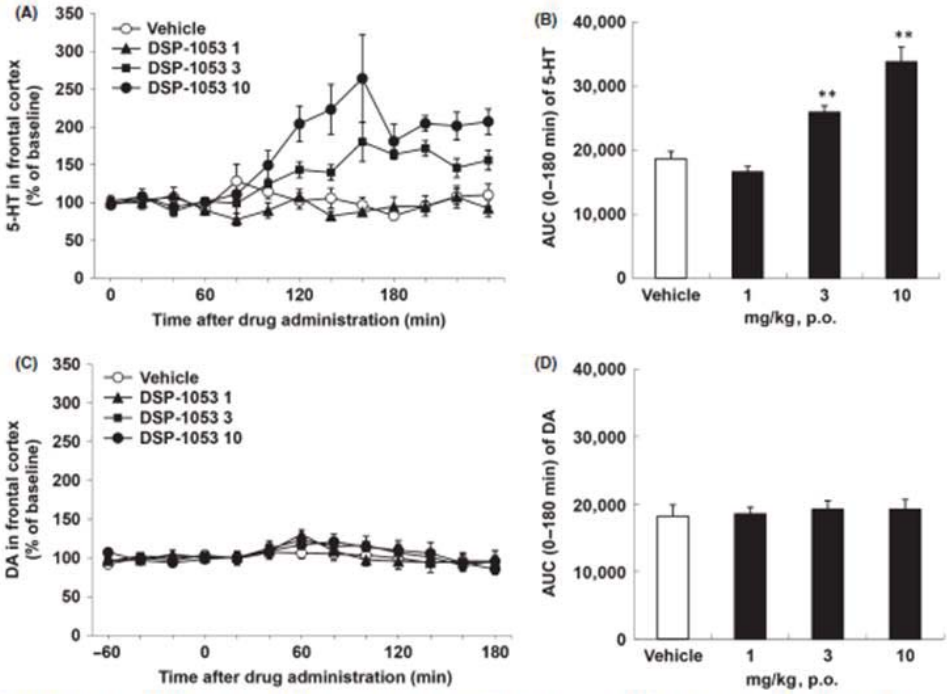


(A) Each point with a vertical bar represents the mean ± SEM of percentage baseline value.
(B) Each column with vertical bar represents the mean ± SEM of AUC of 5-HT percent over 3 h.
*P < 0.05, compared to the vehicle-treated group using parametric Tukey's multiple comparison test. Vehicle group, n = 6; compound 46 and 57 10 mg kg^{-1} groups, n = 3; and compound 55 10 mg kg^{-1} group, n = 2.

Figure 23. ラットマイクロダイアリシスの評価結果
(A) 化合物 **46**, **55**, **57** 経口投与後のラット前頭前皮質におけるセロトニン遊離量の経時変化
(B) 化合物 **46**, **55**, **57** 経口投与後 3 時間のラット前頭前皮質におけるセロトニン遊離量のベースラインに対する変化量(%)の AUC

3-6. **DSP-1053** のラットを用いた in vivo 薬効評価

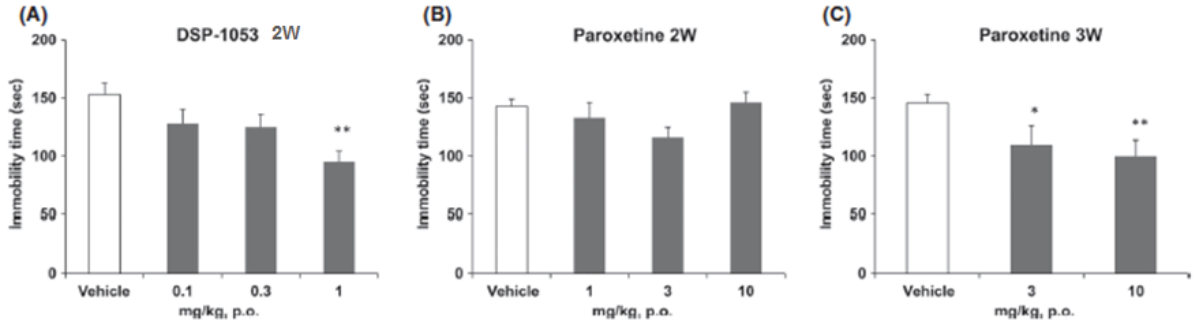
DSP-1053 は、前述の通り、SERT および 5-HT$_{1A}$ に対し、その Ki 値が 10 倍の範囲内という良好なバランスで、かつ、一桁 nM という非常に強い結合阻害活性とセロトニン取り込阻害（SRI）活性を示し、CYP2D6 の代謝寄与率が 60%以下であり、10 mg/kg 経口投与にてラット前頭前皮質における強いセロトニン遊離量上昇作用を示した。そこで、新規抗うつ薬の開発化合物として適しているかどうかを判断するため、**DSP-1053** のラットを用いた各種 in vivo 薬効評価が行われた。[9] まず、神経伝達物質の遊離量に対する **DSP-1053** の影響をより詳細に検討するため、ラットマイクロダイアリシスにて 1, 3, 10 mg/kg 経口投与時の前頭前皮質でのセロトニンとドパミンの遊離量を測定した。その結果、用量依存的に 3 mg/kg から有意なセロトニン遊離量の増加が見られたのに対し、ドパミンについては 10 mg/kg まで変化がなかった。5-HT$_{1A}$ 作動薬（完全作動薬，部分作動薬ともに）はラット前頭前皮質におけるセロトニン遊離量を減少させ、ドパミン遊離量を増加させることが知られている。[10,11] 以上の結果から、前述の通り、**DSP-1053** は in vitro 評価系において 5-HT$_{1A}$ 受容体の部分作動薬であることが示されたが、間接的なデータでさらなる詳細な検討は必要であるものの in vivo においては 5-HT$_{1A}$ 受容体のアンタゴニストとして作用することが示唆された（Figure 24）。



(A and C) Each point with a vertical bar represents the mean ± SEM of percentage baseline value.
(B and D) Each column with vertical bar represents the mean ± SEM of AUC of 5-HT or DA percent over 3 h.
**P < 0.01, compared to the vehicle-treated group using parametric Dunnett's multiple comparison test.
Vehicle group, n = 6; DSP-1053 1 and 3 mg kg^{-1} groups, n = 4; and DSP-1053 10 mg kg^{-1}, n = 3.

Figure 24. **DSP-1053** のラットマイクロダイアリシスの結果 [9]
(A) **DSP-1053** 経口投与後のラット前頭前皮質におけるセロトニン遊離量の経時変化
(B) **DSP-1053** 経口投与後 3 時間のラット前頭前皮質におけるセロトニン遊離量のベースラインに対する変化量(%)の AUC
(C) **DSP-1053** 経口投与後のラット前頭前皮質におけるドパミン遊離量の経時変化
(D) **DSP-1053** 経口投与後 3 時間のラット前頭前皮質におけるドパミン遊離量のベースラインに対する変化量(%)の AUC

続いて、ラット強制水泳試験にて **DSP-1053** の抗うつ様作用を評価した。その結果、**DSP-1053** は 1 mg/kg 経口投与という低用量による 2 週間の連続投与にて、有意な抗うつ様作用を発現した（Figure 25，図 A）。一方、代表的な SSRI の一つであるパロキセチンは、2 週間の連続投与では 10 mg/kg 経口投与まで抗うつ様作用の発現はなく（Figure 25，図 B）、3 週間の連続投与にて 3 mg/kg 経口投与から有意な抗うつ様作用を発現した（Figure 25，図 C）。以上のように、**DSP-1053** はパロキセチンと比較し、期待通りの早い抗うつ様作用のオンセットを低用量から示すことが確認された。



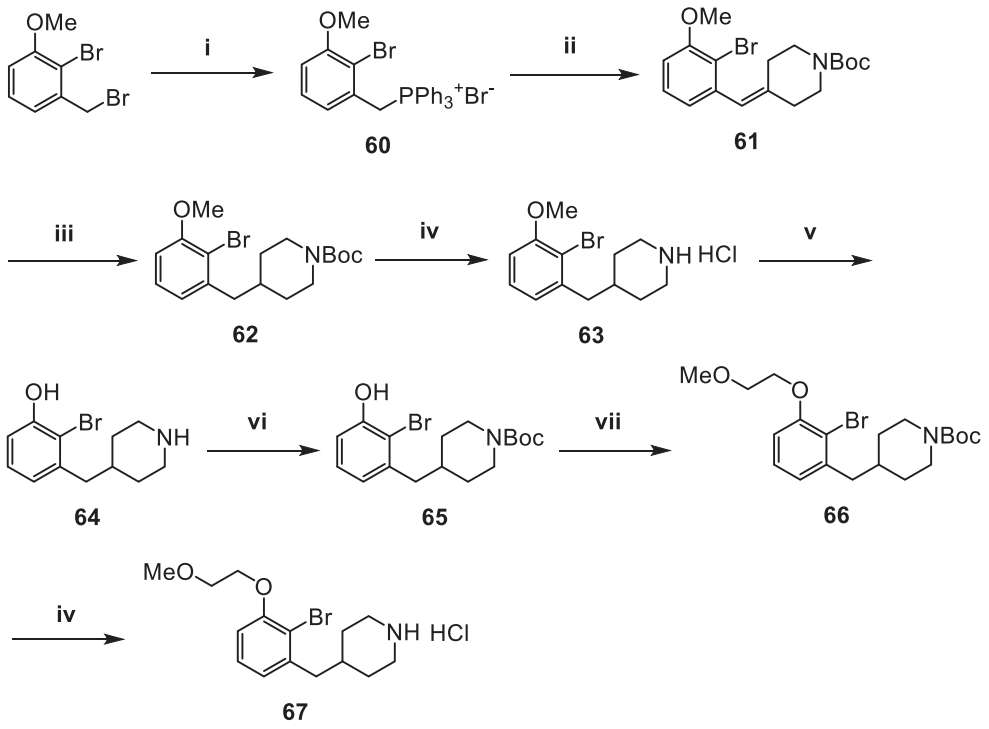
Each bar represents the mean ± SEM of immobility time during a 5 min test session (n = 16–18 per group). *P < 0.05, **P < 0.01, compared to the vehicle-treated group using parametric Dunnett's multiple comparison test.

Figure 25. ラット強制水泳試験の結果 [9]
(A) **DSP-1053** 二週間投与でのラット強制水泳試験時の無動時間
(B) パロキセチン二週間投与でのラット強制水泳試験時の無動時間
(C) パロキセチン三週間投与でのラット強制水泳試験時の無動時間

DSP-1053 は、SERT および 5-HT_{1A} に対し、その Ki 値が 10 倍の範囲内という良好なバランスで、かつ、一桁 nM という非常に強い結合阻害活性を示し、目的とするセロトニン取り込阻害（SRI）活性と 5-HT_{1A} 自己受容体阻害活性を併せ持ち SSRI の問題点の一つである治療オンセットの遅さを克服しうることが確認された。本化合物は、時に SSRI の使用を困難にする吐き気や嘔吐といった副作用の発現が温和であることが確認され、[9] 十分な安全性と優れた薬物動態プロファイルを示したため、開発化合物として臨床試験へと進んだ。

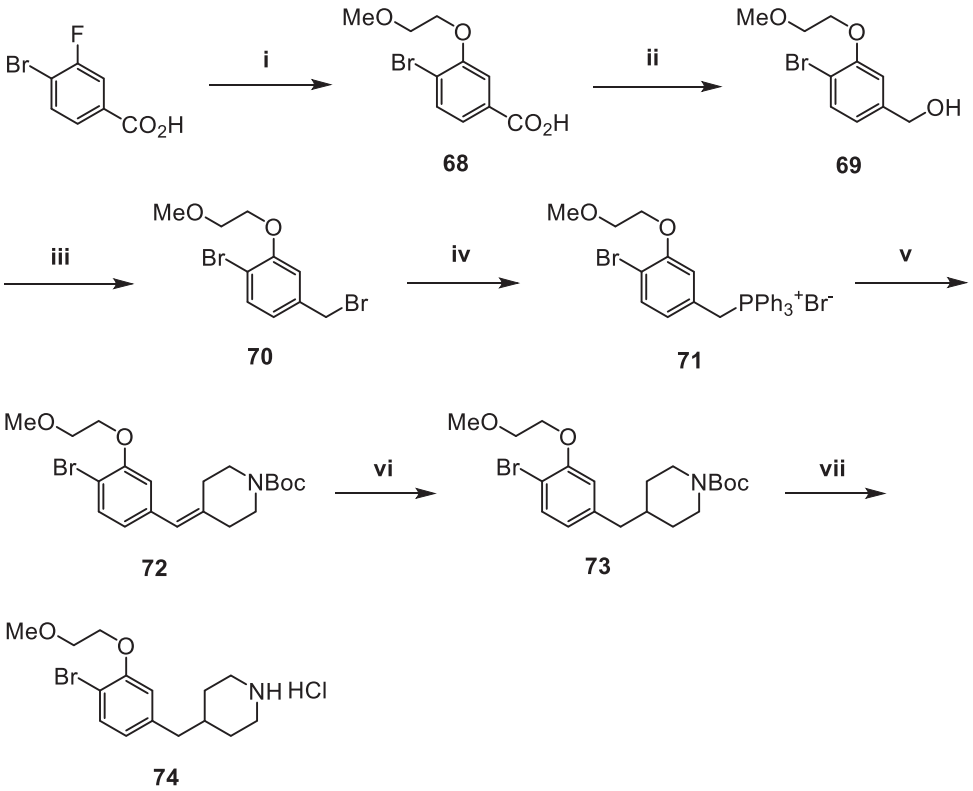
3-7. 化合物の合成

上記化合物は、左側パーツにあたるベンジルピペリジン中間体と右側パーツにあたるトシラート中間体をそれぞれ合成し、アルキル化反応により最終化合物とした。まず、**SMP-304** の左側ベンゼン環上のブロモ基の置換位置の変換検討のために、ベンジルピペリジン中間体 **67, 74, 82** を合成した。2-bromo-3-methoxybenzyl bromide とトリフェニルホスフィンを反応させ、ホスホニウム塩 **60** を得た。続いて、ホスホニウム塩 **60** と *tert*-butyl 4-oxopiperidine-1-carboxylate との Wittig 反応によりオレフィン **61** へと導いた後、ロジウムカーボンを用いた水素添加反応により、副反応の脱ブロモ化を起こすことなく、オレフィン選択的な還元反応を行い、得られた **62** を塩化水素－1,4-ジオキサンで処理することで、中間体 **63** を合成した。続いて、**63** の三臭化ホウ素による脱メチル化反応および窒素原子の Boc 化により、フェノール **65** へと導いた。フェノール 65 の O-アルキル化反応、さらに、塩化水素－1,4-ジオキサンを用いた処理による脱 Boc 化反応を行うことで、ベンジルピペリジン中間体 **67** を得た（Scheme 7）。



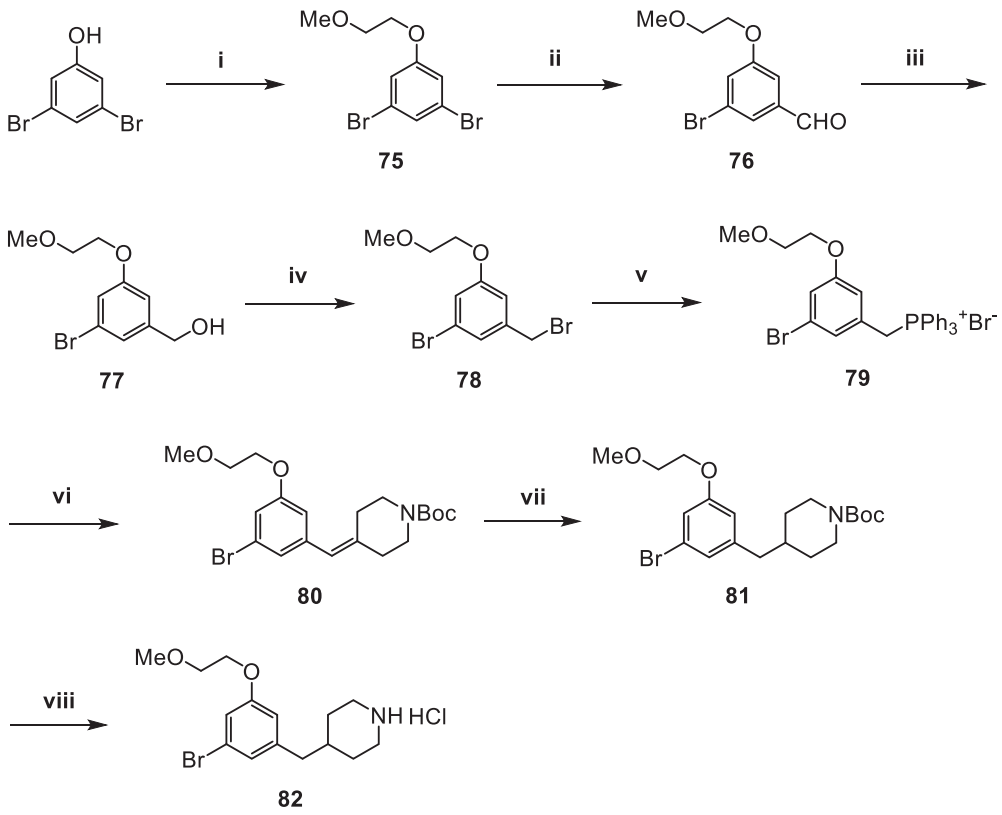
Scheme 7. 試薬と反応条件：(i) PPh_3, toluene, reflux, (ii) *tert*-butyl 4-oxopiperidine-1-carboxylate, K_2CO_3, 2-PrOH, reflux, (iii) H_2, 5% Rh-C, EtOAc, EtOH, 45°C, (iv) 4N HCl/1,4-dioxane, AcOH, 50°C, (v) BBr_3, CH_2Cl_2, 0°C, (vi) $(Boc)_2O$, 1,4-dioxane, H_2O, r.t., (vii) $MeO(CH_2)_2Br$, K_2CO_3, KI, DMF, 80°C

4-Bromo-3-fluorobenzoic acid と 2-methoxyethanol との S_NAr 反応によりカルボン酸 **68** を得た後、水素化ホウ素ナトリウムと三フッ化ホウ素ジエチルエーテル錯体を用いたカルボン酸 **68** の還元反応により、アルコール **69** へと導いた。続いて、アルコール **69** のメタンスルホニル化を経由したブロモ化反応により、ベンジルブロミド **70** を合成した。得られたベンジルブロミド **70** から、2-bromo-3-methoxybenzyl bromide と同様の手法によりベンジルピペリジン中間体 **74** を得た（Scheme 8）。



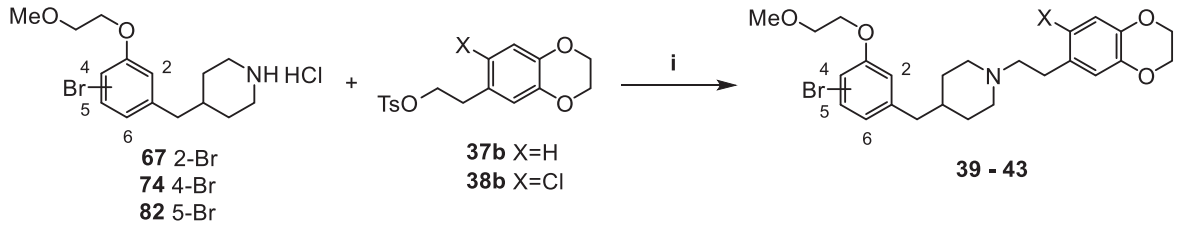
Scheme 8. 試薬と反応条件: (i) $MeO(CH_2)_2OH$, KO^{t}Bu, NMP, 90^{o}C, (ii) $NaBH_4$, BF_3-Et_2O, THF, r.t., (iii) MsCl, Et_3N, Me_3NHCl, toluene, 0^{o}C, then LiBr, THF, reflux, (iv) PPh_3, toluene, reflux, (v) *tert*-butyl 4-oxopiperidine-1-carboxylate, K_2CO_3, 2-PrOH, reflux, (vi) H_2, 5% Rh-C, EtOAc, EtOH, 45^{o}C, (vii) cHCl, AcOH, 50^{o}C

3,5-Dibromophenol と 2-methoxyethyl bromide とのアルキル化反応に、続いて、モノホルミル化反応により、アルデヒド **76** へと導いた後、水素化ホウ素ナトリウムによるアルデヒド **76** の還元反応によりアルコール **77** を得た。得られたアルコール **77** から、アルコール **69** と同様の手法によりベンジルピペリジン中間体 **82** を合成した（Scheme 9）。



Scheme 9. 試薬と反応条件: (i) $MeO(CH_2)_2Br$, K_2CO_3, DMF, 80°C, (ii) *n*-BuLi, *n*-BuMgCl, toluene, 0°C, then DMF, (iii) $NaBH_4$, MeOH, r.t., (iv) MsCl, Et_3N, Me_3NHCl, toluene, 0°C, then LiBr, THF, reflux, (v) PPh_3, toluene, reflux, (vi) *tert*-butyl 4-oxopiperidine-1-carboxylate, K_2CO_3, 2-PrOH, reflux, (vii) H_2, 5% Rh-C, EtOAc, r.t., (viii) 10% HCl/MeOH, MeOH, r.t.

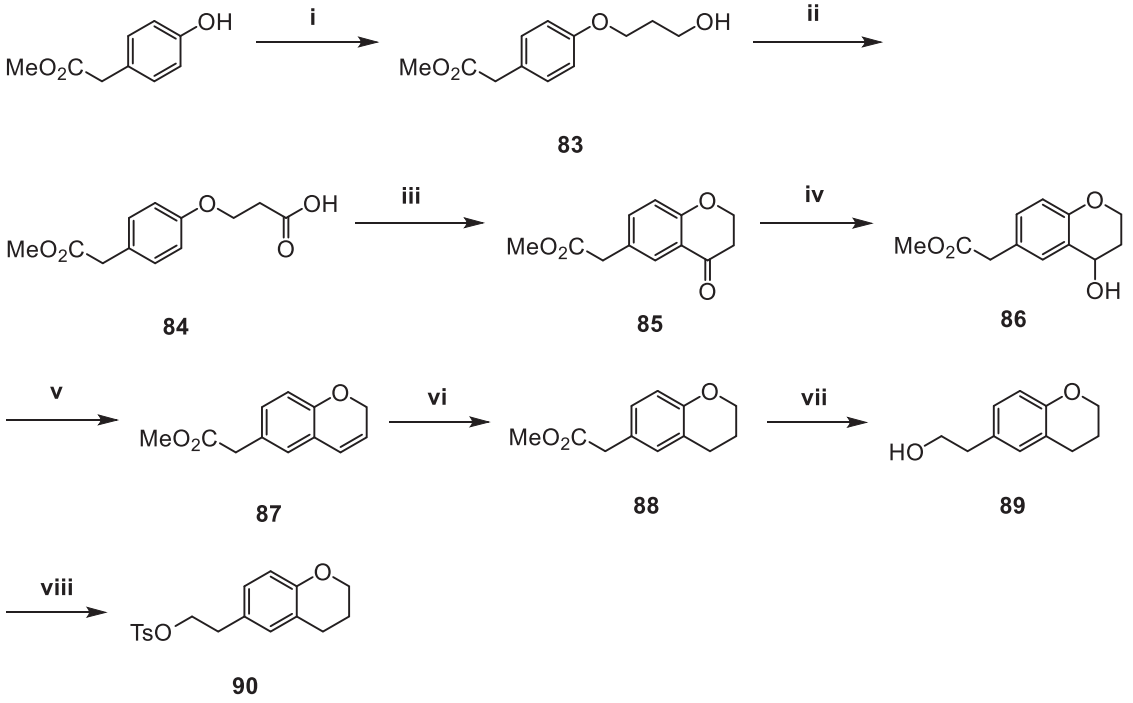
炭酸カリウム存在下、アセトニトリル中でのベンジルピペリジン中間体 **67**, **74**, **82** とトシラート中間体 **37b**, **38b** とのアルキル化反応により、目的とする化合物 **39 – 43** を合成した（Scheme10）。



Scheme 10. 試薬と反応条件: (i) K_2CO_3, MeCN, reflux

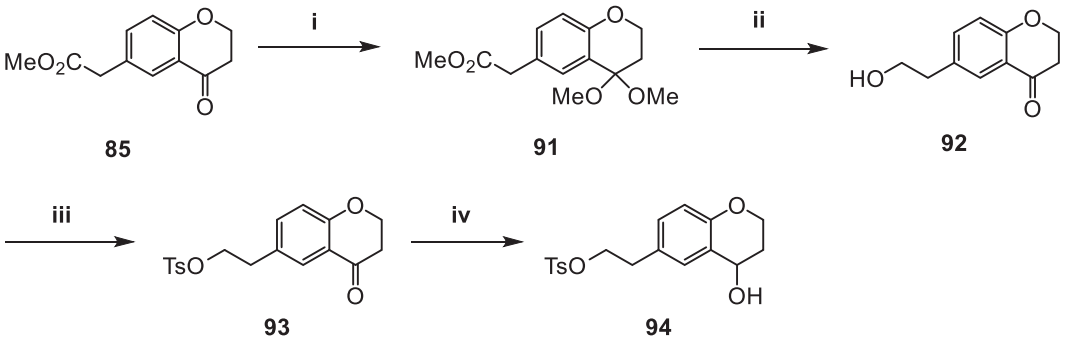
SMP-304 の右側パーツのベンゾジオキサン環の最適化検討のため、トシラート中間体 **90**, **93**, **94**, **102**, **105**, **111**, **117**, **122** を合成した。methyl 4-hydroxyphenylacetate と 3-bromo-1-propanol とのアルキル化反応によりアルコール **83** へと導いた後、PDC を用いた酸化反応、続いて、PPA を用いた環化反応によりアルコール **83** をケトン **85** へと変換した。ケトン **85** を水素化ホウ素ナトリウムにより還元し、アルコール **86** とした後、酸性触媒による脱水反応を行い、さらに、パラジウム炭素を用いた水素添加反応により、エステル **88** を得た。水素化リチウムアルミニウムを用い、エステル **88** を還

元した後、トシル化反応を行うことでトシラート中間体 **90** を合成した（Scheme 11）。



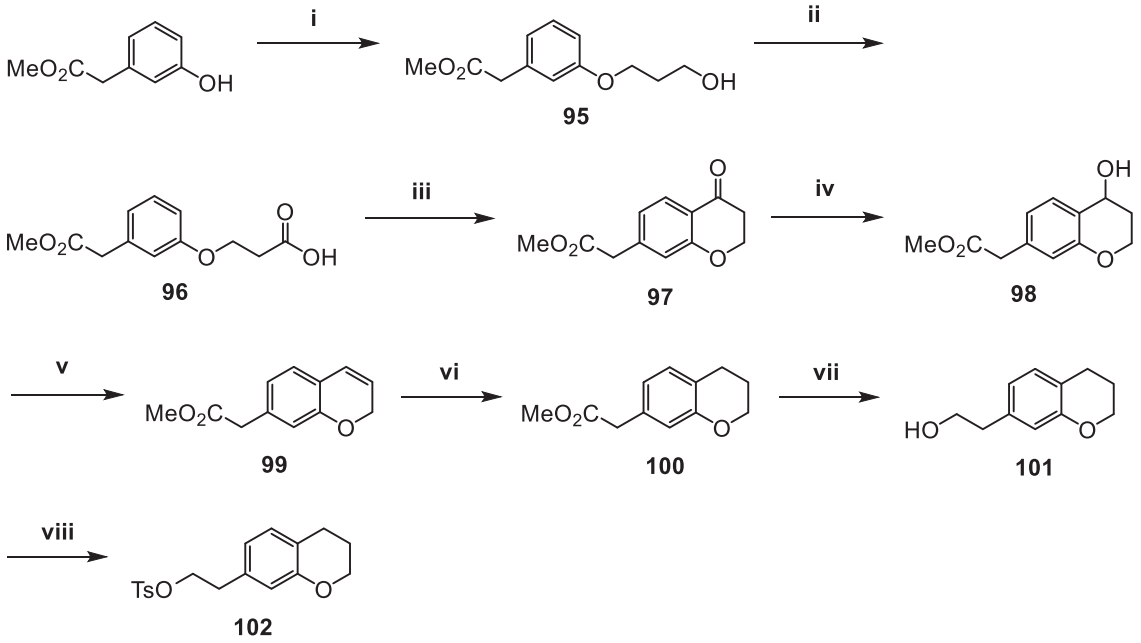
Scheme 11. 試薬と反応条件: (i) $Br(CH_2)_3OH$, K_2CO_3, MeCN, reflux, (ii) PDC, MS3Å, DMF, r.t., (iii) PPA, 80°C, (iv) $NaBH_4$, MeOH, r.t., (v) p-TsOH (cat.), benzene, reflux, (vi) H_2, 10% Pd-C, EtOAc, r.t., (vii) $LiAlH_4$, THF, r.t., (viii) p-TsCl, Et_3N, Me_3NHCl, CH_2Cl_2, 0°C

ケトン **85** をアセタール化反応によりアセタール **91** とし、続いて、水素化リチウムアルミニウムで還元後、トシル化反応を行い、トシラート中間体 **93** を得た。また、トシラート中間体 **93** の水素化ホウ素ナトリウムによる還元反応により、トシラート中間体 **94** を合成した（Scheme 12）。

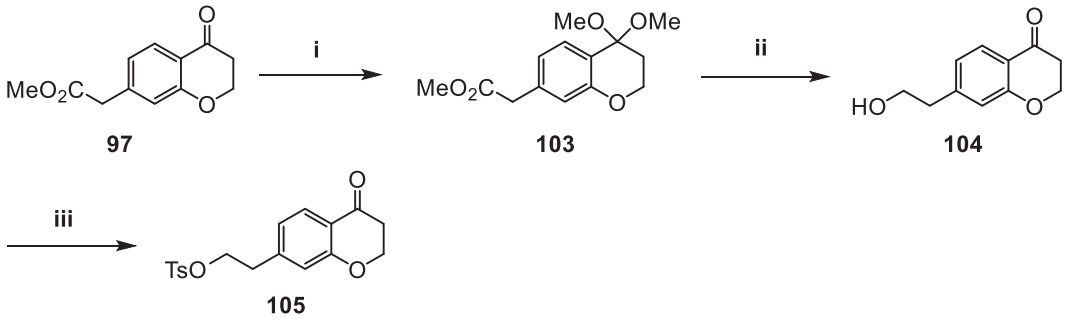


Scheme 12. 試薬と反応条件: (i) $HC(OMe)_3$, p-TsOH, MeOH, r.t., (ii) $LiAlH_4$, THF, r.t., then 2N aq.HCl, acetone, r.t., (iii) TsCl, Et_3N, Me_3NHCl, CH_2Cl_2, 0°C, (v) $NaBH_4$, MeOH, THF, r.t.

トシラート中間体 **102** と **105** は、methyl 2-(3-hydroxyphenyl)acetate からトシラート中間体 **90** および **93** と同様の手法により、それぞれ合成した（Scheme 13, 14）。

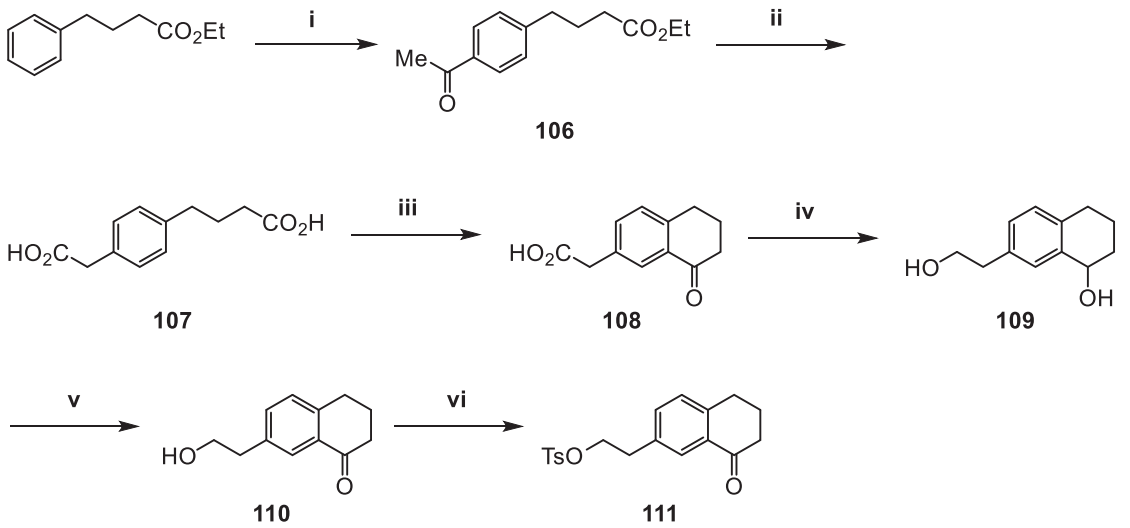


Scheme 13. 試薬と反応条件: (i) $Br(CH_2)_3OH$, K_2CO_3, MeCN, reflux, (ii) PDC, MS3Å, DMF, r.t., (iii) PPA, 80°C, (iv) $NaBH_4$, MeOH, r.t., (v) p-TsOH (cat.), benzene, reflux, (vi) H_2, 10% Pd-C, EtOAc, r.t., (vii) $LiAlH_4$, THF, r.t., (viii) p-TsCl, Et_3N, Me_3NHCl, CH_2Cl_2, 0°C



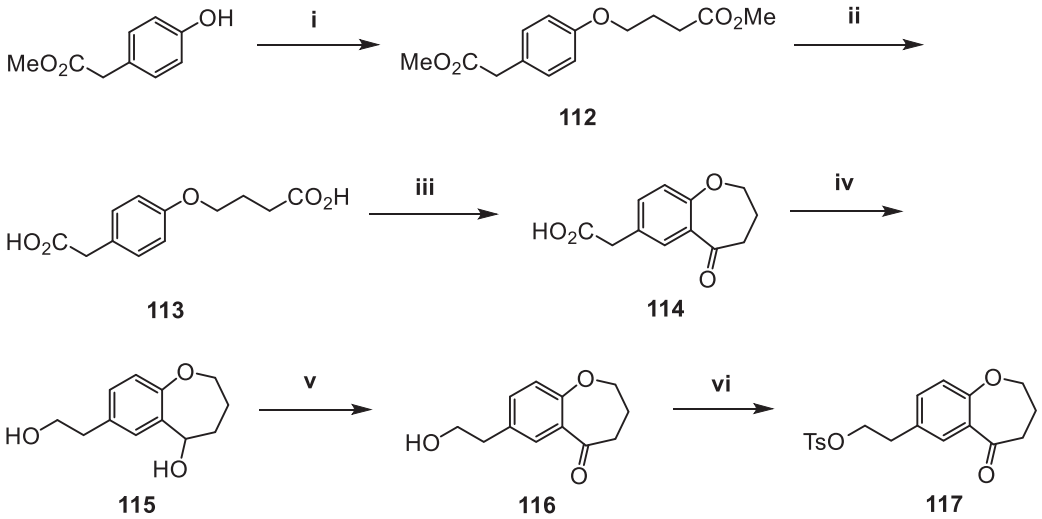
Scheme 14. 試薬と反応条件: (i) $HC(OMe)_3$, p-TsOH, MeOH, r.t., (ii) $LiAlH_4$, THF, r.t., then 2N aq.HCl, acetone, r.t., (iii) TsCl, Et_3N, Me_3NHCl, CH_2Cl_2, 0°C

Ethyl 4-phenylbutyrate のアセチル化反応により **106** を得た後、Willgerodt-Kindler 反応により、ジカルボン酸 **107** とした。[12] PPA を用いた環化反応を行い、さらに、水素化リチウムアルミニウムを用いた還元反応により、アルコール **109** を得た。二酸化マンガンによる選択的なアルコールの酸化により、ケトン **110** とした後、トシル化反応を行い、トシラート中間体 **111** を合成した（Scheme 15）。

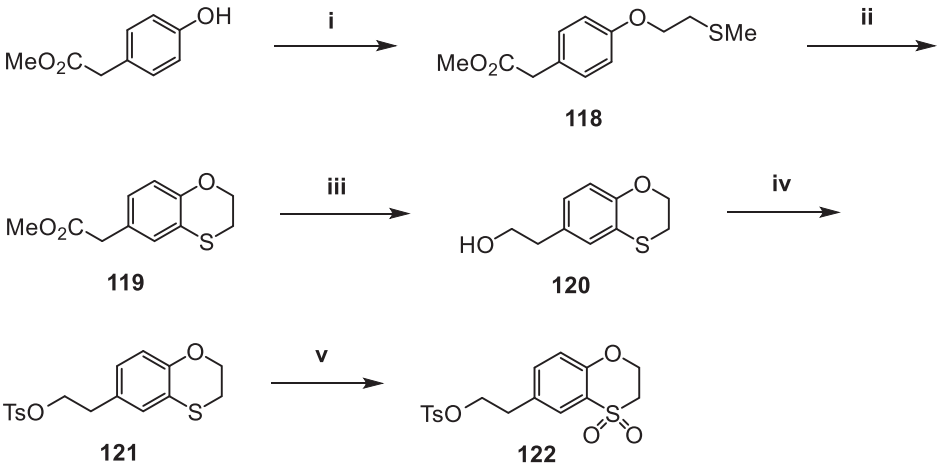


Scheme 15. 試薬と反応条件: (i) AcCl, $AlCl_3$, CH_2Cl_2, r.t., (ii) morpholine, S_8, reflux, then 15% aq.NaOH, 2-PrOH, reflux, (iii) PPA, 80°C, (iv) $LiAlH_4$, THF, reflux, (v) MnO_2, CH_2Cl_2, r.t., (vi) TsCl, Et_3N, Me_3NHCl, CH_2Cl_2, 0°C

ジカルボン酸 **113** を methyl 4-hydroxyphenylacetate のアルキル化反応、さらに、加水分解反応を行い合成した。続いて、得られたジカルボン酸 **113** からトシラート中間体 **111** と同様の手法によりトシラート中間体 **117** を合成した（Scheme 16）。エステル **118** を methyl 4-hydroxyphenylacetate の光延反応により合成し、無水トリフルホロメタンスルホン酸を用いた環状スルホニウム塩を形成する環化反応、続いて、トリエチルアミンによる脱アルキル化反応を行い、環状スルフィド **119** を得た。[13] 環状スルフィド **119** の水素化リチウムアルミニウムを用いた還元反応を行い、さらに、トシル化反応により **121** とした後、スルフィドの酸化反応によりトシラート中間体 **122** を合成した（Scheme 17）。

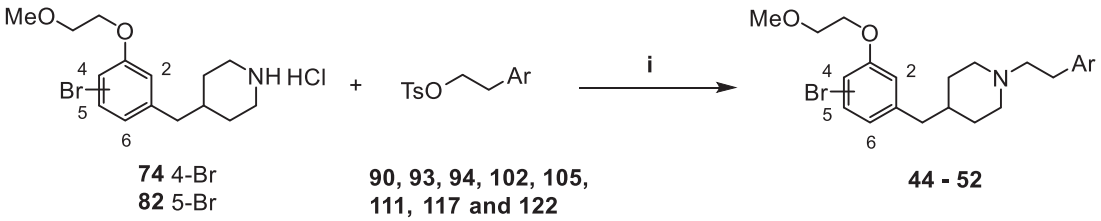


Scheme 16. 試薬と反応条件: (i) $Br(CH_2)_3CO_2Me$, K_2CO_3, MeCN, reflux, (ii) 6N aq.NaOH, MeOH, r.t., (iii) PPA, 80°C, (iv) $LiAlH_4$, THF, reflux, (v) MnO_2, CH_2Cl_2, r.t., (vi) p-TsCl, Et_3N, Me_3NHCl, CH_2Cl_2, 0°C



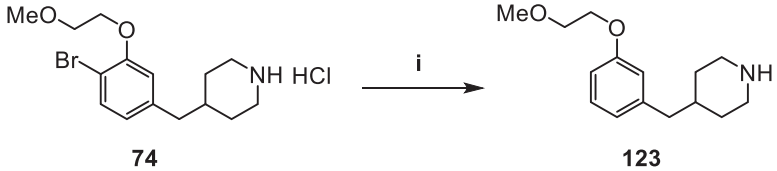
Scheme 17. 試薬と反応条件: (i) $OH(CH_2)_2SMe$, DEAD, PPh_3, THF, r.t., (ii) Tf_2O, CH_2Cl_2, -78°C, then Et_3N, MeCN, r.t., (iii) $LiAlH_4$, THF, r.t., (iv) p-TsCl, Et_3N, pyridine, CH_2Cl_2, r.t., (v) mCPBA, CH_2Cl_2, r.t.

炭酸カリウム存在下、ベンジルピペリジン中間体 **74**, **82** とトシラート中間体 **90**, **93**, **94**, **102**, **105**, **111**, **117**, **122** とのアルキル化反応により、目的とする化合物 **44 – 52** を合成した（Scheme 18）。

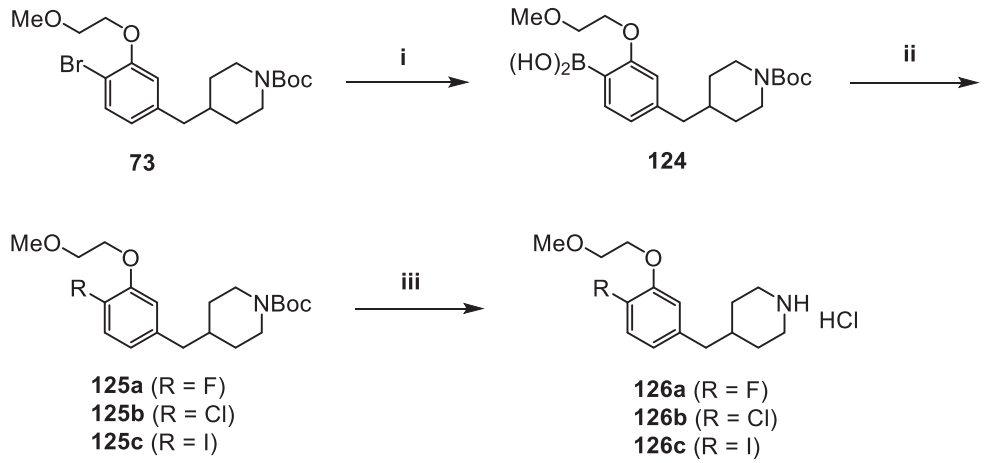


Scheme 18. 試薬と反応条件: (i) K_2CO_3, MeCN, reflux

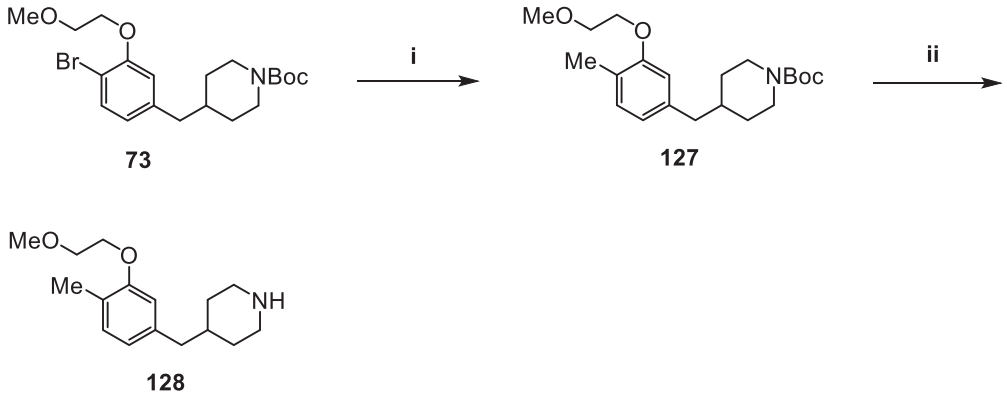
左側メチレンピペリジンに結合するベンゼン環の4位置換基における詳細な構造活性相関の理解のために、ブロモ基が置換した中間体 **73** と **74** からの直接的構造変換によりベンジルピペリジン中間体 **123**, **126a**, **126b**, **126c**, **128**, **131a**, **131b** を合成した。具体的には、ベンジルピペリジン中間体 **123** は、中間体 **74** の加水素分解により得た（Scheme 19）。また、中間体 **73** から得られるボロン酸 **124** のハロゲン化反応、[14] 続いて、塩化水素ー酢酸エチルを用いた脱保護反応によりベンジルピペリジン中間体 **126a**, **126b**, **126c** を合成した（Scheme 20）。ベンジルピペリジン中間体 **128** は、**73** とメチルボロン酸とのカップリング反応および脱保護反応により合成した（Scheme 21）。さらに、中間体 **73** のリチオ化反応を足掛かりとする各求電子種との反応により、**129a**, **129b** へと変換後、加水素分解反応および脱保護反応によりベンジルピペリジン中間体 **131a**, **131b** を得た（Scheme 22）。対応するベンジルピペリジン中間体とトシラート中間体 **93** とのアルキル化反応により、目的とする **53** – **59** を合成した（Scheme 23）。



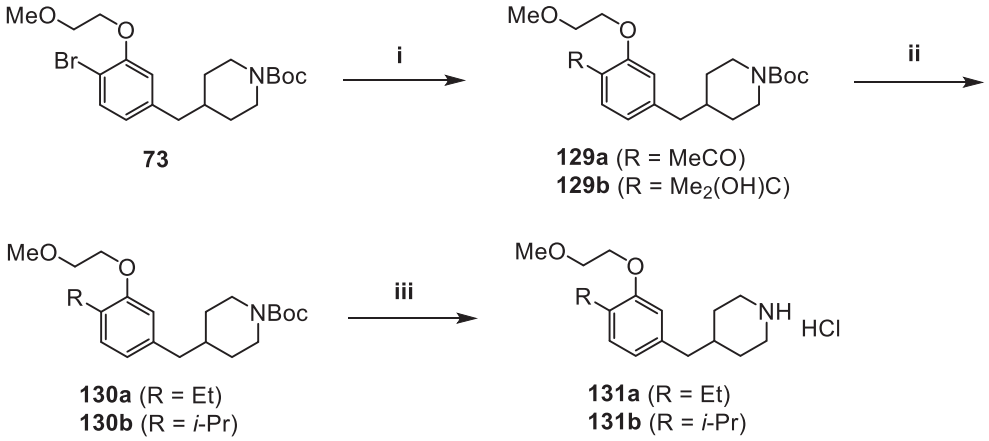
Scheme 19. 試薬と反応条件: (i) H_2, 10%Pd/C, MeOH, r.t.



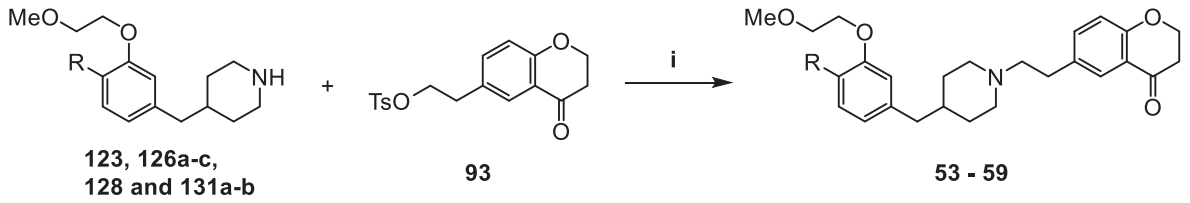
Scheme 20. 試薬と反応条件: (i) *n*-BuLi, THF, -78°C, then $B(OMe)_3$, r.t., (ii) R = F: NaOH, AgOTf, MeOH, 0°C, then F-TEDA-BF_4, MS3Å, acetone, 0°C, R = Cl or I: NCS or NIS, MeCN, r.t., (iii) 4N HCl/EtOAc, $CHCl_3$, r.t.



Scheme 21. 試薬と反応条件: (i) $MeB(OH)_2$, $Pd(PPh_3)_4$, 1.0 M aq.K_2CO_3, 1,4-dioxane, reflux (ii) 10% HCl/MeOH, r.t.



Scheme 22. 試薬と反応条件: (i) *n*-BuLi, THF, -78°C, then DMA or acetone (ii) H_2 (0.4 MPa), 10% Pd-C, c.HCl, MeOH, r.t., (iii) 4N HCl/EtOAc, $CHCl_3$, r.t.



123, 126a-c, 128 and 131a-b　　93　　53 - 59

Scheme 23. 試薬と反応条件: (i) K_2CO_3, MeCN, reflux

3-8. 考察ならびに小括

SMP-304 の CYP2D6 単代謝といった課題と SERT 結合阻害活性の向上を目的とし、**SMP-304** の最適化研究を実施した。左側ピペリジン環の 4 位に置換したベンジル基のベンゼン環に導入した Br 基の置換位置の変換により、SERT 結合阻害活性が向上し、右側二環性部位の変換により CYP2D6 の代謝寄与率の高さが大幅に改善された。化合物 **46** の Br 基の各種ハロゲン基や低級アルキル基への変換結果が示すように、化合物 **46** やその類縁体の SERT への結合阻害活性には、ハロゲン結合の関与が考えられる。**SMP-304** の左側ピペリジン環の 4 位に置換したベンジル基のベンゼン環に導入した Br 基の置換位置の変換により SERT 結合阻害活性が向上したが、SERT とのハロゲン結合を介した相互作用に最適な置換位置へと変換されたことがその要因の一つと考えられる。また、**SMP-304** の右側ベンゾジオキサン環が代謝されるという知見のもと右側パーツを他の二環性部位に変換したところ、多くの化合物で CYP2D6 の代謝寄与率が低減する結果となった。代謝部位を変換するということは、電子密度や脂溶性などのファクターが変化するため、CYP2D6 や他の代謝酵素（CYP 種）による酸化代謝反応の起こりやすさやが変わることが予想され、本研究で実施した構造変換においても CYP2D6 による代謝速度が遅くなる、あるいは、他の CYP 種による代謝速度が速くなることにより、CYP2D6 の代謝寄与率が変化したものと考えられる。In silico シミュレーションや様々な代謝研究等を行うことで、CYP2D6 の代謝寄与率が改善した要因は明らかにできると思われる。より詳細な検討は今後の課題である。

本最適化研究の中で、SERT および 5-HT_{1A} に対し、その Ki 値が 10 倍の範囲内という良好なバランスで、かつ、一桁 nM という非常に強い結合阻害活性を示し、CYP2D6 の代謝寄与率が 60%以下であった化合物 **46**, **55** および **57** のセロトニン取り込み阻害（SRI）活性の評価を実施した。その結果、SRI 活性の強さは、SERT 結合阻害活性の強さと良く相関した。このことより、本研究結果は、SERT 結合阻害活性と SRI 活性は良く相関し、スループット性の高くない SRI 活性評価の代替法として SERT 結合阻害活性評価を使用できることを示す一例であると言える。

これら 3 化合物 **46**, **55** および **57** は、いずれも同様に 5-HT_{1A} 部分作動活性を示したことから、抗うつ作用に非常に重要と考えらえる前頭前皮質におけるセロトニン遊離量の上昇作用をラットマイクロダイアリシスにて評価した。その結果、SERT 結合阻害活性および SRI 活性の強さに従い、化合物 **46** が最も強いセロトニン遊離量の上昇作用を示したため、化合物 **46**（**DSP-1053**）を開発候補化合物として、ラット in vivo 評価にて、生体内での 5-HT_{1A} 自己受容体への拮抗作用の有無、および、目的とする

抗うつ作用の早いオンセットを示しうるかについて検討を行った。その結果、ラットマイクロダイアリシスにて、5-HT_{1A}アゴニストが示す結果とは異なり、ラット前頭前皮質にてセロトニン遊離量を上昇させドパミン遊離量には変化を与えないという結果を **DSP-1053** は示し、本化合物が生体内で 5-HT_{1A} 自己受容体拮抗作用を有することが示唆された。さらに、ラット強制水泳試験にて、**SMP-304** と同様に既存 SSRI であるパロキセチンよりも早い抗うつ様作用のオンセットを示し、期待通り SSRI の課題である抗うつ作用のオンセットの遅さを **DSP-1053** は改善した新規抗うつ薬となりうることが示された。ラットマイクロダイアリシスの結果はあくまで間接的な証拠であり、**DSP-1053** が生体内で 5-HT_{1A} 自己受容体拮抗作用を有することを証明するためには、より詳細な検討が必要であり、今後の課題である。

3-9. 実験の部

3-9-1. Synthesis

Melting points were determined on a Stanford Research System; OptiMelt MPA 100 without correction. NMR spectra were recorded at ambient temperature on a JEOL JNM-AL400 FT NMR spectrometer. Chemical shifts are expressed in δ values (ppm) relative to the internal standard tetramethylsilane, and signals are expressed as: s (singlet), d (doublet), t (triplet), m (multiplet) or br (broad). IR spectra were recorded on a JEOL JIR-SPX60 spectrometer as attenuated total reflection (ATR). High-resolution mass spectra (HRMS) were recorded on a Thermo Fisher Scientific LTQ orbitrap Discovery MS equipment. Elemental analysis was performed on a CE Instrument EA1110 and a Yokokawa analytical system IC7000. In general, reagents and solvents were used as obtained from commercial suppliers without further purification. Reaction progress was determined by thin layer chromatography (TLC) analysis on a Merck silica gel 60 F254 precoated glass plate. Visualization was done with UV light (254 nm) or iodine. Flash column chromatography was conducted using Merck silica gel 60 (70-230 mesh). All reactions were carried out under a nitrogen atmosphere unless otherwise mentioned.

3-9-1-1. (2-bromo-3-methoxybenzyl)(triphenyl)phosphonium bromide (**60**)

To a solution of 2-bromo-3-methoxybenzyl bromide (1.46 g, 5.25 mmol) in toluene (30 mL) was added triphenylphosphine (1.65 g, 6.30 mmol). After reflux for 7 h, the reaction mixture was cooled to room temperature and stirred at 0^{o}C for 20 min. The resulting solid was collected to give 2.70 g (95%) of the title compound as a white solid. ^{1}H NMR (300 MHz, DMSO-D_6) δ: 3.79 (3H, s), 5.16 (2H, d, J = 14.9 Hz), 6.71 (1H, brd, J = 7.3 Hz), 7.09 (1H, brd, J = 8.6 Hz), 7.26 (1H, dd, J = 7.9, 7.9 Hz), 7.54-7.64 (6H, m), 7.67-7.76 (6H, m), 7.91 (3H, brdd, J = 8.3, 8.3 Hz).

3-9-1-2. *tert*-butyl 4-(2-bromo-3-methoxybenzylidene)piperidine-1-carboxylate (**61**)

To a mixture of the phosphonium salt **60** (2.70 g, 4.98 mmol) and potassium carbonate (1.03 g,

7.47 mmol) in 2-PrOH (50 mL) was added 1-(*tert*-Butoxycarbonyl)-4-oxopiperidine (0.992 g, 4.98 mmol). After reflux for 10 h, the reaction mixture was concentrated. EtOAc (150 mL) was added to the residue, and the mixture was washed with H_2O (100 mL), dried over $MgSO_4$, filtered and concentrated. The residue was then purified by silica gel chromatography eluting with 17% EtOAc/hexane to give 1.80 g (94%) of the title compound as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.47 (9H, s), 2.27 (2H, t, J = 6.8 Hz), 2.36 (2H, t, J = 6.8 Hz), 3.39 (2H, t, J = 5.9 Hz), 3.53 (2H, t, J = 5.9 Hz), 3.91 (3H, s), 6.32 (1H, brs), 6.79 (2H, d, J = 7.9 Hz), 7.22 (1H, dd, J = 7.9, 7.9 Hz).

3-9-1-3. *tert*-butyl 4-(2-bromo-3-methoxybenzyl)piperidine-1-carboxylate (**62**)

The olefin intermediate **61** (1.90 g, 5.00 mmol) was dissolved in EtOH (50 mL) and EtOAc (50 mL) and hydrogenated over 5% Rh on carbon (0.760 g) at 45°C for 3 h. The catalyst was removed by filtration, and the filtrate was concentrated to give 1.74 g (91%) of the title compound as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.45 (9H, s), 1.14-1.29 (2H, m), 1.55-1.68 (2H, m), 1.74-1.87 (1H, m),2.57-2.69 (2H, m), 2.71 (2H, d, J = 7.2 Hz), 3.90 (3H, s), 4.00-4.18 (2H, m), 6.76-6.78 (2H, m), 7.18 (1H, dd, J = 7.9, 7.9 Hz).

3-9-1-4. 4-(2-bromo-3-methoxybenzyl)piperidine hydrochloride (**63**)

To a solution of intermediate **62** (1.96 g, 5.10 mmol) in AcOH (40 mL) was added 4 N HCl/1,4-dioxane (5.1 mL). After stirring at 50°C for 2 h, the reaction mixture was evaporated in vacuo. Heptane (10 mL) was added to the residue, and the mixture was concentrated. To the residue was added *i*-Pr_2O (50 mL), and the whole was stirred at 50°C for 20 min. The reaction mixture was cooled to room temperature and stirred at 0°C for 30 min. The resulting solid was collected to give 1.19 g (68%) of the title compound as a white solid. ^{1}H NMR (300 MHz, CD_3OD) δ: 1.50 (2H, brd, J = 11.6 Hz), 1.87 (2H, brd, J = 14.9 Hz), 1.93-2.09 (1H, m), 2.79 (2H, d, J = 7.2 Hz), 2.92 (2H, td, J = 12.8, 3.1 Hz), 3.29-3.40 (2H, m), 3.85 (3H, s), 6.86 (1H, dd, J = 7.7, 1.3 Hz), 6.90 (1H, dd, J = 8.4, 1.6 Hz), 7.24 (1H, dd, J = 7.7, 7.7 Hz).

3-9-1-5. 2-bromo-3-(piperidin-4-ylmethyl)phenol (**64**)

To a solution of intermediate **63** (1.19 g, 3.71 mmol) in CH_2Cl_2 (25 mL) was added dropwise 1N BBr_3 in CH_2Cl_2 solution (3.71 mL, 3.71 mmol) at 0°C for 10min. After stirring at room temperature for 20h, MeOH (10 mL) was added to the reaction mixture. The resulting mixture was stirred at room temperature for 30min and concentrated. Toluene (10 mL) was then added to the residue, and the whole was concentrated. To the residue was added 3.3N aqueous KOH (22.5 mL) and the reaction mixture was stirred at 50°C for 1 h. Next, phosphoric acid (2.92 g) was added, and the whole was stirred at 50°C for 30 min. The reaction mixture was then cooled to room temperature and stirred at 0°C for 30 min to give 0.893 g (89%) of the title compound as a white solid. ^{1}H NMR (300 MHz, DMSO-D_6) δ: 1.07 (2H, brd, J =7.9 Hz), 1.45 (2H, d, J = 13.0 Hz), 1.53-1.71 (1H, m), 2.33 (2H, t, J = 11.6 Hz), 2.56 (2H, d, J = 7.0

Hz), 2.86 (2H, d, J = 12.3 Hz), 3.50-3.99 (1H, m), 6.63 (1H, brd, J = 7.3 Hz), 6.75 (1H, brd, J = 7.3 Hz), 7.02 (1H, dd, J = 7.5, 7.5 Hz).

3-9-1-6. *tert*-butyl 4-(2-bromo-3-hydroxybenzyl)piperidine-1-carboxylate (**65**)

To a mixture of intermediate **64** (0.150 g, 0.555 mmol) in 1,4-dioxane (3.0 mL) and H_2O (3.0 mL) was added di-*tert*-butyl dicarbonate (0.128 g, 0.555 mmol). After stirring at room temperature for 3h, the reaction mixture was treated with H_2O (50 mL) and extracted with EtOAc (50 mL). The organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated to give 0.208 g (quant.) of the title compound as a white solid. ^{1}H NMR (300 MHz, DMSO-D_6) δ: 0.98-1.15 (2H, m), 1.37 (9H, s), 1.44-1.55 (2H, m), 1.64-1.80 (1H, m), 2.59 (2H, d, J = 7.0 Hz), 2.47-2.72 (2H, m), 3.89 (2H, brd, J = 12.2 Hz), 6.69 (1H, d, J = 7.5 Hz), 6.78 (1H, d, J = 7.9 Hz), 7.05 (1H, dd, J = 7.9, 7.9 Hz), 10.1 (1H, s).

3-9-1-7. *tert*-butyl 4-[2-bromo-3-(2-methoxyethoxy)benzyl]piperidine-1-carboxylate (**66**)

To a mixture of intermediate **65** (0.200 g, 0.540 mmol), potassium carbonate (0.149 g, 1.08 mmol) and potassium iodide (0.0897 g, 0.540 mmol) in DMF (5 mL) was added 2-bromoethyl methyl ether (0.113 g, 0.810 mmol). After stirring at 80°C for 5 h, the reaction mixture was treated with H_2O (100 mL) and extracted with EtOAc/toluene = 1/1 (100 mL). The organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by silica gel chromatography eluting with 16% EtOAc/hexane to give 0.203 g (88%) of the title compound as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.12-1.29 (2H, m), 1.45 (9H, s), 1.55-1.67 (2H, m), 1.71-1.90 (1H, m), 2.55-2.70 (2H, m), 2.70 (2H, d, J = 7.0 Hz), 3.50 (3H, s), 3.82 (2H, t, J = 5.1 Hz), 3.99-4.21 (2H, m), 4.16 (2H, t, J = 4.6 Hz), 6.77 (2H, d, J = 7.9 Hz), 7.15 (1H, dd, J = 7.7, 7.7 Hz).

3-9-1-8. 4-[2-bromo-3-(2-methoxyethoxy)benzyl]piperidine hydrochloride (**67**)

The title compound was prepared in a manner similar to that for the preparation of **63** using intermediate **66**. Compound **67** was obtained in 68% yield as a white solid. ^{1}H NMR (300 MHz, DMSO-D_6) δ: 1.41 (2H, brd, J = 11.4 Hz), 1.66 (2H, brd, J = 12.3 Hz), 1.77-1.92 (1H, m), 2.67 (2H, d, J = 7.2 Hz), 2.76 (2H, t, J = 13.4 Hz), 3.20 (2H, brd, J = 12.5 Hz), 3.33 (3H, s), 3.68 (2H, t, J = 4.6 Hz), 4.13 (2H, t, J = 4.2 Hz), 6.88 (1H, d, J = 7.9 Hz), 6.95 (1H, d, J = 8.3 Hz), 7.24 (1H, dd, J = 7.9, 7.9 Hz), 8.71 (2H, s).

3-9-1-9. 4-bromo-3-(2-methoxyethoxy)benzoic acid (**68**)

To a mixture of 2-methoxyethanol (16.5 g, 217 mmol) and potassium *tert*-butoxide (24.3 g, 217 mmol) in N-methyl-2-pyrrolidinone (175 mL) was added 4-bromo-3-fluorobenzoic acid (19.0 g, 86.8 mmol). After stirring at 90°C for 6 h, the reaction mixture was cooled to room temperature and added dropwise to a solution of conc.HCl (25 mL) in H_2O (500 mL) for 40 min. After stirring at room temperature for 1 h, the resulting solid was filtered, rinsed with

H_2O (20 mL x 2) and MeCN (20 mL x 2), and then collected. The white solid was crystallized using MeCN (380 mL) and stirred at room temperature for 1 h. The resulting solid was filtered, rinsed with MeCN (20 mL x 2) and collected to give 20.1 g (85%) of the title compound as a white solid. ^{1}H NMR (300 MHz, DMSO-D_6) δ: 3.33 (3H, s), 3.70 (2H, t, J = 4.2 Hz), 4.23 (2H, t, J = 4.2 Hz), 7.44 (1H, brd, J = 8.1 Hz), 7.54 (1H, brs), 7.70 (1H, d, J = 8.1 Hz), 13.2 (1H, brs).

3-9-1-10. [4-bromo-3-(2-methoxyethoxy)phenyl]methanol (**69**)

To a suspension of sodium borohydride (8.08 g, 214 mmol) in THF (100 mL) was added boron trifluoride diethyl etherate (35.0 mL, 285 mmol). After stirring at room temperature for 1 h, a solution of intermediate **68** (19.5 g, 71.2 mmol) in THF (300 mL) was added dropwise to the reaction mixture for 30 min, and the whole was stirred at room temperature for 3 h. H_2O (200 mL) was then added dropwise to the reaction mixture for 20 min, and the mixture was extracted with toluene (200 mL x 2). The combined organic layer was first washed with 3% aqueous $NaHCO_3$ (200 mL) and H_2O (200 mL), and then concentrated to give 18.2 g (98%) of the title compound as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.70 (1H, t, J = 5.9 Hz), 3.49 (3H, s), 3.82 (2H, t, J = 4.8 Hz), 4.20 (2H, t, J = 4.8 Hz), 4.66 (2H, d, J = 5.7 Hz), 6.83 (1H, brd, J = 8.1 Hz), 6.97 (1H, brs), 7.50 (1H, d, J = 8.1 Hz).

3-9-1-11. 1-bromo-4-(bromomethyl)-2-(2-methoxyethoxy)benzene (**70**)

To a mixture of intermediate **69** (18.0 g, 68.9 mmol), trimethylamine hydrochloride (0.467 g, 7.12 mmol) and triethylamine (19.8 mL, 142 mmol) in toluene (90 mL) was added dropwise methanesulfonyl chloride (8.56 g, 74.7 mmol) at 0°C for 30 min. After stirring at 0°C for 2 h, the reaction mixture was added to 5% aqueous $KHSO_4$ (180 mL) at 0°C. The organic layer was separated, and the water layer was extracted with toluene (90 mL). The combined organic layer was then washed with H_2O (180 mL) and concentrated. To a solution of the residue in THF (100 mL) was added lithium bromide (18.5 g, 214 mmol). After reflux for 1 h, H_2O (100 mL) was added to the reaction mixture, and the whole was extracted with toluene (100 mL x 2). The combined organic layer was washed with 5% aqueous $NaHCO_3$ (100 mL) and H_2O (100 mL) and then concentrated to give 19.5 g (88%) of the title compound as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.49 (3H, s), 3.82 (2H, t, J = 4.6 Hz), 4.20 (2H, t, J = 4.6 Hz), 4.43 (2H, s), 6.87 (1H, dd, J = 8.1, 1.7 Hz), 6.97 (1H, d, J = 1.8 Hz), 7.49 (1H, d, J = 8.3 Hz).

3-9-1-12. [4-bromo-3-(2-methoxyethoxy)benzyl](triphenyl)phosphonium bromide (**71**)

The title compound was prepared in a manner similar to that for the preparation of **60** using intermediate **70**. Compound **71** was obtained in 78% yield as a white solid. ^{1}H NMR (300 MHz, DMSO-D_6) δ: 3.27 (3H, s), 3.52 (2H, t, J = 5.0 Hz), 3.66 (2H, t, J = 4.6 Hz), 5.12 (2H, d, J = 15.8 Hz), 6.55 (1H, brd, J = 7.9 Hz), 6.59 (1H, brs), 7.47 (1H, d, J = 7.9 Hz), 7.60-7.81 (12H, m), 7.90 (3H, brdd, J = 7.7, 7.7 Hz).

3-9-1-13. *tert*-butyl 4-[4-bromo-3-(2-methoxyethoxy)benzylidene]piperidine-1-carboxylate (**72**)

The title compound was prepared in a manner similar to that for the preparation of **61** using intermediate **71**. Compound **72** was obtained quantitatively as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.48 (9H, s), 2.32 (2H, t, J = 5.6 Hz), 2.43 (2H, t, J = 6.7 Hz), 3.40 (2H, t, J = 5.9 Hz), 3.46-3.55 (5H, m), 3.81 (2H, t, J = 5.0 Hz), 4.16 (2H, t, J = 4.4 Hz), 6.28 (1H, brs), 6.68 (1H, dd, J = 8.1, 1.7 Hz), 6.74 (1H, d, J = 2.1 Hz), 7.46 (1H, d, J = 8.1 Hz).

3-9-1-14. *tert*-butyl 4-[4-bromo-3-(2-methoxyethoxy)benzyl]piperidine-1-carboxylate (**73**)

The title compound was prepared in a manner similar to that for the preparation of **62** using intermediate **72**. Compound **73** was obtained in 64% yield as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.04-1.20 (2H, m), 1.45 (9H, s), 1.51-1.70 (3H, m), 2.48 (2H, d, J = 6.6 Hz), 2.62 (2H, brt, J = 13.2 Hz), 3.49 (3H, s), 3.81 (2H, t, J = 5.3 Hz), 4.17 (2H, t, J = 4.6 Hz), 3.98-4.23 (2H, m), 6.63 (1H, dd, J = 7.9, 1.7 Hz), 6.70 (1H, d, J = 1.8 Hz), 7.42 (1H, d, J = 8.1 Hz).

3-9-1-15. 4-[4-bromo-3-(2-methoxyethoxy)benzyl]piperidine hydrochloride (**74**)

The title compound was prepared in a manner similar to that for the preparation of **63** using intermediate **73**. Compound **74** was obtained in 88% yield as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.47-1.94 (5H, m), 2.55 (2H, d, J = 5.5 Hz), 2.79 (2H, t, J = 12.0 Hz), 3.47 (2H, t, J = 13.0 Hz), 3.51 (3H, s), 3.81 (2H, t, J = 4.8 Hz), 4.15 (2H, t, J = 4.8 Hz), 6.62 (1H, dd, J = 7.9, 1.8 Hz), 6.68 (1H, d, J = 1.7 Hz), 7.43 (1H, d, J = 8.1 Hz), 9.50 (2H, brs); Mp: 171-172°C.

3-9-1-16. 1,3-dibromo-5-(2-methoxyethoxy)benzene (**75**)

To a mixture of 3,5-dibromophenol (37.7 g, 150 mmol) and potassium carbonate (41.4 g, 300 mmol) in DMF (150 mL) was added 2-bromoethyl methyl ether (31.2 g, 224 mmol). After stirring at 80°C for 9 h, H_2O (300 mL) was added to the reaction mixture and extracted with EtOAc/toluene = 1/1 (300 mL and 100 mL). The combined organic layer was washed with H_2O (50 mL x 2) and brine (50 mL), dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel chromatography using 5%-6% EtOAc/hexane as eluent to give 44.3 g (95%) of the title compound as a brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.44 (3H, s), 3.73 (2H, t, J = 4.6 Hz), 4.08 (2H, t, J = 4.7 Hz), 7.03 (2H, d, J = 1.7 Hz), 7.25 (1H, dd, J = 1.7, 1.7 Hz).

3-9-1-17. 3-bromo-5-(2-methoxyethoxy)benzaldehyde (**76**)

To a mixture of 1.6 M n-BuLi in n-hexane (126 mL, 198 mmol) and toluene (120 mL) was added dropwise 0.89 M n-BuMgCl in THF (116 mL, 100 mmol) at 0°C for 25 min. After stirring at 0°C for 30 min, a solution of intermediate **75** (46.1 g, 149 mmol) in toluene (420

mL) was added dropwise to the reaction mixture for 1 h, and the whole was stirred at 0°C for 2 h. DMF (28.7 mL, 373 mmol) was then added dropwise to the reaction mixture at 0°C for 40 min. After stirring at 0°C for 2 h, 2N aqueous HCl (300 mL) was added to the reaction mixture, the organic layer was separated, and then the water layer was extracted with toluene (100 mL). The combined organic layer was washed with H_2O (100 mL) and brine (100 mL), dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel chromatography using 9%-13% EtOAc/hexane as eluent to give 28.8 g (74%) of the title compound as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.46 (3H, s), 3.77 (2H, t, J = 4.6 Hz), 4.17 (2H, t, J = 4.6 Hz), 7.33-7.39 (2H, m), 7.59 (1H, dd, J = 1.5, 1.5 Hz), 9.90 (1H, s).

3-9-1-18. [3-bromo-5-(2-methoxyethoxy)phenyl]methanol (**77**)

To a solution of intermediate **76** (28.9 g, 112 mmol) in MeOH (112 mL) was added sodium borohydride (4.22 g, 112 mmol). After stirring at room temperature for 3 h, H_2O (200 mL) was added to the reaction mixture and MeOH was evaporated. The remaining mixture was then extracted with EtOAc (200 mL + 50 ml). The combined organic layer was washed with brine (50 ml), dried over Na_2SO_4, filtered and concentrated to give 28.7 g (98%) of the title compound as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.77 (1H, t, J = 6.1 Hz), 3.45 (3H, s), 3.74 (2H, t, J = 4.6 Hz), 4.10 (2H, t, J = 4.6 Hz), 4.64 (2H, d, J = 5.9 Hz), 6.88 (1H, brs), 7.00 (1H, dd, J = 2.0, 2.0 Hz), 7.10 (1H, brs).

3-9-1-19. 1-bromo-3-(bromomethyl)-5-(2-methoxyethoxy)benzene (**78**)

The title compound was prepared in a manner similar to that for the preparation of **70** using intermediate **77**. Compound **78** was obtained in 93% yield as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.45 (3H, s), 3.74 (2H, t, J = 4.6 Hz), 4.11 (2H, t, J = 4.6 Hz), 4.37 (2H, s), 6.90 (1H, dd, J = 1.8, 1.8 Hz), 7.01 (1H, dd, J = 2.0, 2.0 Hz), 7.13 (1H, dd, J = 1.6, 1.6 Hz).

3-9-1-20. [3-bromo-5-(2-methoxyethoxy)benzyl](triphenyl)phosphonium bromide (**79**)

The title compound was prepared in a manner similar to that for the preparation of **60** using intermediate **78**. Compound **79** was obtained in 87% yield as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.38 (3H, s), 3.63 (2H, t, J = 4.3 Hz), 3.94 (2H, t, J = 4.3 Hz), 5.43 (2H, d, J = 14.4 Hz), 6.39 (1H, brs), 6.99 (1H, dd, J = 4.1, 2.4 Hz), 7.24 (1H, brs), 7.61-7.71 (6H, m), 7.75-7.86 (9H, m).

3-9-1-21. *tert*-butyl 4-[3-bromo-5-(2-methoxyethoxy)benzylidene]piperidine-1-carboxylate (**80**)

The title compound was prepared in a manner similar to that for the preparation of **61** using intermediate **79**. Compound **80** was obtained in 95% yield as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.48 (9H, s), 2.31 (2H, t, J = 5.8 Hz), 2.42 (2H, t, J = 5.5 Hz), 3.40 (2H, t, J =

5.8 Hz), 3.45 (3H, s), 3.49 (2H, t, J = 5.9 Hz), 3.74 (2H, t, J = 4.7 Hz), 4.09 (2H, t, J = 4.6 Hz), 6.24 (1H, s), 6.69 (1H, dd, J = 1.7, 1.7 Hz), 6.91-6.96 (2H, m).

3-9-1-22. *tert*-butyl 4-[3-bromo-5-(2-methoxyethoxy)benzyl]piperidine-1-carboxylate (**81**)

The olefin intermediate **80** (34.6 g, 81.0 mmol) was dissolved in EtOAc (80 mL) and hydrogenated over 5% Rh on carbon (9.74 g) at room temperature for 26 h. The catalyst was removed by filtration, and the filtrate was concentrated to give 34.2 g (98%) of the title compound as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.02-1.20 (2H, m), 1.45 (9H, s), 1.53-1.72 (3H, m), 2.45 (2H, d, J = 6.8 Hz), 2.56-2.70 (2H, m), 3.45 (3H, s), 3.74 (2H, t, J = 4.7 Hz), 3.98-4.19 (4H, m), 6.65 (1H, dd, J = 1.9, 1.9 Hz), 6.89 (1H, dd, J = 1.5, 1.5 Hz), 6.91 (1H, dd, J = 1.9, 1.9 Hz).

3-9-1-23. 4-[3-bromo-5-(2-methoxyethoxy)benzyl]piperidine hydrochloride (**82**)

To a solution of intermediate **81** (34.2 g, 80.0 mmol) in MeOH (34 mL) was added 10 % HCl in MeOH (103 mL). After stirring at room temperature for 24 h, the reaction mixture was concentrated, and the residue was triturated with Et_2O (100 mL), filtered and collected. The solid was recrystallized using MeCN (132 mL) and stirred at 0°C for 1 h. The resulting solid was filtered, rinsed with MeCN (20 mL) and collected to give 22.6 g (85%) of the title compound as a white solid. ^{1}H NMR (300 MHz, DMSO-D_6) δ: 1.25-1.40 (2H, m), 1.67 (2H, d, J = 14.0 Hz), 1.72-1.85 (1H, m), 2.49 (2H, d, J = 6.8 Hz), 2.77 (2H, td, J = 12.0, 2.4 Hz), 3.20 (2H, brd, J = 12.6 Hz), 3.34 (3H, s), 3.63 (2H, t, J = 4.5 Hz), 4.09 (2H, t, J = 4.5 Hz), 6.80 (1H, dd, J = 1.7, 1.7 Hz), 6.96-6.99 (2H, m); Mp: 107-108°C.

3-9-1-24. 4-[2-bromo-3-(2-methoxyethoxy)benzyl]-1-[2-(7-chloro-2,3-dihydro-1,4-benzo-dioxin-6-yl)ethyl]piperidine (**39**)

To a mixture of the benzyl piperidine intermediate **67** (50 mg, 0.137 mmol) and potassium carbonate (41.7 mg, 0.302 mmol) in MeCN (1.0 mL) was added intermediate **38b** (50.6 mg, 0.137 mmol). After reflux for 5 h, EtOAc (10 mL) was added to the reaction mixture and the whole was filtered. The filtrate was evaporated in vacuo, and the residue was purified by silica gel chromatography using 0%-5% MeOH/$CHCl_3$ as eluent to give 60.8 mg (84%) of the title compound as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.15-1.31 (2H, m), 1.45-1.64 (3H, m), 1.80-1.91 (2H, m), 2.34-2.43 (2H, m), 2.60-2.74 (4H, m), 2.81-2.92 (2H, m), 3.33 (3H, s), 3.68 (2H, t, J = 4.6 Hz), 4.13 (2H, t, J = 4.8 Hz), 4.20 (4H, s), 6.84 (1H, s), 6.85 (1H, d, J = 6.8 Hz), 6.88 (1H, s), 6.92 (1H, d, J = 8.4 Hz), 7.21 (1H, dd, J =8.1, 8.1 Hz); HRMS (ESI) m/z calcd for $C_{25}H_{32}BrClNO_4$ $[M+H]^+$ 524.1198; found 524.1204.

3-9-1-25. 4-[4-bromo-3-(2-methoxyethoxy)benzyl]-1-[2-(7-chloro-2,3-dihydro-1,4-benzo-dioxin-6-yl)ethyl]piperidine (**40**)

The title compound was prepared in a manner similar to that for the preparation of **39** using

intermediates **74** and **38b**. Compound **40** was obtained in 95% yield as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.23-1.42 (2H, m), 1.43-1.56 (1H, m), 1.63-1.70 (2H, m), 1.90-2.04 (2H, m), 2.44-2.58 (4H, m), 2.74-2.86 (2H, m), 2.92-3.07 (2H, m), 3.49 (3H, s), 3.81 (2H, t, J = 4.9 Hz), 4.17 (2H, t, J = 4.9 Hz), 4.22 (4H, s), 6.64 (1H, dd, J = 8.0, 1.8 Hz), 6.71 (1H, d, J = 1.8 Hz), 6.73 (1H, s), 6.85 (1H, s), 7.41 (1H, d, J = 8.0 Hz); HRMS (ESI) m/z calcd for $C_{25}H_{32}BrClNO_4$ $[M+H]^+$ 524.1198; found 524.1203.

3-9-1-26. 4-[3-bromo-5-(2-methoxyethoxy)benzyl]-1-[2-(7-chloro-2,3-dihydro-1,4-benzo-dioxin-6-yl)ethyl]piperidine (**41**)

The title compound was prepared in a manner similar to that for the preparation of **39** using intermediates **82** and **38b**. Compound **41** was obtained in 86% yield as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.21-1.40 (2H, m), 1.42-1.56 (1H, m), 1.60-1.69 (2H, m), 1.91-2.05 (2H, m), 2.42-2.56 (4H, m), 2.75-2.85 (2H, m), 2.92-3.04 (2H, m), 3.45 (3H, s), 3.74 (2H, t, J = 4.7 Hz), 4.08 (2H, t, J = 4.7 Hz), 4.22 (4H, s), 6.66 (1H, dd, J = 1.8, 1.8 Hz), 6.73 (1H, s), 6.85 (1H, s), 6.90 (2H, d, J = 1.8 Hz); HRMS (ESI) m/z calcd for $C_{25}H_{32}BrClNO_4$ $[M+H]^+$ 524.1198; found 524.1204.

3-9-1-27. 4-[4-bromo-3-(2-methoxyethoxy)benzyl]-1-[2-(2,3-dihydro-1,4-benzo-dioxin-6-yl)ethyl]piperidine (**42**)

The title compound was prepared in a manner similar to that for the preparation of **39** using intermediates **74** and **37b**. Compound **42** was obtained in 93% yield as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.23-1.40 (2H, m), 1.42-1.55 (1H, m), 1.62-1.70 (2H, m), 1.86-1.99 (2H, m), 2.44-2.57 (4H, m), 2.64-2.74 (2H, m), 2.92-3.02 (2H, m), 3.50 (3H, s), 3.81 (2H, t, J = 4.9 Hz), 4.17 (2H, t, J = 4.9 Hz), 4.23 (4H, s), 6.62-6.68 (2H, m), 6.69-6.72 (2H, m), 6.77 (1H, d, J = 8.3 Hz), 7.41 (1H, d, J = 8.1 Hz); HRMS (ESI) m/z calcd for $C_{25}H_{33}BrNO_4$ $[M+H]^+$ 490.1587; found 490.1591.

3-9-1-28. 4-[3-bromo-5-(2-methoxyethoxy)benzyl]-1-[2-(2,3-dihydro-1,4-benzo-dioxin-6-yl)ethyl]piperidine (**43**)

The title compound was prepared in a manner similar to that for the preparation of **39** using intermediates **82** and **37b**. Compound **43** was obtained in 76% yield as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.23-1.40 (2H, m), 1.43-1.56 (1H, m), 1.64-1.70 (2H, m), 1.87-2.00 (2H, m), 2.46 (2H, d, J = 7.0 Hz), 2.49-2.58 (2H, m), 2.65-2.75 (2H, m), 2.91-3.03 (2H, m), 3.45 (3H, s), 3.74 (2H, t, J = 4.6 Hz), 4.08 (2H, t, J = 4.6 Hz), 4.23 (4H, s), 6.63-6.68 (2H, m), 6.70 (1H, d, J = 1.8 Hz), 6.77 (1H, d, J = 8.1 Hz), 6.90 (2H, d, J = 1.8 Hz); HRMS (ESI) m/z calcd for $C_{25}H_{33}BrNO_4$ $[M+H]^+$ 490.1587; found 490.1596.

3-9-1-29. methyl [4-(3-hydroxypropoxy)phenyl]acetate (**83**)

To a mixture of methyl 4-hydroxyphenylacetate (26.6 g, 160 mmol) and potassium carbonate

(26.5 g, 192 mmol) in MeCN (500 mL) was added 3-bromo-1-propanol (17.4 mL, 192 mmol). After reflux for 14 h, the reaction mixture was filtered, and the filtrate was concentrated. The residue was purified by silica gel chromatography using 34% EtOAc/hexane as eluent to give 23.7 g (66%) of the title compound as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.75 (1H, t, J = 5.5 Hz), 2.00-2.09 (2H, m), 3.57 (2H, s), 3.68 (3H, s), 3.87 (2H, td, J = 5.5, 5.5 Hz), 4.12 (2H, t, J = 5.9 Hz), 6.84-6.90 (2H, m), 7.16-7.22 (2H, m).

3-9-1-30. 3-[4-(2-methoxy-2-oxoethyl)phenoxy]propanoic acid (**84**)

To a mixture of PDC (19.8 g, 52.6 mmol) and MS3Å (18 g) in DMF (60 mL) was added dropwise a solution of intermediate **83** (3.37 g, 15.0 mmol) in DMF (30 mL). After stirring at room temperature for 20 h, Et_2O (600 mL) and celite® (20 g) were added to the reaction mixture. After stirring at room temperature for 10 min, the reaction mixture was filtered, and the filtrate was concentrated. 10% aqueous $NaHCO_3$ (150 mL) was added to the residue, and the aqueous layer was washed with EtOAc (200 mL). 2N aqueous HCl was added to the aqueous layer to adjust pH of the solution to 2 and extracted with EtOAc (400 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated to give 2.35 g (66%) of the title compound as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.85 (2H, t, J = 6.3 Hz), 3.57 (2H, s), 3.69 (3H, s), 4.24 (2H, t, J = 6.3 Hz), 6.87 (2H, d, J = 8.5 Hz), 7.20 (2H, d, J = 8.5 Hz).

3-9-1-31. methyl (4-oxo-3,4-dihydro-2H-chromen-6-yl)acetate (**85**)

A mixture of intermediate **84** (22.4 g, 94.0 mmol) and polyphosphoric acid (112 g) was stirred at 80°C for 2 h. Toluene (300 mL) and H_2O (1000 mL) were then added to the reaction mixture, and the whole was extracted with toluene (500 mL) and Et_2O (500 mL). The combined organic layer was dried over $MgSO_4$, filtered and concentrated, and the residue was purified by silica gel chromatography using 25% EtOAc/hexane as eluent to give 19.0 g (92%) of the title compound as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.81 (2H, t, J = 6.5 Hz), 3.60 (2H, s), 3.70 (3H, s), 4.53 (2H, t, J = 6.5 Hz), 6.96 (1H, d, J = 8.5 Hz), 7.41 (1H, dd, J = 8.5, 2.2 Hz), 7.78 (1H, d, J = 2.2 Hz).

3-9-1-32. methyl (4-hydroxy-3,4-dihydro-2H-chromen-6-yl)acetate (**86**)

To a solution of intermediate **85** (4.00 g, 18.2 mmol) in MeOH (90 mL) was added sodium borohydride (0.757 g, 20.0 mmol) at 0°C. After stirring at room temperature for 4 h, the reaction mixture was added to sat. aqueous NH_4Cl (300 mL), and MeOH was evaporated. The remaining mixture was extracted with EtOAc (500 mL), and the organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by silica gel chromatography using 50% EtOAc/hexane as eluent to give 3.48 g (86%) of the title compound as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.90 (1H, t, J = 4.2 Hz), 2.19 (2H, m), 3.56 (2H, s), 3.69 (3H, s), 4.23-4.30 (2H, m), 4.78 (1H, td, J = 4.2, 4.2 Hz), 6.81 (1H, d, J = 8.4 Hz), 7.12

(1H, dd, J = 8.4, 2.2 Hz), 7.23 (1H, d, J = 2.2 Hz).

3-9-1-33. methyl 2H-chromen-6-ylacetate (**87**)

To a solution of intermediate **86** (3.43 g, 15.4 mmol) in benzene (75 mL) was added p-toluenesulfonic acid (0.0793 g, 0.417 mmol). The mixture was refluxed for 2.5 h during which water was removed azeotropically through a dean-stark. The reaction mixture was then added to sat. aqueous $NaHCO_3$ (300 mL) and extracted with EtOAc (300 mL x 2). The combined organic layer was dried over $MgSO_4$, filtered and concentrated, and the residue was purified by silica gel chromatography using 16% EtOAc/hexane as eluent to give 2.81 g (89%) of the title compound as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.52 (2H, s), 3.69 (3H, s), 4.79-4.82 (2H, m), 5.78 (1H, td, J = 9.7, 3.7 Hz), 6.39 (1H, td, J = 9.7, 1.5 Hz), 6.72 (1H, d, J = 8.3 Hz), 6.88 (1H, d, J = 2.3 Hz), 6.99 (1H, dd, J = 8.3, 2.2 Hz).

3-9-1-34. methyl 3,4-dihydro-2H-chromen-6-ylacetate (**88**)

Intermediate **87** (2.00 g, 9.79 mmol) was dissolved in EtOAc (40 mL) and hydrogenated over 10% Pd on carbon (water ~50%, 0.700 g) at room temperature for 3 h. The catalyst was removed by filtration, and the filtrate was concentrated to give 2.03 g (quant.) of the title compound as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.94-2.04 (2H, m), 2.77 (2H, t, J = 6.6 Hz), 3.52 (2H, s), 3.69 (3H, s), 4.17 (2H, t, J = 5.1 Hz), 6.74 (1H, d, J = 8.1 Hz), 6.93-7.01 (2H, m).

3-9-1-35. 2-(3,4-dihydro-2H-chromen-6-yl)ethanol (**89**)

To a suspension of lithium aluminum hydride (0.558 g, 14.7 mmol) in THF (30 mL) was added dropwise a solution of intermediate **88** (2.02 g, 9.79 mmol) in THF (10 mL). After stirring at room temperature for 1.5 h, water (0.55 mL), 15% aqueous NaOH (0.55 mL) and water (1.7 mL) were added dropwise to the reaction mixture at 0°C. After stirring at 0°C for 30 min, the reaction mixture was filtered, and the filtrate was concentrated to give 1.68 g (97%) of the title compound as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.39 (1H, t, J = 6.0 Hz), 1.95-2.04 (2H, m), 2.74-2.79 (4H, m), 3.82 (2H, td, J = 6.0, 6.0 Hz), 4.17 (2H, t, J = 5.1 Hz), 6.74 (1H, d, J = 8.3 Hz), 6.90 (1H, d, J = 2.0 Hz), 6.94 (1H, dd, J = 8.3, 2.0 Hz).

3-9-1-36. 2-(3,4-dihydro-2H-chromen-6-yl)ethyl 4-methylbenzenesulfonate (**90**)

To a mixture of intermediate **89** (1.18 g, 6.62 mmol), triethlyamine (1.85 mL, 13.2 mmol) and trimethylamine hydrochloride (0.0633 g, 0.662 mmol) in CH_2Cl_2 (30 mL) was added p-toluenesulfonyl chloride (1.89 g, 9.93 mmol) at 0°C. After stirring at 0°C for 1 h, H_2O (50 mL) was added, and the whole was extracted with $CHCl_3$ (50 mL x 2). The combined organic layer was dried over $MgSO_4$, filtered and concentrated, and the residue was purified by silica gel chromatography using 12~25% EtOAc/hexane as eluent to give 1.90 g (86%) of the title compound as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.94-2.02 (2H, m), 2.44 (3H, s),

2.71 (2H, t, J = 6.4 Hz), 2.84 (2H, t, J = 7.2 Hz), 4.08-4.18 (4H, m), 6.66 (1H, d, J = 8.3 Hz), 6.76-6.82 (2H, m), 7.29 (2H, d, J = 8.1 Hz), 7.70 (2H, d, J = 8.1 Hz).

3-9-1-37. methyl (4,4-dimethoxy-3,4-dihydro-2H-chromen-6-yl)acetate (**91**)

To a mixture of intermediate **85** (10.0 g, 45.4 mmol), trimethyl orthoformate (199 mL) and MeOH (106 mL) was added p-toluenesulfonic acid monohydrate (0.864 g, 4.54 mmol). After stirring at room temperature for 22 h, the reaction mixture was added to sat. aqueous $NaHCO_3$ (500 mL) and extracted with EtOAc (500 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated to give 12.1 g (quant.) of the title compound as a pale yellow oil. ^{1}H NMR (300 MHz, CD_3OD) δ: 2.08-2.14 (2H, m), 3.24 (6H, s), 3.56 (2H, s), 3.65 (3H, s), 4.25-4.32 (2H, m), 6.73 (1H, d, J = 8.4 Hz), 7.10 (1H, dd, J = 8.4, 2.1 Hz), 7.41 (1H, d, J = 2.1 Hz).

3-9-1-38. 6-(2-hydroxyethyl)-2,3-dihydro-4H-chromen-4-one (**92**)

To a suspension of lithium aluminum hydrate (2.58 g, 68.1 mmol) in THF (190 mL) was added dropwise a solution of intermediate **91** (12.1 g, 45.4 mmol) for 20 min. After stirring at room temperature for 1 h, H_2O (2.54 mL), 15% aqueous NaOH (2.54 mL) and H_2O (7.63 mL) was added to the reaction mixture at 0°C. After stirring at 0°C for 1 h, the reaction mixture was filtered, and the filtrate was concentrated. To a solution of the residue (10.7 g) in acetone (100 mL) was added 2N aqueous HCl (100 mL). After stirring at room temperature for 1 h, acetone was evaporated from the reaction mixture, and the remaining mixture was extracted with EtOAc (500 mL). The organic layer was washed with H_2O (400 mL), and the aqueous layers were combined and extracted with EtOAc (300 mL). The combined organic layer was dried over $MgSO_4$, filtered and concentrated to give 8.55 g (98%) of the title compound as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.78-2.86 (4H, m), 3.86 (2H, t, J = 6.4 Hz), 4.52 (2H, t, J = 6.4 Hz), 6.94 (1H, d, J = 8.4 Hz), 7.36 (1H, dd, J = 8.4, 2.2 Hz), 7.76 (1H, d, J = 2.2 Hz).

3-9-1-39. 2-(4-oxo-3,4-dihydro-2H-chromen-6-yl)ethyl 4-methylbenzenesulfonate (**93**)

The title compound was prepared in a manner similar to that for the preparation of **90** using intermediate **92**. Compound **93** was obtained in 89% yield as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.44 (3H, s), 2.79 (2H, t, J = 6.5 Hz), 2.91 (2H, t, J = 6.9 Hz), 4.18 (2H, t, J = 6.9 Hz), 4.51 (2H, t, J = 6.4 Hz), 6.88 (1H, d, J = 8.4 Hz), 7.26 (1H, dd, J = 8.5, 2.5 Hz), 7.30 (2H, d, J = 8.5 Hz), 7.60 (1H, d, J = 2.2 Hz), 7.71 (2H, d, J = 8.3 Hz).

3-9-1-40. 2-(4-hydroxy-3,4-dihydro-2H-chromen-6-yl)ethyl 4-methylbenzenesulfonate (**94**)

To a solution of intermediate **93** (0.500 g, 1.44 mmol) in THF (4.0 mL) and MeOH (7.0 mL) was added sodium borohydride (0.0546 g, 1.44 mmol) at 0°C. After stirring at room temperature for 1 h, H_2O (50 mL) was added to the reaction mixture at 0°C, and the whole

was extracted with EtOAc (50 mL x 2). The combined organic layer was dried over $MgSO_4$, filtered and concentrated to give 0.517 g (quant.) of the title compound as a colorless oil. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.79 (1H, d, J = 5.0 Hz), 1.94-2.19 (2H, m), 2.44 (3H, s), 2.89 (2H, t, J = 7.1 Hz), 4.18 (2H, t, J = 7.1 Hz), 4.25 (2H, dd, J = 7.5, 3.7 Hz), 4.73 (1H, td, J = 4.3, 4.3 Hz), 6.73 (1H, d, J = 8.4 Hz), 6.95 (1H, dd, J = 8.4, 2.2 Hz), 7.07 (1H, d, J = 2.2 Hz), 7.30 (2H, d, J = 8.4 Hz), 7.70 (2H, d, J = 8.4 Hz).

3-9-1-41. methyl [3-(3-hydroxypropoxy)phenyl]acetate (**95**)

The title compound was prepared in a manner similar to that for the preparation of **83** using methyl 2-(3-hydroxyphenyl) acetate. Compound **95** was obtained in 62% yield as a colorless oil. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.75 (1H, t, J = 5.7 Hz), 2.00-2.09 (2H, m), 3.60 (2H, s), 3.70 (3H, s), 3.87 (2H, td, J = 5.7, 5.7 Hz), 4.13 (2H, t, J = 5.7 Hz), 6.79-6.90 (3H, m), 7.24 (1H, d, J = 7.3 Hz).

3-9-1-42. 3-[3-(2-methoxy-2-oxoethyl)phenoxy]propanoic acid (**96**)

The title compound was prepared in a manner similar to that for the preparation of **84** using intermediate **95**. Compound **96** was obtained in 67% yield as a white solid. ^1H NMR (300 MHz, $CDCl_3$) δ: 2.85 (2H, t, J = 6.2 Hz), 3.60 (2H, s), 3.69 (3H, s), 4.25 (2H, t, J = 6.2 Hz), 6.79-6.90 (3H, m), 7.24 (1H, d, J = 7.9 Hz).

3-9-1-43. methyl (4-oxo-3,4-dihydro-2H-chromen-7-yl)acetate (**97**)

The title compound was prepared in a manner similar to that for the preparation of **85** using intermediate **96**. Compound **97** was obtained in 71% yield as a pale yellow oil. ^1H NMR (300 MHz, $CDCl_3$) δ: 2.80 (2H, t, J = 6.4 Hz), 3.59 (2H, s), 3.69 (3H, s), 4.53 (2H, t, J = 6.4 Hz), 6.95 (1H, d, J = 8.6 Hz), 7.41 (1H, dd, J= 8.4, 2.4 Hz), 7.78 (1H, d, J = 2.4 Hz).

3-9-1-44. methyl (4-hydroxy-3,4-dihydro-2H-chromen-7-yl)acetate (**98**)

The title compound was prepared in a manner similar to that for the preparation of **86** using intermediate **97**. Compound **98** was obtained in 81% yield as a pale yellow oil. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.81 (1H, d, J = 5.0 Hz), 1.98-2.17 (2H, m), 3.56 (2H, s), 3.69 (3H, s), 4.22-4.31 (2H, m), 4.78 (1H, td, J = 4.4, 4.4 Hz), 6.77 (1H, d, J = 1.8 Hz), 6.84 (1H, dd, J = 7.7, 1.7 Hz), 7.27 (1H, d, J = 7.9 Hz).

3-9-1-45. methyl 2H-chromen-7-ylacetate (**99**)

The title compound was prepared in a manner similar to that for the preparation of **87** using intermediate **98**. Compound **99** was obtained in 95% yield as a colorless oil. ^1H NMR (300 MHz, $CDCl_3$) δ: 3.54 (2H, s), 3.69 (3H, s), 4.81 (2H, dd, J = 1.8, 1.8 Hz), 5.75 (1H, td, J = 9.7, 3.7 Hz), 6.40 (1h, td, J = 9.9, 1.4 Hz), 6.69 (1H, d, J = 1.7 Hz), 6.76 (1H, dd, J = 7.7, 1.8 Hz), 6.90 (1H, d, J = 7.5 Hz).

3-9-1-46. methyl 3,4-dihydro-2H-chromen-7-ylacetate (**100**)

The title compound was prepared in a manner similar to that for the preparation of **88** using intermediate **99**. Compound **100** was obtained in 77% yield as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.96-2.05 (2H, m), 2.76 (2H, t, J = 6.8 Hz), 3.54 (2H, s), 3.68 (3H, s), 4.17 (2H, t, J = 5.2 Hz), 6.71 (1H, d, J = 1.2 Hz), 6.75 (1H, dd, J = 7.6, 1.6 Hz), 6.98 (1H, d, J = 7.6 Hz).

3-9-1-47. 2-(3,4-dihydro-2H-chromen-7-yl)ethanol (**101**)

The title compound was prepared in a manner similar to that for the preparation of **89** using intermediate **100**. Compound **101** was obtained in 95% yield as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.39 (1H, t, J = 6.0 Hz), 1.96-2.03 (2H, m), 2.74-2.81 (4H, m), 3.83 (2H, td, J = 6.0, 6.0 Hz), 4.17 (2H, t, J = 5.2 Hz), 6.67 (1H, d, J = 1.6 Hz), 6.71 (1H, dd, J = 7.6, 1.6 Hz), 6.98 (1H, d, J= 7.6 Hz).

3-9-1-48. 2-(3,4-dihydro-2H-chromen-7-yl)ethyl 4-methylbenzenesulfonate (**102**)

The title compound was prepared in a manner similar to that for the preparation of **90** using intermediate **101**. Compound **102** was obtained in quant. yield as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.96-2.00 (2H, m), 2.44 (3H, s), 2.74 (2H, t, J = 6.8 Hz), 2.86 (2H, t, J = 7.2 Hz), 4.11-4.18 (4H, m), 6.52 (1H, d, J = 1.6 Hz), 6.59 (1H, dd, J = 7.6, 1.6 Hz), 6.91 (1H, d, J = 7.6 Hz), 7.30 (2H, d, J = 8.0 Hz), 7.72 (2H, d, J = 8.4 Hz).

3-9-1-49. methyl (4,4-dimethoxy-3,4-dihydro-2H-chromen-7-yl)acetate (**103**)

The title compound was prepared in a manner similar to that for the preparation of **91** using intermediate **97**. Compound **103** was obtained quantitatively as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.11-2.16 (2H, m), 3.26 (6H, s), 3.56 (2H, s), 3.68 (3H, s), 4.31-4.36 (2H, m), 6.76 (1H, d, J = 2.2 Hz), 6.81 (1H, dd, J = 7.9, 1.7 Hz), 7.47 (1H, d, J = 8.0 Hz).

3-9-1-50. 7-(2-hydroxyethyl)-2,3-dihydro-4H-chromen-4-one (**104**)

The title compound was prepared in a manner similar to that for the preparation of **92** using intermediate **103**. Compound **104** was obtained in 77% yield as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.46 (1H, brs), 2.79 (2H, t, J = 6.4 Hz), 2.87 (2H, t, J = 6.4 Hz), 3.90 (2H, td, J = 5.2, 5.2 Hz), 4.53 (2H, t, J = 6.4 Hz), 6.86 (1H, d, J = 0.8 Hz), 6.90 (1H, dd, J = 8.0, 1.6 Hz), 7.84 (1H, d, J = 8.0 Hz).

3-9-1-51. 2-(4-oxo-3,4-dihydro-2H-chromen-7-yl)ethyl 4-methylbenzenesulfonate (**105**)

The title compound was prepared in a manner similar to that for the preparation of **93** using intermediate **104**. Compound **105** was obtained in 90% yield as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.44 (3H, s), 2.79 (2H, t, J = 6.4 Hz), 2.94 (2H, t, J = 6.8 Hz), 4.23 (2H, t, J = 6.8 Hz), 4.51 (2H, t, J = 6.4 Hz), 6.71-6.77 (2H, m), 7.30 (2H, d, J = 8.0 Hz), 7.71 (2H,

d, J = 8.4 Hz), 7.76 (1H, d, J = 8.0 Hz).

3-9-1-52. ethyl 4-(4-acetylphenyl)butanoate (**106**)

To a mixture of aluminum chloride (38.6 g, 289 mmol) and acetyl chloride (10.3 mL, 145 mmol) in CH_2Cl_2 (200 mL) was added dropwise a solution of ethyl 4-phenylbutyrate (13.9 g, 72.3 mmol) in CH_2Cl_2 (40 mL) at room temperature for 20 min. After stirring at room temperature for 1 h, the reaction mixture was added to ice-water (1000 g) and extracted with $CHCl_3$ (1000 mL). The organic layer was washed with sat. aqueous $NaHCO_3$ (300 mL), dried over $MgSO_4$, filtered and concentrated. The residue was purified by silica gel chromatography using 14% EtOAc/hexane as eluent to give 16.5 g (98%) of the title compound as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.25 (3H, t, J = 7.3 Hz), 1.97 (2H, tt, J = 7.5, 7.5 Hz), 2.32 (2H, t, J = 7.5 Hz), 2.59 (3H, s), 2.71 (2H, t, J = 7.4 Hz), 4.13 (2H, q, J = 7.3 Hz), 7.27 (2H, d, J = 8.1 Hz), 7.89 (2H, d, J = 8.1 Hz).

3-9-1-53. 4-[4-(carboxymethyl)phenyl]butanoic acid (**107**)

A mixture of intermediate **106** (18.5 g, 79.0 mmol), morpholine (24.1 mL, 276 mmol) and sulfur (8.86 g) was first refluxed for 2.5 h, and then concentrated. To a solution of the residue in 2-PrOH (62 mL) was added 15% aqueous NaOH (185 mL), and the whole was refluxed for 8 h. 2-PrOH was then evaporated, and 6N aqueous HCl (180 ml) was added to the remaining reaction mixture. The resulting solid was filtered and dissolved in Acetone (400 mL), and the insoluble matter was removed by filtration. The filtrate was concentrated to give 15.3 g (87%) of the title compound as a pale yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.76 (2H, tt, J = 7.7, 7.7 Hz), 2.19 (2H, t, J = 7.3 Hz), 2.54 (2H, t, J = 7.9 Hz), 3.50 (2H, s), 7.11 (2H, d, J = 8.3 Hz), 7.15 (2H, d, J = 8.1 Hz).

3-9-1-54. (8-oxo-5,6,7,8-tetrahydronaphthalen-2-yl)acetic acid (**108**)

The title compound was prepared in a manner similar to that for the preparation of **85** using intermediate **107**. Compound **108** was obtained in 77% yield as a pale yellow solid. ^{1}H NMR (300 MHz, DMSO-D_6) δ: 1.96-2.08 (2H, m), 2.58 (2H, t, J = 6.4 Hz), 2.91 (2H, t, J = 6.0 Hz), 3.61 (2H, s), 7.28 (1H, d, J = 8.1 Hz), 7.42 (1H, dd, J = 7.9, 2.0 Hz), 7.74 (1H, d, J = 1.8 Hz), 12.37 (1H, s).

3-9-1-55. 7-(2-hydroxyethyl)-1,2,3,4-tetrahydronaphthalen-1-ol (**109**)

To a suspension of lithium aluminum hydride (5.98 g, 158 mmol) in THF (300 mL) was added dropwise a solution of intermediate **108** (9.20 g, 45.0 mmol) in THF (150 mL) at 65°C for 30 min. After reflux for 1 h, water (5.9 mL), 15% aqueous NaOH (5.9 mL) and water (17.7 mL) were added dropwise to the reaction mixture at 0°C. After stirring at 0°C for 30 min, the reaction mixture was filtered, and the filtrate was concentrated to give 8.37 g (97%) of the title compound as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.45 (1H, brs),

1.68-1.82 (2H, m), 1.84-2.04 (3H, m), 2.61-2.90 (4H, m), 3.81-3.91 (2H, m), 4.73-4.81 (1H, m), 7.03-7.11 (2H, m), 7.31 (1H, brs).

3-9-1-56. 7-(2-hydroxyethyl)-3,4-dihydronaphthalen-1(2H)-one (**110**)

To a solution of intermediate **109** (7.00 g, 36.4 mmol) in CH_2Cl_2 (100 mL) was added manganese dioxide (15.8 g, 182 mmol). After stirring at room temperature for 6 h, more manganese dioxide (15.8 g, 182 mmol) was added, and the whole was stirred at room temperature for 15 h. The reaction mixture was then filtered, and the filtrate was concentrated. The residue was purified by silica gel chromatography using 33~50% EtOAc/hexane as eluent to give 4.44 g (64%) of the title compound as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.08-2.19 (2H, m), 2.65 (2H, t, J = 6.6 Hz), 2.89 (2H, t, J = 6.5 Hz), 2.95 (2H, t, J = 6.1 Hz), 3.88 (2H, t, J = 6.5 Hz), 7.22 (1H, d, J = 7.9 Hz), 7.36 (1H, dd, J = 7.9, 2.0 Hz), 7.90 (1H, d, J = 1.8 Hz).

3-9-1-57. 2-(8-oxo-5,6,7,8-tetrahydronaphthalen-2-yl)ethyl 4-methylbenzenesulfonate (**111**)

The title compound was prepared in a manner similar to that for the preparation of **90** using intermediate **110**. Compound **111** was obtained in 99% yield as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.07-2.18 (2H, m), 2.44 (3H, s), 2.64 (2H, t, J = 6.5 Hz), 2.89-3.01 (4H, m), 4.20 (2H, t, J = 7.0 Hz), 7.17 (1H, d, J = 7.9 Hz), 7.27-7.33 (3H, m), 7.71 (2H, d, J = 8.4 Hz), 7.74 (1H, d, J = 1.8 Hz).

3-9-1-58. methyl 4-[4-(2-methoxy-2-oxoethyl)phenoxy]butanoate (**112**)

The title compound was prepared in a manner similar to that for the preparation of **83** using methyl 4-bromobutyrate. Compound **112** was obtained in 92% yield as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.05-2.15 (2H, m), 2.53 (2H, t, J = 7.3 Hz), 3.56 (2H, s), 3.68 (3H, s), 3.69 (3H, s), 3.99 (2H, t, J = 6.1 Hz), 6.84 (2H, d, J = 8.6 Hz), 7.18 (2H, d, J = 8.8 Hz).

3-9-1-59. 4-[4-(carboxymethyl)phenoxy]butanoic acid (**113**)

To a solution of intermediate **112** (6.85 g, 25.7 mmol) in MeOH (60 mL) was added 6N aqueous NaOH (42.8 mL, 257 mmol). After stirring at room temperature for 15 h, MeOH was evaporated, and 2N aqueous HCl (145 mL) was added to the reaction mixture. The resulting solid was filtered and collected to give 5.66 g (92%) of the title compound as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.85-1.97 (2H, m), 2.36 (2H, t, J = 7.2 Hz), 3.46 (2H, s), 3.94 (2H, t, J = 6.4 Hz), 6.84 (2H, d, J = 8.4 Hz), 7.14 (2H, d, J = 8.6 Hz), 12.18 (2H, s).

3-9-1-60. (5-oxo-2,3,4,5-tetrahydro-1-benzoxepin-7-yl)acetic acid (**114**)

The title compound was prepared in a manner similar to that for the preparation of **85** using intermediate **113**. Compound **114** was obtained in 49% yield as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.17-2.27 (2H, m), 2.90 (2H, t, J = 7.0 Hz), 3.64 (2H, s), 4.24 (2H, t, J =

6.6 Hz), 7.05 (1H, d, J = 8.3 Hz), 7.36 (1H, dd, J = 8.3, 2.5 Hz), 7.67 (1H, d, J = 2.2 Hz).

3-9-1-61. 7-(2-hydroxyethyl)-2,3,4,5-tetrahydro-1-benzoxepin-5-ol (**115**)

The title compound was prepared in a manner similar to that for the preparation of **109** using intermediate **114**. Compound **115** was obtained in 81% yield as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.46 (1H, t, J = 5.8 Hz), 1.91-2.06 (3H, m), 2.08-2.22 (1H, m), 2.25 (1H, d, J = 6.4 Hz), 2.84 (2H, t, J = 6.5 Hz), 3.85 (2H, td, J = 6.3, 6.3 Hz), 3.91-4.09 (2H, m), 4.84-4.91 (1H, m), 6.95 (1H, d, J = 8.1 Hz), 7.05 (1H, dd, J = 8.1, 2.2 Hz), 7.23 (1H, d, J = 2.2 Hz).

3-9-1-62. 7-(2-hydroxyethyl)-3,4-dihydro-1-benzoxepin-5(2H)-one (**116**)

The title compound was prepared in a manner similar to that for the preparation of **110** using intermediate **115**. Compound **116** was obtained in 59% yield as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.15-2.27 (2H, m), 2.81-2.95 (4H, m), 3.86 (2H, t, J = 6.5 Hz), 4.23 (2H, t, J = 6.6 Hz), 7.03 (1H, d, J = 8.3 Hz), 7.31 (1H, dd, J = 8.3, 2.3 Hz), 7.63 (1H, d, J = 2.4 Hz).

3-9-1-63. 2-(5-oxo-2,3,4,5-tetrahydro-1-benzoxepin-7-yl)ethyl 4-methylbenzene-sulfonate (**117**)

The title compound was prepared in a manner similar to that for the preparation of **90** using intermediate **116**. Compound **117** was obtained quantitatively as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.15-2.26 (2H, m), 2.44 (3H, s), 2.84-2.97 (4H, m), 4.15-4.24 (4H, m), 6.98 (1H, d, J = 8.3 Hz), 7.21 (1H, dd, J = 8.5, 2.3 Hz), 7.31 (2H, d, J = 8.4 Hz), 7.48 (1H, d, J = 2.4 Hz), 7.72 (2H, d, J = 8.4 Hz).

3-9-1-64. methyl {4-[2-(methylsulfanyl)ethoxy]phenyl}acetate (**118**)

To a mixture of methyl 4-hydroxyphenylacetate (5.00 g, 30.1 mmol), 2-(methylthio)ethanol (2.62 mL, 30.1 mmol) and triphenylphosphine (9.47 g, 36.1 mmol) in THF (100 mL) was added dropwise 40% diethyl azodicarboxylate/toluene (15.7 g, 36.1 mmol) at 0°C for 15 min. After stirring at room temperature for 6.5 h, the reaction mixture was concentrated, and the residue was purified by silica gel chromatography using 16% EtOAc/hexane as eluent to give 4.77 g (66%) of the title compound as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.21 (3H, s), 2.88 (2H, t, J = 6.8 Hz), 3.57 (2H, s), 3.69 (3H, s), 4.14 (2H, t, J = 6.8 Hz), 6.83-6.90 (2H, m), 7.16-7.23 (2H, m).

3-9-1-65. methyl 2,3-dihydro-1,4-benzoxathiin-6-ylacetate (**119**)

To a solution of trifluoromethanesulfonic anhydride (2.51 mL, 15.0 mmol) in CH_2Cl_2 (136 mL) was added dropwise a solution of intermediate **118** (3.27 g, 13.6 mmol) in CH_2Cl_2 (27 mL) at -78°C for 30 min. After stirring at -78°C for 4 h, the reaction mixture was concentrated. To a solution of the residue in MeCN (130 mL) was added dropwise triethylamine (9.48 mL, 68.0 mmol) at 0°C for 20 min. After stirring at room temperature for 16 h, the reaction

mixture was concentrated. H_2O (400 mL) was added to the residue, and the whole was extracted with EtOAc (500 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated, and the residue was purified by silica gel chromatography using 12% EtOAc/hexane as eluent to give 2.56 g (84%) of the title compound as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.09-3.16 (2H, m), 3.50 (2H, s), 3.69 (3H, s), 4.36-4.44 (2H, m), 6.77 (1H, d, J = 8.4 Hz), 6.89 (1H, dd, J = 8.4, 2.0 Hz), 6.95 (1H, d, J = 2.0 Hz).

3-9-1-66. 2-(2,3-dihydro-1,4-benzoxathiin-6-yl)ethanol (**120**)

The title compound was prepared in a manner similar to that for the preparation of **89** using intermediate **119**. Compound **120** was obtained in 94% yield as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.38 (1H, t, J = 6.2 Hz), 2.75 (2H, t, J = 6.2 Hz), 3.10-3.16 (2H, m), 3.81 (2H, td, J = 6.2, 6.2 Hz), 4.36-4.44 (2H, m), 6.77 (1H, d, J = 8.3 Hz), 6.85 (1H, dd, J = 8.3, 2.0 Hz), 6.91 (1H, d, J = 2.0 Hz).

3-9-1-67. 2-(2,3-dihydro-1,4-benzoxathiin-6-yl)ethyl 4-methylbenzenesulfonate (**121**)

To a mixture of intermediate **120** (1.0 g, 5.10 mmol), triethylamine (1.78 mL, 12.8 mmol) and pyridine (0.410 mL, 5.10 mmol) in CH_2Cl_2 (25 mL) was added p-toluenesulfonyl chloride (1.94 g, 10.2 mmol) at 0°C. After stirring at room temperature for 13 h, H_2O (150 mL) was added to the reaction mixture, and the whole was extracted with $CHCl_3$ (100 mL x 2). The combined organic layer was dried over $MgSO_4$, filtered and concentrated, and the residue was purified by silica gel chromatography using 16~20% EtOAc/hexane as eluent to give 1.76 g (98%) of the title compound as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.45 (3H, s), 2.82 (2H, t, J = 7.2 Hz), 3.09-3.15 (2H, m), 4.14 (2H, t, J = 7.2 Hz), 4.36-4.42 (2H, m), 6.69-6.72 (2H, m), 6.74 (1H, brs), 7.27-7.33 (2H, m), 7.68-7.73 (2H, m).

3-9-1-68. 2-(4,4-dioxido-2,3-dihydro-1,4-benzoxathiin-6-yl)ethyl 4-methylbenzene-sulfonate (**122**)

To a solution of intermediate **121** (0.200 g, 0.571 mmol) in CH_2Cl_2 (2.5 mL) was added 75% 3-chloroperoxybenzoic acid (0.394 g, 1.71 mmol) at 0°C. After stirring at room temperature for 2 h, 5% aqueous $Na_2S_2O_3$ (10 mL) was added to the reaction mixture, and the whole was stirred at room temperature for 30 min. H_2O (10 mL) was then added, and the new mixture was extracted with $CHCl_3$ (50 mL x 2). The combined organic layer was washed with sat. aqueous $NaHCO_3$ (40 mL), dried over $MgSO_4$, filtered and concentrated. The residue was purified by silica gel chromatography using 33% EtOAc/hexane as eluent to give 0.203 g (93%) of the title compound as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.45 (3H, s), 2.94 (2H, t, J = 6.7 Hz), 3.46-3.54 (2H, m), 4.18 (2H, t, J = 6.7 Hz), 4.77-4.85 (2H, m), 6.90 (1H, d, J = 8.6 Hz), 7.26 (1H, dd, J = 8.3, 2.1 Hz), 7.33 (2H, d, J = 8.6 Hz), 7.50 (1H, d, J = 2.1 Hz), 7.72 (2H, d, J = 8.3 Hz).

3-9-1-69. 4-[4-bromo-3-(2-methoxyethoxy)benzyl]-1-[2-(3,4-dihydro-2H-chromen-6-yl)-ethyl]piperidine (**44**)

The title compound was prepared in a manner similar to that for the preparation of **39** using intermediates **74** and **90**. Compound **44** was obtained in 86% yield as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.24-1.39 (2H, m), 1.42-1.56 (1H, m), 1.59-1.69 (2H, m), 1.87-2.03 (4H, m), 2.45-2.55 (4H, m), 2.65-2.73 (2H, m), 2.75 (2H, t, J = 6.4 Hz), 2.97 (2H, brd, J = 11.2 Hz), 3.50 (3H, s), 3.81 (2H, t, J = 5.2 Hz), 4.13-4.19 (4H, m), 6.64 (1H, dd, J = 8.0, 2.0 Hz), 6.70 (1H, d, J = 8.4 Hz), 6.71 (1H, d, J = 1.6 Hz), 6.85 (1H, d, J = 2.0 Hz), 6.90 (1H, dd, J = 8.4, 2.0 Hz), 7.41 (1H, d, J = 8.0 Hz); HRMS (ESI) m/z calcd for $C_{26}H_{35}BrNO_3$ [M+H]+ 488.1795; found 488.1800.

3-9-1-70. 6-(2-{4-[4-bromo-3-(2-methoxyethoxy)benzyl]piperidin-1-yl}ethyl)-3,4-dihydro-2H-chromen-4-ol (**45**)

The title compound was prepared in a manner similar to that for the preparation of **39** using intermediates **74** and **94**. Compound **45** was obtained in 53% yield as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.23-1.40 (2H, m), 1.42-1.54 (1H, m), 1.63-1.70 (2H, m), 1.86-2.18 (4H, m), 2.44-2.57 (4H, m), 2.68-2.77 (2H, m), 2.92-3.02 (2H, m), 3.50 (3H, s), 3.81 (2H, t, J = 4.9 Hz), 4.17 (2H, t, J = 4.9 Hz), 4.21-4.28 (2H, m), 4.76 (1H, t, J = 4.0 Hz), 6.64 (1H, dd, J = 8.1, 1.8 Hz), 6.71 (1H, d, J = 1.8 Hz), 6.76 (1H, d, J = 8.3 Hz), 7.03 (1H, dd, J = 8.3, 2.1 Hz), 7.13 (1H, d, J = 2.0 Hz), 7.41 (1H, d, J = 7.9 Hz); HRMS (ESI) m/z calcd for $C_{26}H_{35}BrNO_4$ [M+H]+ 504.1744; found 504.1751.

3-9-1-71. 6-(2-{4-[4-bromo-3-(2-methoxyethoxy)benzyl]piperidin-1-yl}ethyl)-2,3-dihydro-4H-chromen-4-one benzensulfonate (**46**, **DSP-1053**)

The free form of the title compound was prepared in a manner similar to that for the preparation of **39** using intermediates **74** and **93**. The free form of compound **46** was obtained in 99% yield as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.24-1.39 (2H, m), 1.40-1.73 (3H, m), 1.93 (2H, t, J = 10.6 Hz), 2.40-2.61 (2H, m), 2.48 (2H, d, J = 7.2 Hz), 2.66-2.87 (2H, m), 2.79 (2H, t, J = 6.4 Hz), 2.95 (2H, d, J = 11.7 Hz), 3.49 (3H, s), 3.81 (2H, t, J = 4.9 Hz), 4.17 (2H, t, J = 4.9 Hz), 4.51 (2H, t, J = 6.4 Hz), 6.64 (1H, dd, J = 8.1, 1.8 Hz), 6.71 (1H, d, J = 1.8 Hz), 6.89 (1H, d, J = 8.4 Hz), 7.32 (1H, dd, J = 8.4, 2.2 Hz), 7.41 (1H, d, J = 8.1 Hz), 7.70 (1H, d, J = 2.2 Hz); HRMS (ESI) m/z calcd for $C_{26}H_{33}BrNO_4$ [M+H]+ 502.1587; found 502.1591. The product was converted to the title compound quantitatively by treated with bensensulfonic acid monohydrate (1.0 eq.) in 2-PrOH at room temperature for 1h. Mp: 142-143°C. Anal. Calcd for $C_{32}H_{38}BrNO_7S$: C, 58.18; H, 5.80; N, 2.12; S, 4.85; Br, 12.10. Found: C, 58.09; H, 5.80; N, 2.25; S, 4.85; Br, 12.10.

3-9-1-72. 4-[4-bromo-3-(2-methoxyethoxy)benzyl]-1-[2-(3,4-dihydro-2H-chromen-7-yl)-ethyl]piperidine (**47**)

The title compound was prepared in a manner similar to that for the preparation of **39** using intermediates **74** and **102**. Compound **47** was obtained in 82% yield as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.23-1.38 (2H, m), 1.43-1.57 (1H, m), 1.60-1.68 (2H, m), 1.87-2.03 (4H, m), 2.48 (2H, d, J = 6.8 Hz), 2.51-2.58 (2H, m), 2.67-2.78 (4H, m), 2.96 (2H, brd, J = 11.6 Hz), 3.49 (3H, s), 3.81 (2H, t, J = 5.2 Hz), 4.16 (2H, t, J = 4.8 Hz), 4.17 (2H, t, J = 4.4 Hz), 6.62-6.65 (2H, m), 6.67 (1H, dd, J = 7.6, 1.6 Hz), 6.71 (1H, d, J = 1.6 Hz), 6.94 (1H, d, J = 7.6 Hz), 7.41 (1H, d, J = 8.0 Hz); HRMS (ESI) m/z calcd for $C_{26}H_{35}BrNO_3$ [M+H]+ 488.1795; found 488.1799.

3-9-1-73. 7-(2-{4-[4-bromo-3-(2-methoxyethoxy)benzyl]piperidin-1-yl}ethyl)-2,3-dihydro-4H-chromen-4-one (**48**)

The title compound was prepared in a manner similar to that for the preparation of **39** using intermediates **74** and **105**. Compound **48** was obtained in 82% yield as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.23-1.38 (2H, m), 1.43-1.57 (1H, m), 1.60-1.69 (2H, m), 1.95 (2H, t, J = 10.0 Hz), 2.49 (2H, d, J = 6.8 Hz), 2.52-2.61 (2H, m), 2.74-2.83 (4H, m), 2.95 (2H, d, J = 11.2 Hz), 3.50 (3H, s), 3.81 (2H, t, J = 4.8 Hz), 4.17 (2H, t, J = 4.8 Hz), 4.51 (2H, t, J = 6.4 Hz), 6.64 (1H, dd, J = 8.0, 2.0 Hz), 6.71 (1H, d, J = 1.6 Hz), 6.80 (1H, d, J = 1.2 Hz), 6.85 (1H, dd, J = 8.0, 1.6 Hz), 7.41 (1H, d, J = 8.0 Hz), 7.80 (1H, d, J = 8.0 Hz); HRMS (ESI) m/z calcd for $C_{26}H_{33}BrNO_4$ [M+H]+ 502.1587; found 502.1592.

3-9-1-74. 7-(2-{4-[4-bromo-3-(2-methoxyethoxy)benzyl]piperidin-1-yl}ethyl)-3,4-dihydro-naphthalen-1(2H)-one (**49**)

The title compound was prepared in a manner similar to that for the preparation of **39** using intermediates **74** and **111**. Compound **49** was obtained in 95% yield as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.23-1.40 (2H, m), 1.42-1.55 (1H, m), 1.61-1.70 (2H, m), 1.88-2.02 (2H, m), 2.07-2.17 (2H, m), 2.49 (2H, d, J = 6.8 Hz), 2.51-2.59 (2H, m), 2.64 (2H, t, J = 6.4 Hz), 2.77-2.85 (2H, m), 2.89-3.01 (4H, m), 3.49 (3H, s), 3.81 (2H, t, J = 4.8 Hz), 4.17 (2H, t, J = 4.9 Hz), 6.64 (1H, dd, J = 7.9, 1.8 Hz), 6.71 (1H, d, J = 1.7 Hz), 7.17 (1H, d, J = 7.9 Hz), 7.31 (1H, dd, J = 7.7, 2.0 Hz), 7.41 (1H, d, J = 7.9 Hz), 7.85 (1H, d, J = 1.7 Hz); HRMS (ESI) m/z calcd for $C_{27}H_{35}BrNO_3$ [M+H]+ 500.1795; found 500.1801.

3-9-1-75. 7-(2-{4-[4-bromo-3-(2-methoxyethoxy)benzyl]piperidin-1-yl}ethyl)-3,4-dihydro-1-benzoxepin-5(2H)-one (**50**)

The title compound was prepared in a manner similar to that for the preparation of **39** using intermediates **74** and **117**. Compound **50** was obtained in 99% yield as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.21-1.40 (2H, m), 1.42-1.56 (1H, m), 1.62-1.71 (2H, m), 1.88-2.01 (2H, m), 2.14-2.25 (2H, m), 2.49 (2H, d, J = 7.0 Hz), 2.51-2.59 (2H, m), 2.72-2.83

(2H, m), 2.89 (2H, t, J = 6.9 Hz), 2.92-3.03 (2H, m), 3.49 (3H, s), 3.81 (2H, t, J = 4.9 Hz), 4.14-4.24 (4H, m), 6.64 (1H, dd, J = 8.0, 1.9 Hz), 6.71 (1H, d, J = 1.8 Hz), 6.99 (1H, d, J = 8.4 Hz), 7.27 (1H, dd, J = 8.2, 2.5 Hz), 7.41 (1H, d, J = 7.9 Hz), 7.58 (1H, d, J = 2.4 Hz); HRMS (ESI) m/z calcd for $C_{27}H_{35}BrNO_4$ [M+H]+ 516.1744; found 516.1752.

3-9-1-76. 4-[4-bromo-3-(2-methoxyethoxy)benzyl]-1-[2-(4,4-dioxido-2,3-dihydro-1,4-benzoxathiin-6-yl)ethyl]piperidine (**51**)

The title compound was prepared in a manner similar to that for the preparation of **39** using intermediates **74** and **122**. Compound **51** was obtained in 67% yield as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.21-1.38 (2H, m), 1.42-1.55 (1H, m), 1.63-1.69 (2H, m), 1.87-1.99 (2H, m), 2.45-2.57 (4H, m), 2.72-2.82 (2H, m), 2.90-2.99 (2H, m), 3.47-3.53 (5H, m), 3.81 (2H, t, J = 4.9 Hz), 4.17 (2H, t, J = 4.9 Hz), 4.77-4.83 (2H, m), 6.64 (1H, dd, J = 8.1, 1.8 Hz), 6.71 (1H, d, J = 1.8 Hz), 6.90 (1H, d, J = 8.6 Hz), 7.29 (1H, dd, J = 8.6, 2.2 Hz), 7.41 (1H, d, J = 8.1 Hz), 7.61 (1H, d, J = 2.0 Hz); HRMS (ESI) m/z calcd for $C_{25}H_{33}BrNO_5S$ [M+H]+ 538.1257; found 538.1260.

3-9-1-77. 6-(2-{4-[3-bromo-5-(2-methoxyethoxy)benzyl]piperidin-1-yl}ethyl)-2,3-dihydro-4H-chromen-4-one (**52**)

The title compound was prepared in a manner similar to that for the preparation of **39** using intermediates **82** and **93**. Compound **52** was obtained in 99% yield as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.23-1.39 (2H, m), 1.42-1.56 (1H, m), 1.62-1.70 (2H, m), 1.86-2.01 (2H, m), 2.46 (2H, d, J = 7.0 Hz), 2.49-2.58 (2H, m), 2.71-2.84 (4H, m), 2.90-3.01 (2H, m), 3.45 (3H, s), 3.71-3.76 (2H, m), 4.06-4.11 (2H, m), 4.51 (2H, t, J = 6.5 Hz), 6.66 (1H, t, J = 1.8 Hz), 6.87-6.92 (3H, m), 7.32 (1H, dd, J = 8.5, 2.3 Hz), 7.70 (1H, d, J = 2.2 Hz); HRMS (ESI) m/z calcd for $C_{26}H_{33}BrNO_4$ $[M+H]^+$ 502.1587; found 502.1593.

3-9-1-78. 4-[3-(2-methoxyethoxy)benzyl]piperidine (**123**)

The intermediate **74** (0.500 g, 1.37 mmol) was dissolved in MeOH (10 mL) and hydrogenated over 10% Pd on carbon (water ~50%, 0.250 g) at room temperature for 24 h. The catalyst was removed by filtration, and the filtrate was concentrated. Sat. aqueous $NaHCO_3$ (20 mL) was then added to the residue, and the whole was extracted with $CHCl_3$ (20 mL x 2). The combined organic layer was dried over Na_2SO_4, filtered and concentrated to give 0.328 g (96%) of the title compound as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.05-1.22 (2H, m), 1.56-1.64 (3H, m), 2.45-2.58 (4H, m), 2.99-3.08 (2H, m), 3.46 (3H, s), 3.73-3.78 (2H, m), 4.09-4.14 (2H, m), 6.71-6.78 (3H, m), 7.14-7.22 (1H, m).

3-9-1-79. [4-{[1-(*tert*-butoxycarbonyl)piperidin-4-yl]methyl}-2-(2-methoxyethoxy)phenyl]-boronic acid (**124**)

To a solution of intermediate **73** (1.00 g, 2.33 mmol) in THF (10 mL) was added dropwise

2.6M n-BuLi in n-hexane (0.99 mL, 2.57 mmol) at -78°C. After stirring at -78°C for 30 min, trimethyl borate (0.779 mL, 6.99 mmol) was added to the reaction mixture, and the whole was stirred at room temperature for 5 h. 10% aqueous $KHSO_4$ (20 mL) was added to the reaction mixture, and the new mixture was extracted with EtOAc (20 mL x 2). The combined organic layer was dried over $MgSO_4$, filtered and concentrated, and the residue was purified by silica gel chromatography using 32~53% EtOAc/hexane as eluent to give 0.360 g (39%) of the title compound as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.06-1.22 (2H, m), 1.45 (9H, s), 1.60-1.71 (3H, m), 2.53 (2H, d, J = 7.0 Hz), 2.56-2.70 (2H, m), 3.46 (3H, s), 3.75-3.79 (2H, m), 3.99-4.14 (2H, m), 4.18-4.23 (2H, m), 5.84 (2H, brs), 6.67 (1H, d, J = 1.3 Hz), 6.83 (1H, dd, J = 7.5, 1.3 Hz), 7.73 (1H, d, J = 7.5 Hz).

3-9-1-80. *tert*-butyl 4-[4-fluoro-3-(2-methoxyethoxy)benzyl]piperidine-1-carboxylate (**125a**)
To a mixture of intermediate **124** (0.100 g, 0.254 mmol) and NaOH (0.0122 g, 0.305 mmol) in MeOH (1.0 mL) was added silver trifluoromethanesulfonate (0.196 g, 0.762 mmol) at 0°C. After stirring at 0°C for 30 min, the reaction mixture was concentrated and the residual MeOH was removed by co-evaporation with acetone (1.0 mL x 3). To a solution of the residue in acetone (2.0 mL) was added MS3Å (0.127 g) and 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(trifluoroborate) (0.0945 g, 0.267 mmol) at 0°C. After stirring at 0°C for 1.5 h, the reaction mixture was filtered and concentrated. H_2O (30 mL) was then added to the residue, and the whole was extracted with $CHCl_3$ (30 mL x 2). The combined organic layer was dried over $MgSO_4$, filtered and concentrated, and the residue was purified by silica gel chromatography using 11~32% EtOAc/hexane as eluent to give 0.0251 g (27%) of the title compound as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.03-1.21 (2H, m), 1.45 (9H, s), 1.53-1.67 (3H, m), 2.47 (2H, d, J = 6.6 Hz), 2.56-2.70 (2H, m), 3.46 (3H, s), 3.74-3.79 (2H, m), 3.99-4.14 (2H, m), 4.16-4.20 (2H, m), 6.63-6.69 (1H, m), 6.76 (1H, dd, J = 8.0, 2.1 Hz), 6.96 (1H, dd, J = 11.3, 8.2 Hz).

3-9-1-81. 4-[4-fluoro-3-(2-methoxyethoxy)benzyl]piperidine hydrochloride (**126a**)
To a solution of intermediate **125a** (0.0251 g, 0.0680 mmol) in $CHCl_3$ (1.0 mL) was added 4N HCl/EtOAc (2.0 mL). After stirring at room temperature for 30 min, the reaction mixture was concentrated to give 0.0258 g (quant.) of the title compound as a colorless oil. ^{1}H NMR (300 MHz, CD_3OD) δ: 1.31-1.50 (2H, m), 1.80-1.93 (3H, m), 2.58 (2H, d, J = 7.0 Hz), 2.87-2.99 (2H, m), 3.32-3.40 (2H, m), 3.43 (3H, s), 3.72-3.77 (2H, m), 4.14-4.18 (2H, m), 6.71-6.78 (1H, m), 6.93 (1H, dd, J = 8.2, 2.1 Hz), 7.00 (1H, dd, J = 11.4, 8.3 Hz).

3-9-1-82. *tert*-butyl 4-[4-chloro-3-(2-methoxyethoxy)benzyl]piperidine-1-carboxylate (**125b**)
To a solution of intermediate **124** (0.100 g, 0.254 mmol) in MeCN (1.0 mL) was added N-chlorosuccinimide (0.0340 g, 0.254 mmol) and cuprous chloride (0.0252 g, 0.254 mmol). After reflux for 3 h, H_2O (20 mL) was added to the reaction mixture, and the whole was

extracted with EtOAc (30 mL x 2). The combined organic layer was dried over $MgSO_4$, filtered and concentrated, and the residue was purified by silica gel chromatography using 20~41% EtOAc/hexane as eluent to give 0.0775 g (79%) of the title compound as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.05-1.19 (2H, m), 1.45 (9H, s), 1.52-1.69 (3H, m), 2.49 (2H, d, J = 6.8 Hz), 2.56-2.69 (2H, m), 3.48 (3H, s), 3.80 (2H, t, J = 4.9 Hz), 3.97-4.14 (2H, m), 4.17 (2H, t, J = 4.9 Hz), 6.68 (1H, dd, J = 8.0, 2.0 Hz), 6.71 (1H, d, J = 7.8 Hz), 6.73 (1H, d, J = 2.0 Hz).

3-9-1-83. 4-[4-chloro-3-(2-methoxyethoxy)benzyl]piperidine hydrochloride (**126b**)

The title compound was prepared in a manner similar to that for the preparation of **126a** using intermediate **126b**. Compound **126b** was obtained quantitatively as a white solid. ^{1}H-NMR (400 MHz, DMSO-D_6) δ: 1.26-1.39 (2H, m), 1.65-1.72 (2H, m), 1.74-1.84 (1H, m), 2.52 (2H, d, J = 4.9 Hz), 2.73-2.82 (2H, m), 3.18-3.25 (2H, m), 3.34 (3H, s), 3.67-3.70 (2H, m), 4.15-4.18 (2H, m), 6.77 (1H, dd, J = 8.0, 1.7 Hz), 6.99 (1H, d, J = 1.7 Hz), 7.32 (1H, d, J = 8.0 Hz), 8.64 (1H, br s).

3-9-1-84. *tert*-butyl 4-[4-iodo-3-(2-methoxyethoxy)benzyl]piperidine-1-carboxylate (**125c**)

To a solution of intermediate **124** (0.100 g, 0.254 mmol) in MeCN (2.0 mL) was added N-iodosuccinimide (0.0571 g, 0.254 mmol). After stirring at room temperature for 16 h, the reaction mixture was concentrated, and the residue was purified by silica gel chromatography using 11~32% EtOAc/hexane as eluent to give 0.104 g (86%) of the title compound as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.04-1.21 (2H, m), 1.45 (9H, s), 1.59-1.71 (3H, m), 2.48 (2H, d, J = 6.8 Hz), 2.55-2.71 (2H, m), 3.51 (3H, s), 3.82 (2H, t, J = 4.9 Hz), 4.00-4.12 (2H, m), 4.15 (2H, t, J = 5.0 Hz), 6.52 (1H, dd, J = 7.9, 1.7 Hz), 6.62 (1H, d, J = 1.7 Hz), 7.64 (1H, d, J = 7.9 Hz).

3-9-1-85. 4-[4-iodo-3-(2-methoxyethoxy)benzyl]piperidine hydrochloride (**126c**)

The title compound was prepared in a manner similar to that for the preparation of **126a** using **125c**. Compound **126c** was obtained quantitatively as a colorless oil. ^{1}H NMR (300 MHz, CD_3OD) δ: 1.32-1.49 (2H, m), 1.79-1.94 (3H, m), 2.59 (2H, d, J = 7.0 Hz), 2.86-2.99 (2H, m), 3.32-3.40 (2H, m), 3.48 (3H, s), 3.77-3.82 (2H, m), 4.13-4.17 (2H, m), 6.59 (1H, dd, J = 8.0, 1.9 Hz), 6.80 (1H, d, J = 1.8 Hz), 7.66 (1H, d, J = 7.9 Hz).

3-9-1-86. *tert*-butyl 4-[3-(2-methoxyethoxy)-4-methylbenzyl]piperidine-1-carboxylate (**127**)

A mixture of intermediate **73** (5.00 g, 11.7 mmol), methylboronic acid (0.978 g, 16.0 mmol), tetrakis(triphenylphosphine)palladium (0.674 g, 0.583 mmol), 1M aqueous K_2CO_3 (35 mL) and 1,4-dioxane (80 mL) was refluxed for 4 h. After evaporation of 1,4-dioxane from the reaction mixture, H_2O was added, and the whole was extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated, and the residue

was purified by silica gel chromatography using 20% EtOAc/hexane as eluent to give 3.46 g (82%) of the title compound as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.05-1.19 (2H, m), 1.45 (9H, s), 1.58-1.68 (3H, m), 2.20 (3H, s), 2.48 (2H, d, J = 6.6 Hz), 2.57-2.69 (2H, m), 3.47 (3H, s), 3.77 (2H, t, J = 4.9 Hz), 4.00-4.13 (4H, m), 6.60 (1H, d, J = 1.2 Hz), 6.64 (1H, dd, J = 7.4, 1.6 Hz), 7.03 (1H, d, J = 7.8 Hz).

3-9-1-87. 4-[3-(2-methoxyethoxy)-4-methylbenzyl]piperidine (**128**)

A mixture of intermediate **127** (3.46 g, 9.50 mmol) and 10% HCl/MeOH (40 mL) was stirred at room temperature for 12 h and then concentrated. Sat. aqueous $NaHCO_3$ was added to the residue, and the whole was extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4, filtered and concentrated to give 2.56 g (quant.) of the title compound as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.39-1.58 (2H, m), 1.58-1.74 (1H, m), 1.77 (2H, d, J = 13.9 Hz), 2.20 (3H, s), 2.52 (2H, d, J = 7.1 Hz), 2.70 (2H, t, J = 12.6 Hz), 3.33 (2H, d, J = 12.4 Hz), 3.47 (3H, s), 3.77 (2H, t, J = 4.8 Hz), 4.10 (2H, t, J = 4.8 Hz), 6.59 (1H, s), 6.63 (1H, d, J = 7.6 Hz), 7.03 (1H, d, J = 7.6 Hz).

3-9-1-88. *tert*-butyl 4-[4-acetyl-3-(2-methoxyethoxy)benzyl]piperidine-1-carboxylate (**129a**)

To a solution of intermediate **73** (1.00 g, 2.33 mmol) in THF (10 mL) was added 2.6M n-BuLi in n-hexane (0.99 mL, 2.57 mmol) at -78°C. After stirring at -78°C for 30 min, N,N-dimethylacetamide (0.259 mL, 2.80 mmol) was added to the reaction mixture, and the whole was stirred at -78°C for 30 min. 10% aqueous $KHSO_4$ (20 mL) was then added, and the new mixture was extracted with EtOAc (30 mL x 2). The combined organic layer was dried over $MgSO_4$, filtered and concentrated, and the residue was purified by silica gel chromatography using 22~43% EtOAc/hexane as eluent to give 0.128 g (14%) of the title compound as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.03-1.23 (2H, m), 1.45 (9H, s), 1.60-1.74 (3H, m), 2.54 (2H, d, J = 7.0 Hz), 2.57-2.70 (5H, m), 3.45 (3H, s), 3.78-3.83 (2H, m), 4.00-4.12 (2H, m), 4.18-4.23 (2H, m), 6.70 (1H, d, J = 1.7 Hz), 6.79 (1H, dd, J = 7.9, 1.5 Hz), 7.70 (1H, d, J = 7.9 Hz).

3-9-1-89. *tert*-butyl 4-[4-ethyl-3-(2-methoxyethoxy)benzyl]piperidine-1-carboxylate (**130a**)

A mixture of intermediate **129a** (0.128 g, 0.327 mmol), c.HCl (0.0338 g, 0.343 mmol) and 10% Pd on carbon (water ~50%, 0.100 g) in MeOH (5.0 mL) was hydrogenated at room temperature under 0.4 MPa. After 36 h, the catalyst was removed by filtration, and the filtrate was concentrated. The residue was purified by silica gel chromatography using 0~21% EtOAc/hexane as eluent to give 0.0524 g (43%) of the title compound as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.04-1.22 (5H, m), 1.45 (9H, s), 1.59-1.69 (3H, m), 2.48 (2H, d, J = 6.6 Hz), 2.56-2.70 (4H, m), 3.47 (3H, s), 3.77 (2H, t, J = 5.0 Hz), 3.98-4.14 (4H, m), 6.61 (1H, d, J = 1.5 Hz), 6.67 (1H, dd, J = 7.5, 1.5 Hz), 7.05 (1H, d, J = 7.5 Hz).

3-9-1-90. 4-[4-ethyl-3-(2-methoxyethoxy)benzyl]piperidine hydrochloride (**131a**)

The title compound was prepared in a manner similar to that for the preparation of **126a** using intermediate **130a**. Compound **131a** was obtained quantitatively as a colorless oil. ^{1}H NMR (300 MHz, CD_3OD) δ: 1.14 (3H, t, *J* = 7.4 Hz), 1.28-1.49 (2H, m), 1.80-1.94 (3H, m), 2.53-2.64 (4H, m), 2.86-2.99 (2H, m), 3.32-3.40 (2H, m), 3.44 (3H, s), 3.74-3.78 (2H, m), 4.08-4.12 (2H, m), 6.69 (1H, dd, *J* = 7.3, 1.5 Hz), 6.73 (1H, d, *J* = 1.5 Hz), 7.04 (1H, d, *J* = 7.3 Hz).

3-9-1-91. *tert*-butyl 4-[4-(2-hydroxypropan-2-yl)-3-(2-methoxyethoxy)benzyl]-piperidine-1-carboxylate (**129b**)

The title compound was prepared in a manner similar to that for the preparation of **129a** using acetone. Compound **129b** was obtained in 30% yield as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.05-1.22 (2H, m), 1.45 (9H, s), 1.59-1.67 (9H, m), 2.49 (2H, d, J = 6.8 Hz), 2.56-2.69 (2H, m), 3.45 (3H, s), 3.75-3.80 (2H, m), 3.98-4.11 (2H, m), 4.18-4.23 (2H, m), 4.63 (1H, s), 6.67 (1H, d, J = 1.7 Hz), 6.71 (1H, dd, J = 8.0, 1.6 Hz), 7.19 (1H, d, J = 7.9 Hz).

3-9-1-92. *tert*-butyl 4-[3-(2-methoxyethoxy)-4-(propan-2-yl)benzyl]piperidine-1-carboxylate (**130b**)

The title compound was prepared in a manner similar to that for the preparation of **130a** using intermediate **129b**. Compound **130b** was obtained in 37% yield as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.05-1.18 (2H, m), 1.20 (6H, d, J = 7.0 Hz), 1.45 (9H, s), 1.58-1.69 (3H, m), 2.48 (2H, d, J = 6.6 Hz), 2.57-2.70 (2H, m), 3.25-3.36 (1H, m), 3.46 (3H, s), 3.77 (2H, t, J = 4.9 Hz), 3.98-4.14 (4H, m), 6.61 (1H, d, J = 1.5 Hz), 6.70 (1H, dd, J = 7.6, 1.4 Hz), 7.10 (1H, d, J = 7.5 Hz).

3-9-1-93. 4-[3-(2-methoxyethoxy)-4-(propan-2-yl)benzyl]piperidine hydrochloride (**131b**)

The title compound was prepared in a manner similar to that for the preparation of **126a** using intermediate **130b**. Compound **131b** was obtained quantitatively as a colorless oil. ^{1}H NMR (300 MHz, CD_3OD) δ: 1.18 (6H, d, J = 7.0 Hz), 1.32-1.49 (2H, m), 1.83-1.93 (3H, m), 2.57 (2H, d, J = 6.8 Hz), 2.86-2.98 (2H, m), 3.24-3.40 (3H, m), 3.44 (3H, s), 3.74-3.79 (2H, m), 4.08-4.12 (2H, m), 6.71-6.75 (2H, m), 7.10 (1H, d, J = 7.9 Hz).

3-9-1-94. 6-(2-{4-[3-(2-methoxyethoxy)benzyl]piperidin-1-yl}ethyl)-2,3-dihydro-4H-chromen-4-one (**53**)

The title compound was prepared in a manner similar to that for the preparation of **39** using intermediates **93** and **123**. Compound **53** was obtained quantitatively as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.23-1.38 (2H, m), 1.44-1.56 (1H, m), 1.62-1.69 (2H, m), 1.87-1.99 (2H, m), 2.47-2.56 (4H, m), 2.72-2.82 (4H, m), 2.90-2.99 (2H, m), 3.46 (3H, s), 3.73-3.78 (2H, m), 4.09-4.14 (2H, m), 4.51 (2H, t, J = 6.4 Hz), 6.72-6.78 (3H, m), 6.89 (1H, d,

J = 8.6 Hz), 7.14-7.21 (1H, m), 7.32 (1H, dd, J = 8.5, 2.3 Hz), 7.70 (1H, d, J = 2.2 Hz); HRMS (ESI) m/z calcd for $C_{26}H_{34}NO_4$ $[M+H]^+$ 424.2482; found 424.2490.

3-9-1-95. 6-(2-{4-[4-fluoro-3-(2-methoxyethoxy)benzyl]piperidin-1-yl}ethyl)-2,3-dihydro-4H-chromen-4-one (**54**)

The title compound was prepared in a manner similar to that for the preparation of **39** using intermediates **93** and **126a**. Compound **54** was obtained in 59% yield as a colorless oil. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.22-1.38 (2H, m), 1.40-1.54 (1H, m), 1.62-1.69 (2H, m), 1.89-2.00 (2H, m), 2.45-2.57 (4H, m), 2.72-2.83 (4H, m), 2.92-3.01 (2H, m), 3.46 (3H, s), 3.77 (2H, t, J = 4.8 Hz), 4.19 (2H, t, J = 4.9 Hz), 4.51 (2H, t, J = 6.4 Hz), 6.64-6.71 (1H, m), 6.77 (1H, dd, J = 8.2, 2.1 Hz), 6.89 (1H, d, J = 8.4 Hz), 6.96 (1H, dd, J = 11.4, 8.3 Hz), 7.32 (1H, dd, J = 8.4, 2.4 Hz), 7.70 (1H, d, J = 2.2 Hz); HRMS (ESI) m/z calcd for $C_{26}H_{33}FNO_4$ [M+H]+ 442.2388; found 442.2396.

3-9-1-96. 6-(2-{4-[4-chloro-3-(2-methoxyethoxy)benzyl]piperidin-1-yl}ethyl)-2,3-dihydro-4H-chromen-4-one (**55**)

The title compound was prepared in a manner similar to that for the preparation of **39** using intermediates **93** and **126b**. Compound **55** was obtained in 98% yield as a colorless oil. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.23-1.37 (2H, m), 1.43-1.56 (1H, m), 1.60-1.78 (2H, m), 1.94 (2H, t, J = 12.0 Hz), 2.49 (2H, d, J = 7.2 Hz), 2.50-2.55 (2H, m), 2.72-2.80 (2H, m), 2.79 (2H, t, J = 6.4 Hz), 2.96 (2H, d, J = 11.5 Hz), 3.49 (3H, s), 3.81 (2H, t, J = 4.8 Hz), 4.18 (2H, t, J = 4.8 Hz), 4.51 (2H, t, J = 6.4 Hz), 6.69 (1H, dd, J = 8.0, 1.8 Hz), 6.74 (1H, d, J = 1.8 Hz), 6.89 (1H, d, J = 8.5 Hz), 7.24 (1H, d, J = 8.0 Hz), 7.31 (1H, dd, J = 8.5, 2.2 Hz), 7.70 (1H, d, J = 2.2 Hz); HRMS (ESI) m/z calcd for $C_{26}H_{33}ClNO_4$ $[M+H]^+$ 458.2093; found 458.2100.

3-9-1-97. 6-(2-{4-[4-iodo-3-(2-methoxyethoxy)benzyl]piperidin-1-yl}ethyl)-2,3-dihydro-4H-chromen-4-one (**56**)

The title compound was prepared in a manner similar to that for the preparation of **39** using intermediates **93** and **126c**. Compound **56** was obtained in 68% yield as a colorless oil. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.23-1.39 (2H, m), 1.42-1.54 (1H, m), 1.62-1.69 (2H, m), 1.88-1.99 (2H, m), 2.46-2.56 (4H, m), 2.72-2.83 (4H, m), 2.91-3.00 (2H, m), 3.51 (3H, s), 3.82 (2H, t, J = 4.9 Hz), 4.16 (2H, t, J = 4.9 Hz), 4.51 (2H, t, J = 6.4 Hz), 6.53 (1H, dd, J = 7.9, 1.8 Hz), 6.63 (1H, d, J = 1.8 Hz), 6.89 (1H, d, J = 8.4 Hz), 7.32 (1H, dd, J = 8.5, 2.3 Hz), 7.64 (1H, d, J = 7.9 Hz), 7.70 (1H, d, J = 2.2 Hz); HRMS (ESI) m/z calcd for $C_{26}H_{33}INO_4$ $[M+H]^+$ 550.1449; found 550.1453.

3-9-1-98. 6-(2-{4-[3-(2-methoxyethoxy)-4-methylbenzyl]piperidin-1-yl}ethyl)-2,3-dihydro-4H-chromen-4-one (**57**)

The title compound was prepared in a manner similar to that for the preparation of **39** using

intermediates **93** and **128**. Compound **57** was obtained in 93% yield as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.24-1.37 (2H, m), 1.44-1.54 (1H, m), 1.61-1.69 (2H, m), 1.89-1.97 (2H, m), 2.20 (3H, s), 2.46-2.55 (4H, m), 2.72-2.82 (4H, m), 2.91-2.98 (2H, m), 3.47 (3H, s), 3.77 (2H, t, J = 4.9 Hz), 4.12 (2H, t, J = 4.9 Hz), 4.51 (2H, t, J = 6.5 Hz), 6.61 (1H, d, J = 1.2 Hz), 6.65 (1H, dd, J = 7.3, 1.5 Hz), 6.89 (1H, d, J = 8.5 Hz), 7.03 (1H, d, J = 7.6 Hz), 7.32 (1H, dd, J = 8.5, 2.4 Hz), 7.70 (1H, d, J = 2.2 Hz); HRMS (ESI) m/z calcd for $C_{27}H_{36}NO_4$ $[M+H]^+$ 438.2639; found 438.2643.

3-9-1-99. 6-(2-{4-[4-ethyl-3-(2-methoxyethoxy)benzyl]piperidin-1-yl}ethyl)-2,3-dihydro-4H-chromen-4-one (**58**)

The title compound was prepared in a manner similar to that for the preparation of **39** using intermediates **93** and **131a**. Compound **58** was obtained in 65% yield as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.19 (3H, t, J = 7.5 Hz), 1.24-1.40 (2H, m), 1.42-1.56 (1H, m), 1.63-1.71 (2H, m), 1.89-2.00 (2H, m), 2.46-2.57 (4H, m), 2.63 (2H, q, J = 7.5 Hz), 2.72-2.83 (4H, m), 2.91-3.00 (2H, m), 3.47 (3H, s), 3.77 (2H, t, J = 4.9 Hz), 4.12 (2H, t, J = 4.9 Hz), 4.51 (2H, t, J = 6.4 Hz), 6.62 (1H, d, J = 1.1 Hz), 6.69 (1H, dd, J = 7.6, 1.2 Hz), 6.89 (1H, d, J = 8.4 Hz), 7.05 (1H, d, J = 7.5 Hz), 7.32 (1H, dd, J = 8.4, 2.2 Hz), 7.70 (1H, d, J = 2.2 Hz); HRMS (ESI) m/z calcd for $C_{28}H_{38}NO_4$ $[M+H]^+$ 452.2795; found 452.2799.

3-9-1-100. 6-(2-{4-[3-(2-methoxyethoxy)-4-(propan-2-yl)benzyl]piperidin-1-yl}ethyl)-2,3-dihydro-4H-chromen-4-one (**59**)

The title compound was prepared in a manner similar to that for the preparation of **39** using intermediates **93** and **131b**. Compound **59** was obtained in 71% yield as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.20 (6H, d, J = 6.8 Hz), 1.25-1.41 (2H, m), 1.45-1.55 (1H, m), 1.64-1.72 (2H, m), 1.89-2.02 (2H, m), 2.46-2.58 (4H, m), 2.73-2.83 (4H, m), 2.92-3.01 (2H, m), 3.25-3.36 (1H, m), 3.47 (3H, s), 3.78 (2H, t, J = 5.0 Hz), 4.12 (2H, t, J = 5.0 Hz), 4.51 (2H, t, J = 6.4 Hz), 6.62 (1H, d, J = 1.5 Hz), 6.71 (1H, dd, J = 7.7, 1.5 Hz), 6.89 (1H, d, J = 8.6 Hz), 7.10 (1H, d, J = 7.7 Hz), 7.32 (1H, dd, J = 8.4, 2.4 Hz), 7.70 (1H, d, J = 2.4 Hz); HRMS (ESI) m/z calcd for $C_{29}H_{40}NO_4$ $[M+H]^+$ 466.2952; found 466.2957.

3-9-2. Biological tests

3-9-2-1. Materials

All radioligands were purchased from Perkin Elmer Japan (Kanagawa, Japan). Cell membranes expressing human serotonin transporter and 5-HT_{1A} receptor were purchased from Perkin Elmer Japan. Chinese hamster ovary cells expressing human serotonin transporter used for [^{3}H]5-HT uptake assay were established in the Pharmacology Research Laboratories at Sumitomo Dainippon Pharma Co., Ltd.

3-9-2-2. Radioreceptor binding assay

In a total volume of 500 μL, 2.5 μL of test substance solution or dimethyl sulfoxide, 50 μL of [^{3}H]citalopram or [^{3}H]8-OH-DPAT solution, and 447.5 μL of cell membranes were mixed. Cell membranes expressing human serotonin transporter and 5-HT_{1A} receptor were diluted with the reaction buffer to a final concentration of 1 unit/447.5 μL beforehand. All samples were reacted at 25°C for 0.5 h (for 5-HT_{1A}) or 1 h (for serotonin transporter) in an incubator. The reaction was terminated by addition of 4 mL ice-cold reaction buffer, and the cell membranes were collected by vacuum filtration through GF/B glass filters. The glass filters were then washed with 4 mL of ice-cold reaction buffer and placed in scintillation vials with scintillation fluid. After more than 3 h, the radioactivity in each sample was measured with a liquid scintillation counter for 2 min, and the calculated dpm value was used for data analysis. In the serotonin transporter binding assay, GF/B glass filters were soaked in 0.05% polyethylenimine solution for more than 15 min before use. The inhibition constant (Ki) was calculated in Microsoft® Office Excel 2003 (Microsoft Corporation) using the Cheng–Prusoff equation [$Ki = IC_{50}/(1 + ([L]/Kd)]$, where L is the concentration of radioligand in the assay and Kd is the dissociation constant of the radioligand for the receptor.

3-9-2-3. [^{3}H]5-HT uptake assay

Phosphate-buffered saline containing 0.1 mmol/L $CaCl_2$ and 1 mmol/L $MgCl_2$ was used as reaction buffer. Dimethyl sulfoxide (1 μL) or test substance was mixed with reaction buffer (199 μL), and 50 μL of the mixture was added to human serotonin transporter-expressing CHO cells cultured in 96-well assay plates. The plates were pre-incubated at 37°C for 10 min. During that time, dimethyl sulfoxide or test substance was diluted with [^{3}H]5-HT solution in another 96-well plate. After cells pre-incubation, 50 μL of the prepared [^{3}H]5-HT solution containing dimethyl sulfoxide or test substance was added to the wells, and the mixture was incubated at 37°C for 20 min. After incubation, the liquid layer was discarded, and the cells were rinsed twice with 200 μL reaction buffer before being lysed with 100 μL of the solvable solution. Radioactivity in each lysate sample was measured as described in the previous section.

3-9-2-4. Guanosine 5'-(γ-thio) Triphosphate, [^{35}S]-(GTPγS) assay for 5-HT_{1A} receptor

To make up a total volume of 500 μL, 2.5 μL of test compound, GTPγS (2 mM, to measure nonspecific binding), DMSO (to measure basal [^{35}S]GTPγS binding) or serotonin (20 mM, to measure maximal [^{35}S]GTPγS binding); 50 μL of reaction buffer [HEPES-NaOH buffer (20 mM, pH 7.4) containing 100 mM NaCl, 10 mM MgCl2, 0.1 mM DTT, and 1 μM GDP] containing 0.5 nM [^{35}S]GTPγS; and 447.5 μL of cell membranes expressing human 5-HT_{1A} receptors were mixed. The following manipulation was carried out as described in the above 5-HT transporter binding assay. Intrinsic activity was expressed as relative value of the activity of 100 μM serotonin, which was considered to be 100%.

3-9-2-4-1. Data analyses

The following formulae were used:

1) Basal [^{35}S]GTPγS binding

Basal [^{35}S]GTPγS binding (dpm) = Binding activity in the DMSO group (dpm) − Binding activity in the GTPγS group (dpm)

2) Maximal [^{35}S]GTPγS binding

Maximal [^{35}S]GTPγS binding (dpm) = Binding activity in the serotonin group (dpm) − Binding activity in the GTPγS group (dpm)

3) Specific binding of the test substance

Specific binding of the test substance (dpm) = Binding activity in test substance group (dpm) − Binding activity in GTPγS group (dpm)

4) Maximal specific binding

Maximal specific binding of the test substance was determined using Dx calculation (logistic curve fitting) with the "measurement value input" function in Stat Preclinica Client Version 1.0. The direct estimation method was used. Maximal specific binding was calculated using the logistic curve of test substance concentrations and the specific binding values.

5) Intrinsic activity of the test substance

When increment in maximal [^{35}S]GTPγS binding (Maximal [^{35}S]GTPγS binding − Basal [^{35}S]GTPγS binding) was considered as 100%, intrinsic activity of the test substance, which is equivalent to percentage increment in maximal specific binding of the test substance (Maximal specific binding of test substance − Basal [^{35}S]GTPγS binding), was calculated using the following formula.

Intrinsic activity of the test substance (%) = 100 × {[Maximal specific binding of test substance (dpm) − Basal [^{35}S]GTPγS binding (dpm)]／[Maximal [^{35}S]GTPγS binding (dpm) − Basal [^{35}S]GTPγS binding (dpm)]}

3-9-2-5. Assay for CYP2D6 metabolic contribution in human liver microsomes

Potassium phosphate buffer solution (0.2 mL, 50 mM, pH7.4) containing NADPH (final concentration: 3 mM, Oriental Yeast Co., Ltd.), 1 mg/mL human liver microsomes (XENOTECH, LLC), and a 1 μM test substance was heated in a water bath set at 37°C. After 15 ~ 30 minutes, methanol was added in a volume 3 times that of the reaction solution, and the mixture was stirred to terminate the reaction. The reaction solution was next centrifuged for protein precipitation, and the supernatant was collected and subjected to LC-MS/MS analysis. The results were as follows.

- The test substance was quantified, and time-dependent change in the amount of the substance remaining was logarithmically plotted. Metabolic rate was calculated from the slope
- The ratio of metabolic rate obtained by addition of quinidine (final concentration: 4 μM) to the reaction solution to metabolic rate obtained without quinidine addition was used as the

rate of metabolic contribution of enzymes other than CYP2D6. The rate of metabolic contribution obtained by subtracting the rate of contribution of other enzymes from the total was used as the rate of CYP2D6 metabolic contribution. Specifically, it was calculated according to the equation [rate of contribution (%) = {1 - (metabolic rate [with quinidine] / metabolic rate [without quinidine])} x 100].

3-9-2-6. Rat microdialysis

3-9-2-6-1. Surgery

This experiment was performed using 5–6-week old male rats (Crlj:WI). A vertical guide cannula (AG-04; EICOM) was implanted in the right side of the frontal cortex (3.7 mm anterior, 3.0 mm lateral, and 1.5 mm ventral from the bregma) of the rat under pentobarbital anesthesia [80 mg kg-1, intraperitoneal (i.p.)]. Microdialysis was conducted on the day after surgery. A dialysis probe (A-I-4-03; EICOM) was inserted into the guide cannula under light anesthesia with isoflurane and continuously perfused by Ringer solution (147 mmol/L NaCl, 4 mmol/L KCl, 2.3 mmol/L $CaCl_2$) at 2 lL min-1 using a microsyringe pump. Microdialysate samples (10 lL) were continuously collected for 5 min at 20-min intervals and automatically injected into the HPLC system. Compounds or vehicle were orally administered to the rats at least 3 h after the start of perfusion, that is, when stable HPLC baseline values for 5-HT were obtained in the dialysate samples. Measurement continued for 3 h after drug or vehicle administration.

3-9-2-6-2. Chromatography

The collected microdialysate samples (10 μL) were separated by HPLC using a PP-ODS column (EICOM) and a mobile phase containing 0.1 mol/L phosphate buffer (pH 6.0), 1% methanol, 50 mg L^{-1} ethylenediamine tetraacetic acid disodium, and 500 mg L^{-1} sodium 1-decanesulfonateat a flow rate of 0.5 mL min^{-1}. The peaks corresponding to 5-HT and dopamine were amperometrically detected using a graphite electrode set at 400 mV with an Ag/AgCl reference electrode (RE-100; EICOM). Online data acquisition was performed using PowerChrom software (Version 2.2; AD Instruments Pty Ltd., Nagoya, Aichi, Japan). Before performing the microdialysis, the retention time of the HPLC peak for 5-HT was determined using a standard solution. The peak height (mV) of 5-HT at each measurement was converted into a percentage of the average of the last 4 pre-drug baseline values (percentage of baseline).

3-10. 引用文献

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第 4 章　**DSP-1053** の重要中間体クロマン－４－オン誘導体の製法検討

4-1. 重要中間体 **93** の探索研究時の合成法とその課題

セロトニン取り込阻害（SRI）活性と 5-HT_{1A} 自己受容体阻害活性を併せ持つ薬剤の探索研究の結果、**DSP-1053** に代表されるクロマン－４－オン構造を有する誘導体がセロトニントランスポーター（SERT）と 5-HT_{1A} 受容体に対する強力な結合阻害活性を併せ持つことを見出した。そこで、共通の重要中間体 **93** のスケールアップ可能な実践的合成方法が必要となった（Figure 27）。一般的にクロマン－４－オンの構築には強酸性または強塩基性条件下での環化反応が必要である。また、既報の合成法はいずれも中程度の収率であり、実践的な合成法の報告はなかった（Scheme 24）。[1-4] 安定性が懸念される中間体 **93** の製造においては、いかに温和な条件下でクロマノン環を構築するかがポイントとなる。その点を考慮し、重要中間体 **93** の合成法検討を開始した。

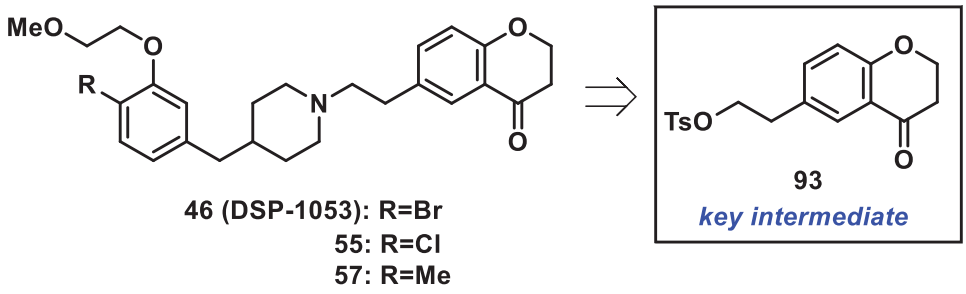
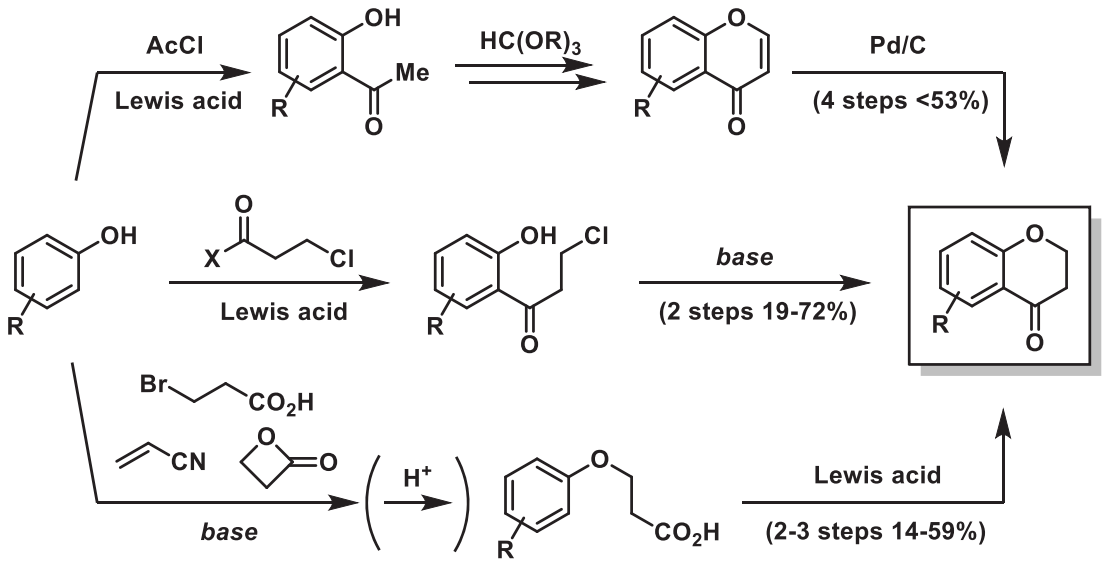


Figure 27. SERT/5-HT_{1A} dual inhibitors と重要中間体 **93**



Scheme 24. クロマン－４－オンの一般的合成法

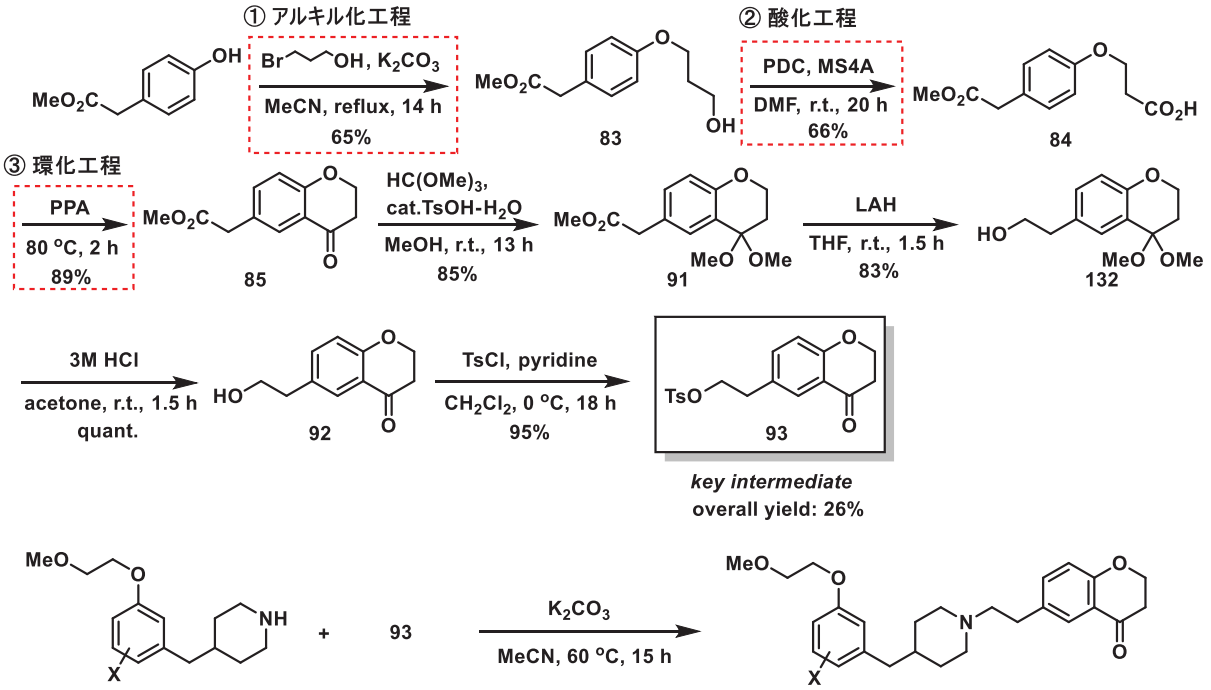
以下に探索研究時の合成法を示す。methyl 4-hydroxyphenylacetate を出発原料とし、3-bromo-1-propanol とのアルキル化反応によりアルコール **83** へと導いた後、PDC を用いた酸化反応によりカルボン酸 **84** へと変換した。続いて、カルボン酸 **84** の PPA を用いた環化反応によりケトン **85** を得た。ケトン **85** からアセタール保護を行い、水素化

リチウムアルミニウムによる還元反応、塩酸による脱保護反応、さらに、トシル化反応を行うことで重要中間体 **93** を合成するという方法である（Scheme 25）。本合成法での課題は、以下の通りである。

① アルキル化工程（methyl 4-hydroxyphenylacetate→**83**）：
収率が中適度である（65%）。また、不均一反応系であるため収率・反応終了時間等の再現性が低い。

② 酸化工程（**83**→**84**）：
収率が中程度である（66%）。毒性の高い PDC を使用している。また、そのため、大量の廃クロムが発生し、煩雑な後処理が必要である。

③ 環化工程（**84**→**85**）：
PPA の高い粘性により煩雑な仕込み・後処理が必要となる。また、大量のリン酸廃液が発生する。



Scheme 25. 探索研究時の合成法

課題の改善に向け、まず、反応試剤の最適化を中心に検討を開始した。

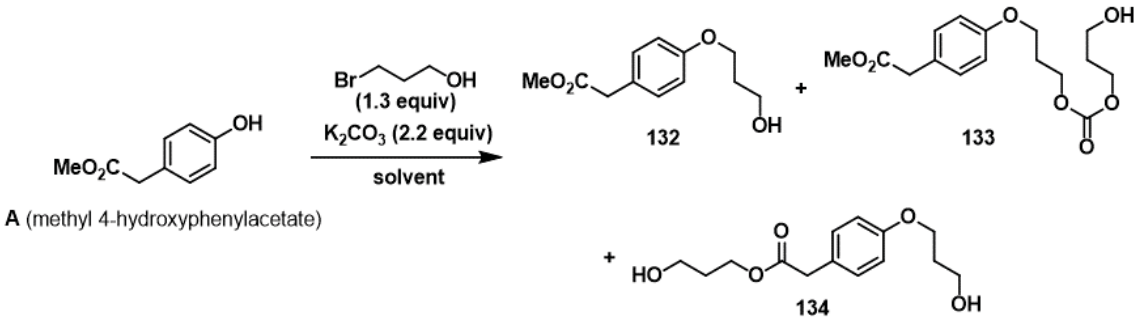
4-2. 重要中間体 **93** の第一世代合成法の確立

4-2-1. アルキル化工程（methyl 4-hydroxyphenylacetate→**83**）の検討

Methyl 4-hydroxyphenylacetate から **83** へのアルキル化工程における収率の向上・低い再現性の改善を目的とし、まず、溶媒の検討を行った（Table 8）。アセトン、２－ブタノン、THF、DMF、DMSO といった種々の溶媒への変更検討を行ったが、いずれの溶媒においても副生成物 **133** と **134** の生成量が増え、[5] アセトニトリルを上回る結果は得られなかった（Table 8, entry 1 ~ 6）。続いて、溶媒にアセトニトリルを用いた場合に、新たに開封した炭酸カリウムを使用したところ反応の遅延や副生成物 **133** と

134 の生成量の増大が見られ、用いる炭酸カリウムの開封時からの期間に応じて反応の進行速度や副生成物の生成量に違いが生じることがわかった（Table 8, entry 7）。炭酸カリウムは吸湿性を有し、本アルキル化工程のような不均一系の反応においてしばしば再現性の低さの原因となることが知られている。そこで、反応系中の含水量をコントロールすることで再現性の低さを改善できると考えた。反応系中の含水量 0.5%, 1.0%, 2.0%の各条件で反応を行ったところ、含水量 1.0%において、最も反応が速やかに進行し、副生成物 **133** と **134** の生成量が抑えられた（Table 8, entry 8 ~ 10）。また、反応系中の含水量を 1.0%にコントロールすることで、反応スケールを 1 g から 75 g に上げても再現性良く反応が進行することを確認した（Table 8, entry 11）。

Table 8. アルキル化工程の検討



entry[a]	solvent	Temp., °C	HPLC purity @ 3 h (%)			
			132	A	133	134
1	CH_3CN	reflux	92	-	3.9	1.4
2	acetone	reflux	55	41	-	0.49
3	2-butanone	reflux	79	17	1.8	0.91
4	THF	reflux	12	82	-	-
5	DMF	80	61	17	16	0.62
6	DMSO	80	54	25	13	1.4
7[b,c]	CH_3CN	reflux	75	17	4.8	0.20
	(crude)[e]	reflux	83	0.43	12	0.24
8[b]	0.5% (v/v)-H_2O / CH_3CN	reflux	94	0.32	1.9	1.7
9[b]	1.0% (v/v)-H_2O / CH_3CN	reflux	96	-	1.2	1.7
10[b]	2.0% (v/v)-H_2O / CH_3CN	reflux	93	-	0.46	2.4
11[b,d]	1.0% (v/v)-H_2O / CH_3CN	reflux	95	-	0.66	0.44

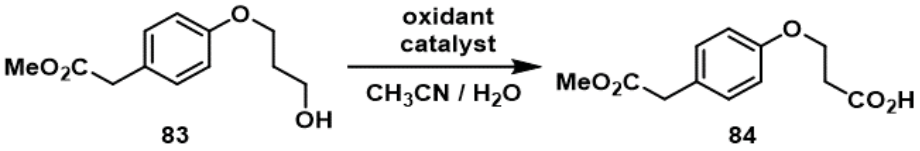
[a] Screening was performed at a 1 g scale in freshly opened CH_3CN.
[b] Freshly opened potassium carbonate was used. [c] 50 g scale. [d] 75 g scale. [e] Refluxed for 9 h

4-2-2. 酸化工程（**83**→**84**）の検討

探索研究時の合成法では、**83** から **84** への酸化工程において、PDC を用いており、

収率が中程度であり、大量の廃クロムが発生し煩雑な後処理を必要とするといった課題があった。そこで、本課題の改善のため、酸化剤の変更検討を行った（Table 9）。まず、$NaIO_4$ と $RuCl_3$ を用いた酸化反応を検討したところ、PDC を用いた場合と比べ収率が向上した（Table 9, entry 2）。[6] しかし、高価なルテニウムを使用しており、また、環境負荷の観点からさらなる改善が必要と考え、2,2,6,6-tetramethylpiperidine-1-oxyl（TEMPO）を触媒として用いる種々の酸化反応を検討した。酸化剤として、(diacetoxyiodo)benzene（DAIB）[7]、trichloroisocyanuric acid（TCCA）[8]、$NaClO_2$ を用いそれぞれ反応を行った結果、TEMPO/NaClO を触媒とし $NaClO_2$ を酸化剤として用いる酸化反応において極めて高い収率で目的の **84** が得られることがわかった（Table 9, entry 3~5）。[9]

Table 9. 酸化工程の検討

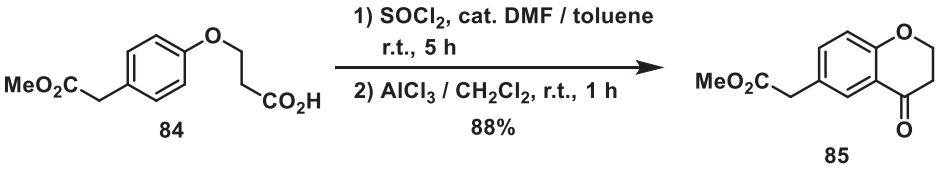


entry[a]	oxidant (equiv)	catalyst (mol%)	yield (%)[b]
1	PDC (3.5)	none	66
2	$NaIO_4$ (4)	$RuCl_3$ (3)	85
3	DAIB (2)	TEMPO (10)	30
4	TCCA (2)	TEMPO (1)-NaBr (5)	<5
5	$NaClO_2$ (2)	TEMPO (7)-NaClO (2)	98

[a] Chromatographic purified alcohol **83** was used. [b] Isolated yield.

4-2-3. 環化工程（**84**→**85**）の検討

84 から **85** の探索研究時の環化工程は PPA を用いており、その高い粘性により煩雑な仕込みと後処理が必要となり、また、大量のリン酸廃液が発生するといった課題があった。そこで、$SOCl_2$ を用い **84** を酸塩化物へと変換した後、$AlCl_3$ と作用させることで速やかに環化体 **85** が高収率で得られることがわかった（Scheme 26）。

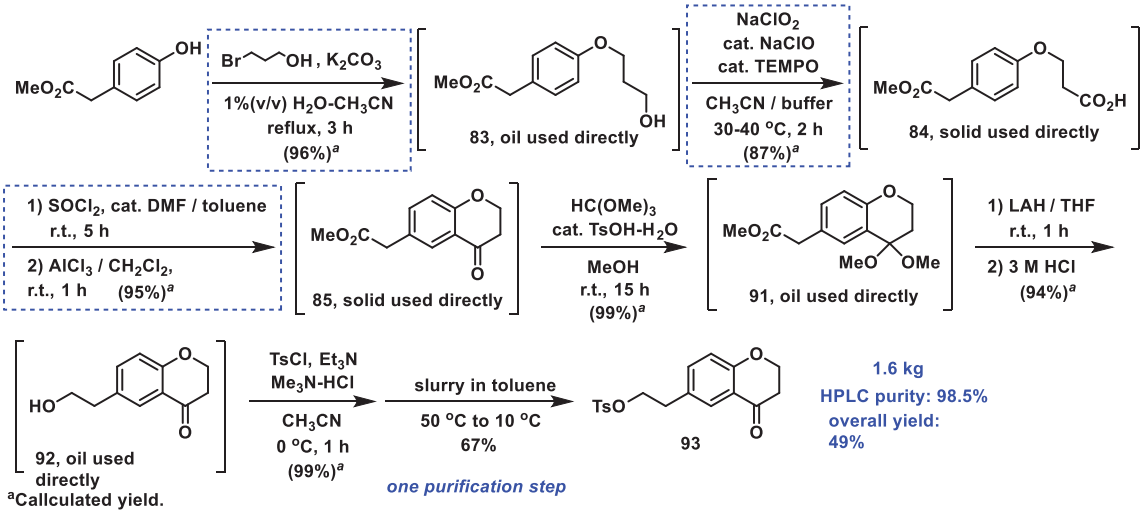


Scheme 26. 環化工程の検討

4-2-4. 第一世代合成法による重要中間体 **93** の合成

課題のあったアルキル化工程・酸化工程・環化工程における改善検討結果を踏まえ、

methyl 4-hydroxyphenylacetate を出発原料とした重要中間体 **93** のキログラムスケールでの合成を実施した。いずれの工程においても反応は再現性良く速やかに進行し、アルコール **92** のトシル化反応後のトルエン中でのトリチュレーションといった精製ステップを一度行うのみで、高純度（HPLC 純度:98.5%）の **93** を総収率 49%で 1.6 kg 合成することができた（Scheme 27）。探索研究時の合成法と本第一世代合成法を比較すると、28%から 49%へと総収率の大幅な向上に成功した。

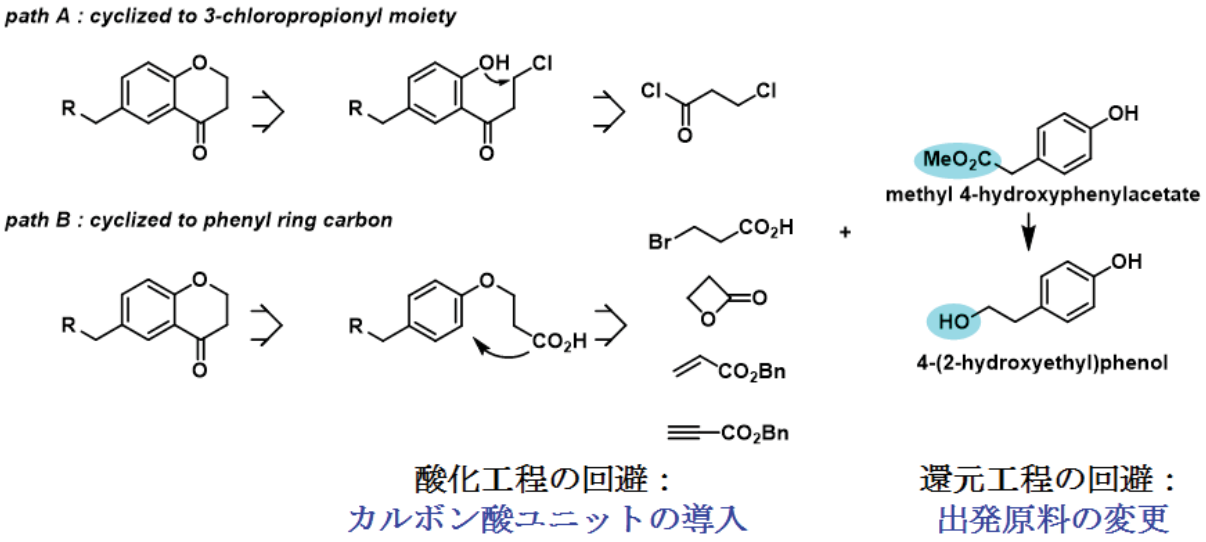


Scheme 27. 第一世代合成法による **93** の合成

4-3. 重要中間体 **93** の第二世代合成法の確立

4-3-1. 第二世代合成法確立のためのルート戦略

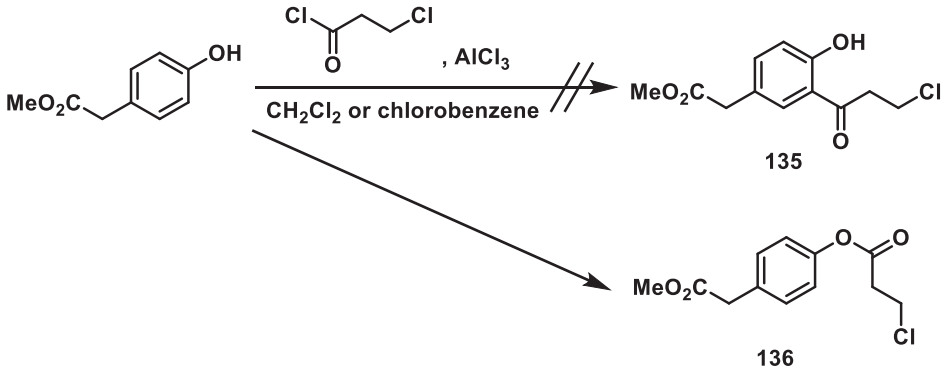
キログラムスケールで重要中間体 **93** を合成可能とする第一世代合成法を確立したが、保護・脱保護工程（**85**→**91**, **91**→**92**）や酸化・還元工程の回避といったさらなる改善すべき課題があった。マルチキログラムスケールに適応可能な合成法の確立を目指し、ルート短縮や総収率の向上によるさらなる合成の効率化のため、抜本的な製法変更に着手した。保護・脱保護工程や酸化・還元工程の回避のためのルート戦略を Scheme 28 に記した。すなわち、3-bromopropan-1-ol のようなアルコール原料ではなく、カルボン酸・酸塩化物・エステルといったカルボン酸ユニットを有する原料を用いクロマンー４ーオン環が構築できれば酸化工程の回避が可能となる。ルートとしては 3-chloropropionyl chloride を原料とするベンゼン環へのアシル化反応を経た *O*-アルキル化により環を構築する path A と 3-bromopropanoic acid、β-propiolactone、benzyl acrylate、あるいは、benzyl propiolate を原料とする *O*-アルキル化反応の後に Friedel-Crafts 反応により環を構築する path B が考えられる。さらに、出発原料を methyl 4-hydroxyphenylacetate から 4-(2-hydroxyethyl)phenol に変更できれば還元工程の回避が可能となると考えた。



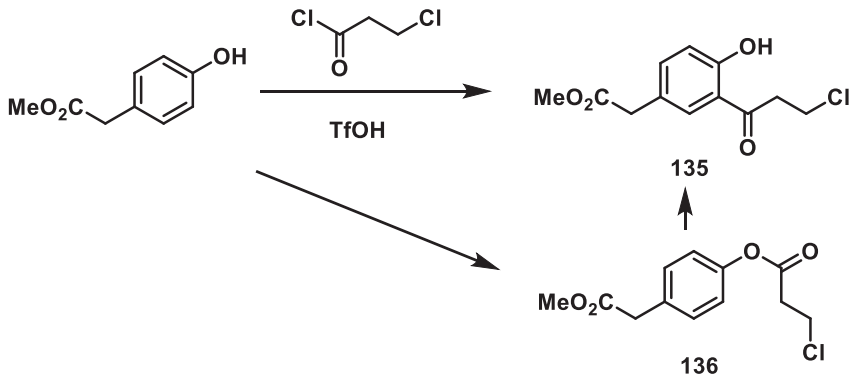
Scheme 28. 抜本的な製法変更のためのルート戦略

4-3-2. Path A（3-chloropropionyl chloride を用いたクロマン－4－オン環構築）の検討

Methyl 4-hydroxyphenylacetate を出発原料とする path A の検討を行った。$AlCl_3$ 存在下、methyl 4-phenylacetate と 3-chloropropionyl chloride を CH_2Cl_2 または chlorobenzene 中で加熱還流したところ、目的とする **135** は得られず、*O*-アシル化反応が進行した **136** が得られるのみであった（Scheme 29）。[10] また、methyl 4-hydroxyphenylacetate と 3-chloropropionyl chloride を TfOH 中で作用させたところ、**136** からの Fries 転位を経て目的の **135** が得られた（Scheme 30）。しかし、強酸の TfOH 中での反応であることから、複数の副生成物が生成し、反応が複雑になり、カラムクロマトグラフィーによる精製が必要であり、単離収率も 37%と低収率であった。本検討結果より、マルチキログラムスケールでの製造には本合成ルートは不適であると判断し、検討を中断した。



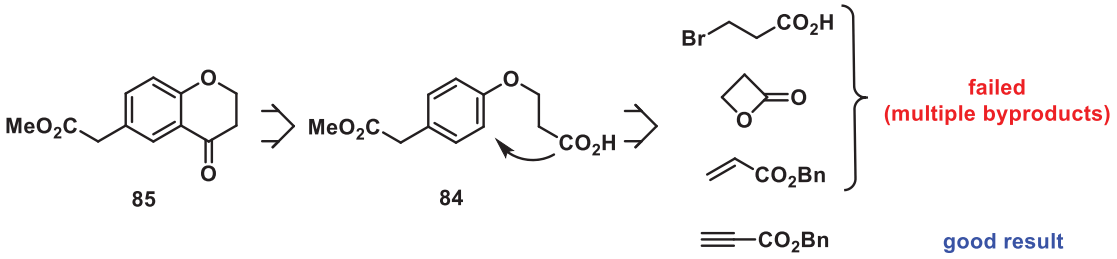
Scheme 29. path A の検討 - 1



Scheme 30. path A の検討 - 2

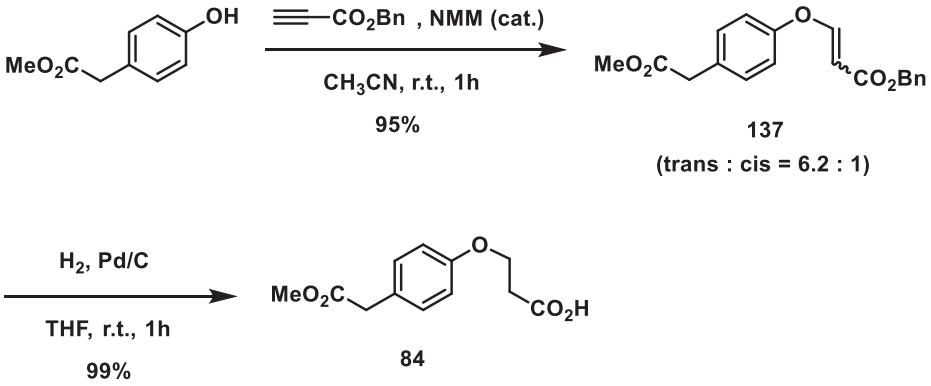
4-3-2. Path B（*O*-アルキル化－Friedel-Crafts 反応によるクロマン－4－オン環構築）の検討

Methyl 4-hydroxyphenylacetate を出発原料とする path B の検討を行った。まず、methyl 4-hydroxyphenylacetate と 3-bromopropanoic acid [3] あるいは β-propiolactone [11] との塩基性条件下でのアルキル化反応や benzyl acrylate との Michael 付加反応を試みたが、methyl 4-phenylacetate の活性メチレンへの反応等の副反応が進行し、反応は複雑になった。[12] 一方、benzyl propiolate との *N*-methyl morpholine (NMM)を塩基として用いた Michael 付加反応は速やかに進行し、大きな副反応が進行することもなかった（Scheme 31）。[13] そこで、benzyl propiolate を原料とする path B の詳細な検討を開始した。



Scheme 31. path B の検討

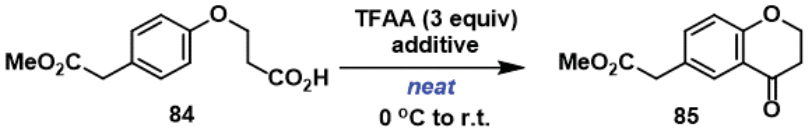
Methyl 4-hydroxyphenylacetate と benzyl propiolate との Michael 付加反応は、アセトニトリル中、NMM を触媒量用いることで速やかに進行し、高収率で **137** を得ることができた。続いて、THF 中、パラジウム炭素による水素添加反応を行うことで **137** をカルボン酸 **84** へと高収率で変換することができ、酸化工程を回避した methyl 4-hydroxyphenylacetate からの **84** の合成法を確立することができた（Scheme 32）。



Scheme 32. 酸化工程を経ない **84** の合成

第一世代合成法では、カルボン酸 **84** から酸塩化物を経由し $AlCl_3$ を用いた Friedel-Crafts 反応により環化体 **85** を得ていた。酸塩化物を経由しない **84** の直接的かつ温和な環化反応条件を見出すことができれば、さらなる合成の効率化が可能となる。そこで、**84** の環化工程の検討を行った（Table 10）。**84** と TFAA（3 当量）を作用させたところ、目的の **85** は生成するものの反応の進行は遅かった。[14,15] しかしながら、そこへ TFA（3 当量）を加えると反応が劇的に加速されることがわかった（Table 10, entry 2~3）。次に、添加する酸の当量を減らすべく種々の酸を検討した。その結果、添加する酸の当量を 0.1 当量に減らしたところ TFA や AcOH では反応の加速効果はあまり見られないが、H_3PO_4 では、0.1 当量に添加する量を減らしても反応の加速効果が見られ、速やかにかつ高収率で **85** が得られることがわかった（Table 10, entry 4~6）。さらに、本検討で見出された **84** と TFAA との混合物に 0.1 当量の H_3PO_4 を添加する環化反応条件は、非常に温和なため、**84** のメチルエステル基がトシラート基に変わった **139** においても問題なく適応可能であることがわかった。

Table 10. 環化工程の検討

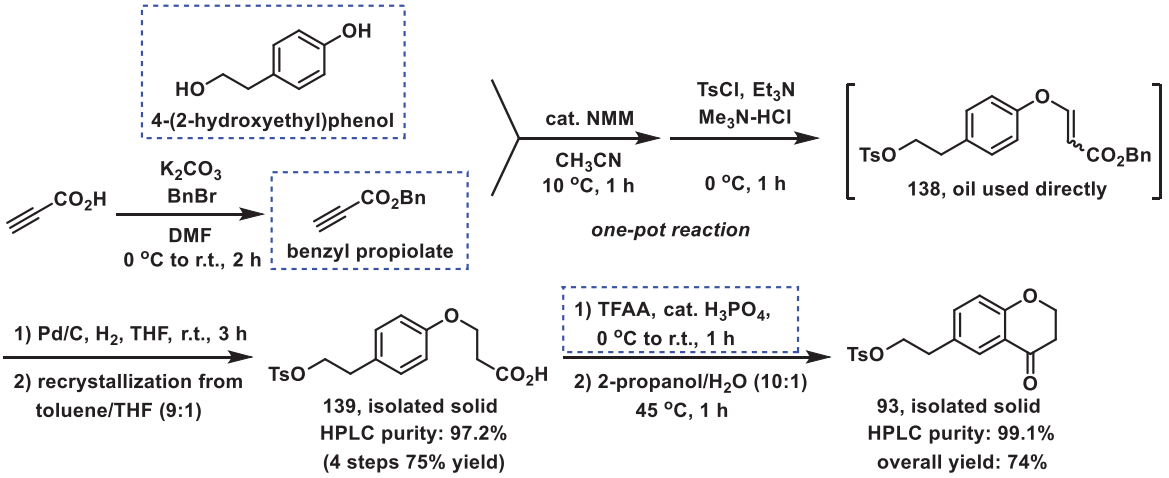


entry	additive (equiv)	HPLC purity 85 / 84 @ 1h (%)	yield (%)[c]
1[a]	-	94.6 / -	88
2	none	2.72 / 95.2[b]	-
3	TFA (3)	90.2 / 7.41	-
4	TFA (0.1)	7.14 / 90.8[b]	-
5	H_3PO_4 (0.1)	98.1 / -	97
6	AcOH (0.1)	5.12 / 92.9[b]	-

[a] Data obtained by the use of $AlCl_3$ in first-generation synthesis.
[b] Reaction did not reach completion after 8 h. [c] Isolated yield.

4-3-3. 第二世代合成法による重要中間体 **93** の合成

上記検討により見出された TFAA-H_3PO_4(cat.)による環化反応を鍵反応とする 4-(2-hydroxyethyl)phenol からの重要中間体 **93** の合成を行った（Scheme 33）。Propiolic acid から調整可能な benzyl propiolate と 4-(2-hydroxyethyl)phenol との Michael 付加反応は、phenol の酸素原子上に高選択的かつ速やかに進行し、さらに、トシル化反応を行うことで、one-pot で 4-(2-hydroxyethyl)phenol から **138** を得ることができた。続いて、**138** のパラジウム炭素による水素添加反応を行い、トルエン/THF の混合溶媒中で再結晶を行うことで、高純度の **139** を高収率（4-(2-hydroxyethyl)phenol からの 4 段階収率: 75%）で合成することができた。さらに、TFAA 中、触媒量の H_3PO_4 を用いた **139** の環化反応を行い、得られた生成物を 2ープロパノール/水の混合溶媒中でトリチュレーションすることで、高純度の **93** を得ることができた。本第二世代合成法は、第一世代合成法よりも、総収率が 49%から 74%へと向上し、全 8 段階から 5 段階へと合成ルートの短縮化を可能とした。本第二世代合成法は、高効率かつ簡便な合成法であるため、マルチキログラムスケールでの **93** の合成にも適応可能であり、より詳細に条件を最適化した本合成法により、**DSP-1053** の臨床開発用原薬の合成が実施された。



Scheme 33. 第二世代合成法による **93** の合成

4-4. 考察ならびに小括

セロトニン取り込阻害（SRI）活性と 5-HT_{1A} 自己受容体阻害活性を併せ持つ薬剤の探索研究の結果、**DSP-1053** に代表されるクロマンー４ーオン構造を有する誘導体がセロトニントランスポーター（SERT）と 5-HT_{1A} 受容体に対する強力な結合阻害活性を併せ持つことを見出した。そこで、共通の重要中間体 **93** のスケールアップ可能な実践的合成方法の構築のため、検討を開始した。探索研究時の合成方法の課題であった①アルキル化工程・②酸化工程・③環化工程の各ステップは、①反応系の水分含量の精密なコントロール・②酸化剤を $NaClO_2$-TEMPO/NaClO(cat.)へ変更・③酸塩化物を経由する $AlCl_3$ を用いた環化反応へと主に反応試剤を変更することで改善することができた（重要中間体 **93** の第一世代合成法）。さらに、第一世代合成法からのさらなる総収率の向上・反応工程の短縮化を目指し、抜本的なルート変更を検討した。出発

原料を Methyl 4-hydroxyphenylacetate から 4-(2-hydroxyethyl)phenol へと変更することで、第一世代合成法では必須であったメチルエステルの還元工程を回避することができた。また、クロマンー４ーオン構造の構築にはカルボン酸ユニットの導入が必要であるが、アルコール 3-bromopropan-1-ol からエステル benzyl propiolate へと変更することで、アルコールの酸化工程を回避することができた。さらに、TFAA-H_3PO_4(cat.)を用いた温和な環化反応を見出すことでカルボン酸から直接クロマンー４ーオン構造を構築することができるようになった。以下に、第一世代合成法・第二世代合成法の構築による改善効果をまとめる。

・探索研究時の合成法から第一世代合成法への変更による改善効果

総収率：28%から 49%へ向上。

精製工程：カラム精製を回避し、かつ、**93** のトルエン中でのトリチュレーションのみで高純度の **93** が合成可能。

・第一世代合成法から第二世代合成法への変更による改善効果

総収率：49%から 71%へ向上。

全反応工程数：8 段階から 5 段階へ短縮。

治療薬創出による世の中への貢献、また、特許期間を考慮し可能な限り長い独占販売期間の確保といった観点で、創薬研究開発では、できる限りの早期の上市を目指すために開発化合物を見出してから非臨床試験における安全性試験までの期間を短くする必要がある。非臨床試験における安全性試験を早期に行うためには探索研究時の合成法や知見を大いに活用し、キログラムスケールでの実施可能な合成法を構築し、そしてサンプル製造を行い、検討期間を確保した後、商業化を見越した抜本的なルート改善検討を行うのが望ましい。私が実践した重要中間体 **93** の製法検討は、まさに理想的な形で実施でき、**DSP-1053** の研究開発の促進に大いに貢献することができた。

4-5. 実験の部

4-5-1. General

HPLC was performed on a Shimadzu UFLC system; shim-pack XR-ODS column (3.0 mm i.d. × 100 mm); gradient elution (0.05% TFA·H_2O/0.05% TFA·CH_3CN 90:10 to 10:90 over 6 min, hold 2 min); flow rate = 1.0 mL/min, T = 40 °C, UV detection at 220 nm. The purity listed is determined by area %. Melting points were determined on an electrothermal apparatus without correction. NMR spectra were recorded on a JEOL JNM-LA300 spectrometer. Chemical shifts (δ) are given in parts per million, and tetramethylsilane was used as the internal standard for spectra obtained in $CDCl_3$ or DMSO-d_6. IR spectra were recorded on a JEOL JIR-SPX60 spectrometer as ATR. High-resolution MS spectra were recorded on a Thermo Fisher Scientific LTQ orbitrap Discovery MS equipment. Elemental analysis was performed on a CE Instrument EA1110 and a Yokokawa analytical system IC7000. Column chromatography was carried out using a Yamazen W-prep system. All reactions were carried out under nitrogen atmosphere unless otherwise mentioned. Reagents and solvents were used as obtained from commercial suppliers without further purification.

4-5-2. First-Generation Synthesis (Scheme 27)

4-5-2-1. Methyl [4-(3-Hydroxypropoxy)phenyl]acetate (**83**)

To a solution of methyl (4-hydroxyphenyl)acetate (964 g, 5.80 mol) in CH_3CN (15.2 kg) was added water (193 kg), and the mixture was stirred for 15 min at ambient temperature; then potassium carbonate (1.76 kg, 12.8 mol, 2.2 equiv) and 3-bromo-1-propanol (1.05 kg, 7.55 mol, 1.3 equiv) were added. The mixture was refluxed for 3 h and then allowed to cool to ambient temperature. The precipitate was filtered off and washed with CH_3CN (3.80 kg). The combined filtrates were concentrated in vacuo. Then toluene (8.34 kg) and water (4.82 kg) were added to the residue, and the layers were separated. The aqueous layer was extracted with toluene (3.58 kg). The combined organic layers were washed with 0.5 M NaOH (1.93 kg) and 1% aqueous $KHSO_4$ solution (1.93 kg) and then concentrated in vacuo to give **83** (1.27 kg) as a yellow oil. In total 2.09 kg of **83** was prepared according to the above-described procedure. It was used for the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.13 (d, J = 8.6 Hz, 2H), 6.82 (d, J = 8.6 Hz, 2H), 4.00 (t, J = 6.1 Hz, 2H), 3.72 (t, J = 5.9 Hz, 2H), 3.62 (s, 3H), 3.51 (s, 2H), 3.44 (br, 1H), 1.94 (t, J = 6.1 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.2, 157.6, 129.9, 125.6, 114.1, 64.6, 58.9, 51.6, 39.7, 31.6; IR (ATR) 3390, 1732, 1512 cm^{-1}; HRMS (ESI) m/z calcd for $C_{12}H_{17}O_4$ $[M + H]^+$ 225.1121, found 225.1117.

4-5-2-2. Methyl [4-(3-{[(3-Hydroxypropoxy)carbonyl]oxy}propoxy)-phenyl]acetate (**133**)

Analytically pure **133** was obtained by silica gel chromatography. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.18 (d, J = 8.4 Hz, 2H), 6.84 (d, J = 8.3 Hz, 2H), 4.33 (t, J = 6.2 Hz, 2H), 4.27 (t, J = 6.2 Hz, 2H), 4.04 (t, J = 6.0 Hz, 2H), 3.73−3.65 (m, 2H), 3.67 (s, 3H), 3.56 (s, 2H), 2.42 (br, 1H), 2.13 (quin, J = 6.1 Hz, 2H), 1.89 (quin, J = 6.1 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.3, 157.6, 155.2, 130.1, 126.0, 114.4, 64.8, 64.6, 63.7, 58.6, 51.9, 40.1, 31.4, 28.4; IR (ATR) 3446, 1736, 1512 cm^{-1}; HRMS (ESI) m/z calcd for $C_{16}H_{23}O_7$ $[M + H]^+$ 327.1438, found 327.1433.

4-5-2-3. 3-Hydroxypropyl [4-(3-Hydroxypropoxy)phenyl]acetate (**134**)

Analytically pure **134** was obtained by silica gel chromatography. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16 (d, J = 8.8 Hz, 2H), 6.84 (d, J = 8.8 Hz, 2H), 4.20 (t, J = 6.2 Hz, 2H), 4.06 (t, J = 6.1 Hz, 2H), 3.79 (t, J = 5.4 Hz, 2H), 3.63−3.55 (m, 2H), 3.54 (s, 2H), 2.80−2.60 (m, 2H), 1.99 (quin, J = 6.1 Hz, 2H), 1.81 (quin, J = 6.2 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.3, 157.8, 130.1, 126.0, 114.5, 65.3, 61.8, 59.8, 58.8, 40.3, 31.8, 31.4; IR (ATR) 3358, 1718, 1512 cm^{-1}; HRMS (ESI) m/z calcd for $C_{14}H_{21}O_5$ $[M + H]^+$ 269.1384, found 269.1379.

4-5-2-4. 3-[4-(2-Methoxy-2-oxoethyl)phenoxy]propanoic Acid (**84**)

To a solution of KH_2PO_4 (319 g, 2.34 mol) and $Na_2HPO_4 \cdot 12H_2O$ (838 g, 2.34 mol) in water (18.1 kg) were successively added **83** (1.27 kg, 5.65 mol) in CH_3CN (14.7 kg), TEMPO (111 g, 0.708 mol, 0.13 equiv), and NaClO (5% solution) (169 g, 0.113 mol, 0.02 equiv). After the

mixture was heated to 30−40 °C (internal temperature), a solution of $NaClO_2$ (80%) (1.28 kg, 11.3 mol, 2 equiv) in water (5.63 kg) was slowly added over 1 h. Then the mixture was kept at 30−40°C for 2.5 h and cooled to 0−5 °C. A solution of 20% aqueous $NaHSO_3$ solution (9.38 kg) was added dropwise over 1 h, keeping the temperature lower than 10 °C during the addition (CAUTION: generated sulfur dioxide should be trapped by aqueous NaOH solution.). The mixture was warmed to room temperature, and then the biphasic system was separated. The aqueous layer was extracted with EtOAc (8.46 kg), and the combined organic layers were concentrated in vacuo. The resultant precipitate was added to water (2.82 kg), collected by filtration, washed with water (2 × 1.41 kg), and dried in vacuo to give **84** (1.11 kg) as a white solid. In total 1.93 kg of **84** was prepared according to the above-described procedure. It was used for the next step without further purification. Mp 112 °C; ^{1}H NMR (300 MHz, DMSO-D_6) δ 12.39 (br, 1H), 7.17 (d, J = 8.4 Hz, 2H), 6.87 (d, J = 8.6 Hz, 2H), 4.14 (t, J = 6.0 Hz, 2H), 3.60 (s, 3H), 3.60 (s, 2H), 2.69 (t, J = 6.1 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 172.4, 172.0, 157.3, 130.5, 126.5, 114.3, 63.6, 51.7, 39.3, 34.2; IR (ATR) 1734, 1691, 1514 cm^{-1}; HRMS (ESI) m/z calcd for $C_{12}H_{15}O_5$ $[M + H]^+$ 239.0914, found 239.0910; Anal. Calcd for $C_{12}H_{14}O_5$: C, 60.50; H, 5.92. Found: C, 60.50; H, 5.83.

4-5-2-5. Methyl (4-Oxo-3,4-dihydro-2H-chromen-6-yl)acetate (**85**)

Thionyl chloride (1.16 kg, 9.74 mol, 1.2 equiv) was added dropwise over 0.5 h to a suspension of **84** (1.93 kg, 8.11 mol) in toluene (16.7 kg) and DMF (18.3 g). The mixture was stirred at ambient temperature for 5 h and concentrated in vacuo. Toluene (8.60 kg) was added to the residue, and the resultant solution was concentrated in vacuo (twice) to give the corresponding acid chloride (2.08 kg) as a yellow oil. The residue was dissolved in CH_2Cl_2 (8.28 kg), and then added dropwise over 1 h to a suspension of $AlCl_3$ (2.16 kg, 16.2 mol, 2 equiv) in CH_2Cl_2 (19.3 kg) at ambient temperature. The mixture was stirred for 1.5 h and added dropwise over 2 h to cooled (0−5 °C) 2 M HCl (20.8 kg), keeping the temperature lower than 20 °C during the addition. The mixture was warmed to ambient temperature, and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (5.52 kg). The combined organic layers were successively washed with water (4.15 kg), 5% aqueous $NaHCO_3$ solution (4.16 kg), and water (4.16 kg), and then concentrated in vacuo. MeOH (3.28 kg) was added to the residue, and the mixture was concentrated in vacuo to give **85** (1.71 kg) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77 (d, J = 2.4 Hz, 1H), 7.41 (dd, J = 8.5, 2.3 Hz, 1H), 6.95 (d, J = 8.4 Hz, 1H), 4.52 (t, J = 6.5 Hz, 2H), 3.69 (s, 3H), 3.59 (s, 2H), 2.80 (t, J = 6.4 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 191.5, 171.6, 160.9, 136.9, 127.4, 127.0, 121.0, 118.1, 66.9, 52.0, 39.9, 37.5; IR (ATR) 1720, 1682, 1140 cm^{-1}; HRMS (ESI) m/z calcd for $C_{12}H_{13}O_4$ $[M + H]^+$ 221.0808, found 221.0805.

4-5-2-6. 6-(2-Hydroxyethyl)-2,3-dihydro-4H-chromen-4-one (**92**)

To a suspension of **85** (1.71 kg, 7.72 mol) in MeOH (4.03 kg) were added trimethyl

orthoformate (9.89 kg) and $TsOH \cdot H_2O$ (146 g, 0.766 mol, 0.1 equiv), and the reaction mixture was stirred 15 h at room temperature. After 5% aqueous $NaHCO_3$ solution (8.50 kg) was cooled down to 0−10 °C, the reaction mixture was added dropwise to that solution over 1 h. After the reaction mixture was warmed to room temperature, toluene (7.36 kg) was added, and then the layers were separated. The aqueous layer was washed with toluene (4.42 kg), and the combined organic layers were washed with water (3.40 kg) and then concentrated in vacuo. Toluene (1.50 kg) was added to the residue and concentrated in vacuo to give **91** (2.05 kg) as a yellow oil. ^{1}H NMR (300 MHz, DMSO-D_6) δ 7.36 (d, J = 2.2 Hz, 1H), 7.11 (dd, J = 8.4, 2.2 Hz, 1H), 6.75 (d, J = 8.4 Hz, 1H), 4.25 (t, J = 5.7 Hz, 2H), 3.61 (s, 2H), 3.59 (s, 3H), 3.17 (s, 6H), 2.09 (t, J = 5.8 Hz, 2H).

Obtained **91** (2.05 kg, 7.70 mol) in THF (3.64 kg) was added dropwise over 40 min to a suspension of LAH (439 g, 11.6 mol, 1.5 equiv) in THF (23.7 kg) at such a rate that the reaction temperature stayed in the range 20−30 °C. The mixture was stirred for 2 h and cooled down to around 5 °C. Water (294 g) in THF (145 g) and 3 M HCl (20.5 kg) were successively added dropwise over 2.5 h, keeping the reaction temperature lower than 15 °C during the addition. Toluene (17.7 kg) was added to the reaction mixture, and the layers were separated. The aqueous layer was washed with toluene (17.7 kg), and the combined organic layers were washed with 3 M HCl (4.1 kg) and water (8.2 kg) and then concentrated in vacuo to give **92** (1.39 kg) as a brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.70 (d, J = 2.2 Hz, 1H), 7.35 (dd, J = 8.4, 2.4 Hz, 1H), 6.90 (d, J = 8.4 Hz, 1H), 4.48 (t, J = 6.4 Hz, 2H), 3.81 (t, J = 6.7 Hz, 2H), 2.86 (br, 1H), 2.81 (t, J = 6.7 Hz, 2H), 2.75 (t, J = 6.5 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 192.1, 160.3, 136.9, 131.7, 126.6, 120.8, 117.8, 66.7, 63.0, 37.9, 37.5; IR (ATR) 3404, 1682, 1616 cm−1; HRMS (ESI) m/z calcd for $C_{11}H_{13}O_3$ $[M + H]^+$ 193.0859, found 193.0856.

4-5-2-7. 2-(4-Oxo-3,4-dihydro-2H-chromen-6-yl)ethyl 4-methylbenzenesulfonate (**93**)

To a solution of **92** (1.39 kg, 7.21 mol) in CH_3CN (10.9 kg) were added $Me_3N \cdot HCl$ (68.9 g, 0.721 mol, 0.1 equiv) and triethylamine (1.46 kg, 14.4 mol, 2 equiv). The mixture was cooled down to around 5 °C, and then tosyl chloride (1.65 kg, 8.64 mol, 1.2 equiv) in CH_3CN (5.44 kg) was added dropwise over 1 h. The reaction mixture was stirred for an additional 2 h, and then 5% aqueous $NaHCO_3$ solution (10.4 kg) was added dropwise over 1 h. Toluene (8.98 kg) and water (6.92 kg) were added, and the layers were separated. The aqueous layer was washed with toluene (8.98 kg), and the combined organic layers were washed with 1% aqueous $KHSO_4$ solution (10.4 kg) and 10% aqueous NaCl solution (10.3 kg) and then were dried over $MgSO_4$ (750 g). The solid was filtered off and washed with toluene, and then the filtrate was concentrated in vacuo to 7.2-fold volumes of estimated quantity of **93** (slurry in 6.46 kg of toluene). The suspension was then stirred at 50 °C for 1 h and cooled down to 10 °C over 1 h, held for 2 h, and then filtered. The solid was washed with cooled (5−10 °C) toluene (2 × 1.30 kg) and dried in a vacuum oven at 50 °C. The target compound **93** (1.64 kg, 49% yield) was obtained as a pale-yellow solid (98.5% area purity by HPLC). Mp 125 °C; ^{1}H

NMR (300 MHz, $CDCl_3$) δ 7.70 (d, J = 8.3 Hz, 2H), 7.60 (d, J = 2.2 Hz, 1H), 7.30 (d, J = 8.1 Hz, 2H), 7.25 (dd, J = 8.4, 2.4 Hz, 1H), 6.87 (d, J = 8.4 Hz, 1H), 4.51 (t, J = 6.4 Hz, 2H), 4.18 (t, J = 6.9 Hz, 2H), 2.90 (t, J = 6.9 Hz, 2H), 2.78 (t, J = 6.4 Hz, 2H), 2.44 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 191.5, 160.7, 144.7, 136.7, 132.7, 129.7, 129.2, 127.7, 126.8, 121.0, 118.1, 70.2, 66.9, 37.6, 34.2, 21.5; IR (ATR) 1684, 1493, 1169 cm^{-1}; HRMS (ESI) m/z calcd for $C_{18}H_{19}O_5S$ $[M + H]^+$ 347.0948, found 347.0938.

4-5-3. Second-Generation Synthesis (Scheme 33)

4-5-3-1. 3-[4-(2-{[(4-Methylphenyl)sulfonyl]oxy}ethyl)phenoxy]propanoicAcid (**139**)

To a suspension of potassium carbonate (12.0 g, 86.9 mmol, 1.2 equiv) in DMF (40 mL) were added propiolic acid (6.09 g, 86.9 mmol, 1.2 equiv) in DMF (20 mL) at 0−5 °C, and the reaction mixture was stirred for 10 min. Benzyl bromide (12.4 g, 72.4 mmol, 1.0 equiv) was added, and the mixture was warmed to 25 °C and stirred for 2 h. Then water (90 mL) was added to the residue at 0−5 °C. EtOAc·hexane (1:1) (60 mL) was added to the residue at 25 °C, and the layers were separated. The aqueous layer was extracted with EtOAc·hexane (1:1) (30 mL). The combined organic layers were washed with 5% aqueous NaCl solution (2 × 30 mL) and dried over Na_2SO_4. After filtration, the solvent was removed in vacuo to give benzyl propiolate as a yellow oil.

To a solution of 4-(2-hydroxyethyl)phenol (10.0 g, 72.4 mmol) in CH_3CN (100 mL) were added NMM (796 μL, 7.24 mmol, 0.10 equiv) and a solution of obtained benzyl propiolate in CH_3CN (20 mL) at 10 °C, and the reaction mixture was stirred for 1 h at 25 °C. The mixture was cooled down to 0−5 °C, and then triethylamine (20.1 mL, 145 mmol, 2.0 equiv), Me_3N·HCl (346 mg, 3.62 mmol, 0.050 equiv), and tosyl chloride (16.6 g, 86.9 mmol, 1.2 equiv) were added. The reaction mixture was stirred for additional 1 h at 0−5 °C, and then 3% aqueous $NaHCO_3$ solution (100 mL) was added. The organic solvent was removed in vacuo, toluene (100 mL) was added to the residue, and the layers were then separated. The aqueous layer was extracted with toluene (50 mL). The combined organic layers were washed with 5% aqueous NaCl solution (50 mL), 5% aqueous $KHSO_4$ solution (50 mL), and 5% aqueous NaCl solution (50 mL), and then dried over $MgSO_4$. After filtration, the solvent was removed in vacuo to give **138** as a yellow oil. To a solution of obtained **138** in THF (300 mL) was added 10% Pd/C (50% wet) (6.00 g), and the mixture was stirred under hydrogen atmosphere (1 atm) at 25 °C for 3 h. The reaction mixture was filtered through Celite, and the filtrate was concentrated in vacuo. The resulting solid was purified by recrystallization from toluene·THF (9:1) (300 mL) at 70 °C, and cooled down to 0−5 °C over 2 h and held for 1 h. The solid was filtered, washed with cooled (<5 °C) toluene, and dried in vacuo to give **139** (19.8 g, 75% yield) as a white solid (97.2% area purity by HPLC). Mp 119 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.68 (d, J = 8.3 Hz, 2H), 7.28 (d, J = 8.1 Hz, 2H), 7.01 (d, J = 8.4 Hz, 2H), 6.78 (d, J = 8.6 Hz, 2H), 4.21 (t, J = 6.1 Hz, 2H), 4.16 (t, J = 7.1 Hz, 2H), 2.88 (t, J = 6.8 Hz, 2H), 2.84 (t, J = 6.1 Hz, 2H), 2.43 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.9, 157.3, 144.6, 132.9,

129.9, 129.7, 128.7, 127.8, 114.7, 70.8, 63.0, 34.4, 34.3, 21.6; IR (ATR) 1695, 1512, 1171 cm^{-1}; HRMS (ESI) m/z calcd for $C_{18}H_{21}O_6S$ $[M + H]^+$ 365.1053, found 365.1045.

4-5-3-2. 2-(4-Oxo-3,4-dihydro-2H-chromen-6-yl)ethyl 4-methylbenzenesulfonate (**93**)

To a solution of TFAA (26.8 mL) and phosphoric acid (85%) (316 mg, 2.74 mmol, 0.10 equiv) was added **139** (10.0 g, 27.4 mmol) at 0−5 °C, and the mixture was stirred at 25 °C for 1 h. The reaction mixture was concentrated in vacuo. Toluene·THF (2:1) (150 mL) and water (100 mL) were added to the residue, and the layers were separated. The aqueous layer was extracted with toluene (50 mL). The combined organic layers were washed with 5% aqueous $NaHCO_3$ solution (2 × 50 mL) and 5% aqueous NaCl solution (50 mL) and dried over $MgSO_4$. After filtration, the solvent was removed in vacuo. To the resulting solid was added 2-propanol·H_2O (10:1) (55 mL), and the suspension was warmed to 45 °C and held for 1 h. After the mixture was cooled to 25 °C, water (95 mL) was added and stirred at 25 °C for 1 h. The mixture was cooled to 0−5 °C, held for 1 h, and then filtered. The solid was washed with water (20 mL) and dried in vacuo to give **93** (9.32 g, 98% yield) as a white solid (99.1% area purity by HPLC). Obtained analytical data are in complete accord with that of **93** obtained through first-generation synthesis.

4-6. 引用文献

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第５章　結語

大うつ病は、全世界人口の４％が罹患する世界で最も問題となっている疾患の一つであり、パロキセチンやフルオキセチンに代表されるセロトニン選択的再取り込阻害剤（SSRI）が、うつ病治療の第一選択薬として世界で広く使われている。脳内セロトニンの不足によるセロトニン神経伝達の低下がうつ病の原因の一つとするセロトニン仮説があり、SSRIはセロトニントランスポーター（SERT）を阻害することにより脳内セロトニン量を上げることで抗うつ作用を発揮すると考えられている。しかしながら、SSRIは、投薬開始から治療効果の発現まで２～３週間かかるという治療オンセットの遅さや薬物治療を受けたうつ病患者のおよそ３分の１に治療効果が表れないという問題点があり、これらの問題点を克服した新規抗うつ薬が望まれている。そんな中、5-HT_{1A}受容体阻害活性を持つピンドロールをSSRIと併用すると治療オンセットが早まるという臨床研究結果が報告された。SSRIの治療オンセットの遅さの原因として次の機構が考えられる。SSRI投与によりシナプス間隙で上昇したセロトニンが、シナプス前細胞に発現する5-HT_{1A}自己受容体に作用しネガティブフィードバック機構が働きセロトニン分泌が抑制される。従って、5-HT_{1A}自己受容体が脱感作して初めて脳内セロトニン量の上昇が起こるため、SSRIの連続投与が必要となる。ピンドロールは5-HT_{1A}自己受容体を拮抗するため、セロトニン神経のネガティブフィードバック機構が阻害されSSRIの治療オンセットが早まったと考えられる。そこで、SSRIの治療オンセットの遅さという問題点を改善しうるセロトニン取り込阻害（SRI）活性と5-HT_{1A}自己受容体阻害活性を併せ持つ薬剤の創薬研究を開始した。

大日本住友製薬（株）では、古くからセロトニンやドパミンといった神経伝達物質の受容体やトランスポーターに作用する薬剤の研究開発が行われており、代表化合物として、タンドスピロン、ペロスピロン、ルラシドン、ブロナンセリンがある。これらのプロジェクトを通じ、ピペリジンやピペラジンといった環状アミン構造を中心に左側と右側にリンカーを介して環状構造を有する化合物を多く化合物ライブラリーとして保有している。その化合物ライブラリーの中で、化合物 **1** が、5-HT_{1A} 受容体に対し強い結合阻害活性を有し、弱いながらも SERT に対しても結合阻害活性を有することが分かった。化合物 **1** の SERT 結合阻害活性の向上を目的とし、公知情報を参考に化合物 **1** のピペリジン環 4 位に置換したベンジル基のベンゼン環の 6 位に各種ハロゲン原子の導入を行った。その結果、Br 基を導入した化合物 **4** において最も SERT 阻害活性が向上し、また、同時に 5-HT_{1A} 受容体阻害活性の大幅な向上にも成功した。化合物 **4** のピペリジン 1 位にリンカーを介して置換したベンゼン環及びピペリジン環 4 位に置換したベンジル基のベンゼン環上アルコキシ基の最適化を行い、比較的強い SERT 結合阻害活性と 5-HT_{1A} 結合阻害活性をバランスよく併せ持つ **SMP-304** を見出した（Figure 28）。機能評価の結果から、**SMP-304** は、期待通り SRI 活性を示し、5-HT_{1A} の弱い部分作動薬であり 5-HT_{1A} 自己受容体への阻害活性を有しうることから、ラット強制水泳試験にて抗うつ様作用の評価を行ったところ、既存 SSRI であるパロキセ

チンよりも早いオンセットを示し、私が目的とする SSRI の課題の一つである抗うつ作用のオンセットの遅さを克服しうる化合物であることがわかった。

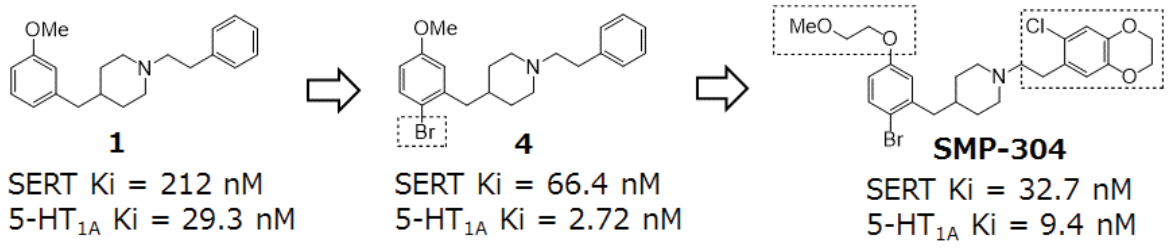

Figure 28. **SMP-304** の創製

比較的強いSERT結合阻害活性と5-HT_{1A}結合阻害活性を併せ持ち、ラット強制水泳試験にて既存のSSRIであるパロキセチンより抗うつ様効果の早いオンセットを示した**SMP-304**だが、その後の検討で、CYP2D6のみで代謝される単代謝であることがわかった。肝臓での代謝の大部分を占める薬物代謝酵素であるシトクロームP450（CYP）の分子種の一つであるCYP2D6には遺伝子多型があり、代謝活性の強いextensive metabolizer（EM）と代謝活性の弱いpoor metabolizer（PM）が存在することが知られている。EMとPMでは時に代謝速度が10倍以上異なることがあり、CYP2D6の単代謝である薬物は血中コントロールが難しく極めて高い安全性が要求される。そこで、**SMP-304**のCYP2D6の単代謝という課題の克服とSERT結合阻害活性の向上を目的とし、最適化研究を行った。その結果、ピペリジン環4位に置換したベンゼン環に置換したBr基の置換位置の変換により、SERT結合阻害活性が向上し、右側二環性部位の変換によりCYP2D6の代謝寄与率の高さが大幅に改善された。SERTおよび5-HT_{1A}に対し、そのKi値が10倍の範囲内という良好なバランスで、かつ、一桁nMという非常に強い結合阻害活性を示し、CYP2D6の代謝寄与率が60%以下であった化合物**46**が見出され、また、化合物**46**のBr基の他のハロゲン基や低級アルキル基への変換検討から化合物**55**と**57**が見出された。これら3化合物のSERTおよび5-HT_{1A}に対する機能評価を行った結果、SERT結合阻害活性の強さの順序に従い、強いSRI活性を示し、いずれの化合物も同様に5-HT_{1A}部分作動活性を示した。抗うつ作用の発現に非常に重要な前頭前皮質でのセロトニン遊離量の上昇作用をラットマイクロダイアリシスにて評価した。その結果、いずれの化合物もセロトニン遊離量の上昇作用を示し、SERT結合阻害活性およびSRI活性の強さと同様に、化合物**46**が最も強いセロトニン遊離量の上昇作用を示した（Figure 29）。

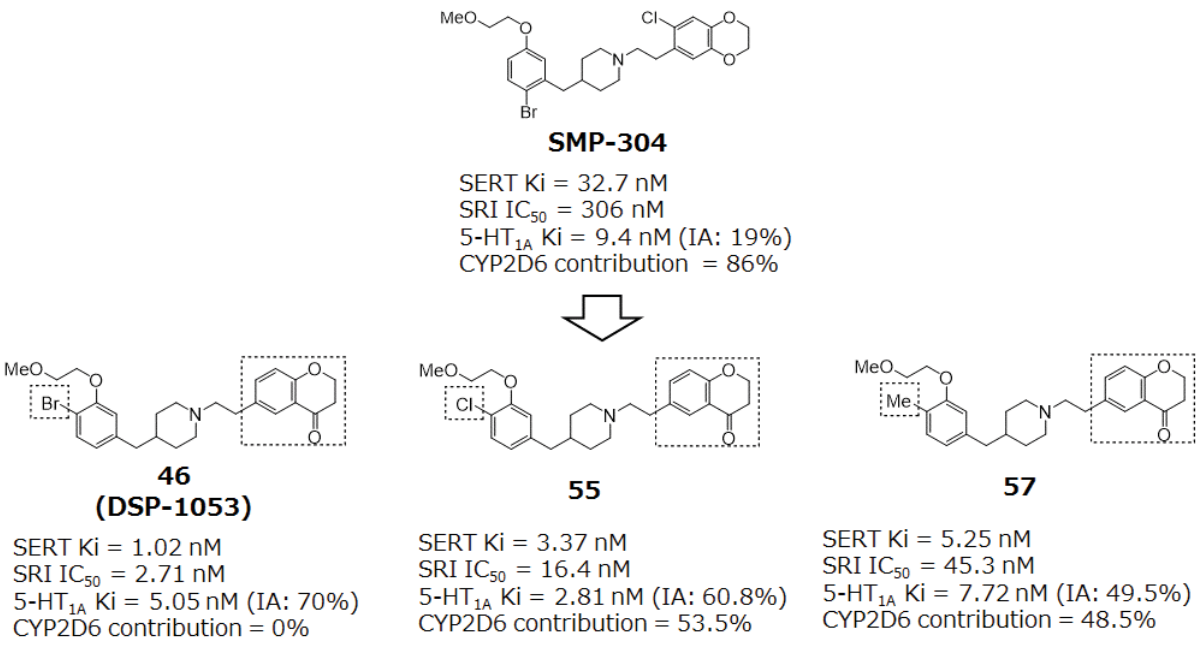

Figure 29. **DSP-1053** の創製

化合物**46**（**DSP-1053**）を有望な開発候補化合物として、さらなる詳細なラットin vivo評価を行った。ラットマイクロダイアリシスの結果から、**DSP-1053**は生体内で5-HT_{1A}自己受容体阻害活性を有することが示唆された。また、ラット強制水泳試験の結果、**SMP-304**と同様に既存SSRIであるパロキセチンよりも早い抗うつ様作用のオンセットを示した。以上のように、**DSP-1053**は、SERTおよび5-HT_{1A}に対し、そのKi値が10倍の範囲内という良好なバランスで、かつ、一桁nMという非常に強い結合阻害活性を示し、目的とするセロトニン取り込阻害（SRI）活性と5-HT_{1A}自己受容体阻害活性を併せ持ちSSRIの問題点の一つである治療オンセットの遅さを克服しうることが確認された。本化合物は、時にSSRIの使用を困難にする吐き気や嘔吐といった副作用の発現が温和であることが確認され、十分な安全性と優れた薬物動態プロファイルを示したため、開発化合物として臨床試験へと進んだ。

上記の通り、**DSP-1053**に代表されるクロマン－４－オン構造を有する誘導体がセロトニントランスポーター（SERT）と5-HT_{1A}受容体に対する強力な結合阻害活性を併せ持つことを見出した。そこで、共通の重要中間体**93**のスケールアップ可能な実践的合成方法の構築のため、検討を開始した。探索研究時の合成方法の課題であった①アルキル化工程・②酸化工程・③環化工程の各ステップを、①反応系の水分含量の精密なコントロール・②酸化剤を$NaClO_2$-TEMPO/NaClO(cat.)へ変更・③酸塩化物を経由する$AlCl_3$を用いた環化反応へと主に反応試剤を変更することで改善することができた。そして、総収率が28%から49%へと向上した重要中間体**93**の第一世代合成法を確立し、キログラムスケールでの合成を実施した。さらに、第一世代合成法からのさらなる総収率の向上・反応工程の短縮化を目指し、抜本的なルート変更を検討した。出発原料をMethyl 4-hydroxyphenylacetate から4-(2-hydroxyethyl)phenolへと変更することで、第一世代合成法では必須であったメチルエステルの還元工程を回避することができた。

また、クロマン－４－オン構造の構築にはカルボン酸ユニットの導入が必要であるが、アルコール3-bromopropan-1-olからエステルbenzyl propiolateへと変更することで、アルコールの酸化工程を回避することができた。さらに、TFAA-H_3PO_4(cat.)を用いた温和な環化反応を見出すことでカルボン酸から直接クロマン－４－オン構造を構築することができるようになった。第一世代合成法と比べ、総収率が49%から71%へ向上し、全反応工程数が8段階から5段階へと短縮した第二世代合成法を確立した。本第二世代合成法は、マルチキログラムスケールでの**93**の合成にも適応可能であり、より詳細に条件を最適化した本合成法により、**DSP-1053**の臨床開発用原薬の合成が実施された。

以上のように、本研究により、リード化合物の創出・最適化、および、開発化合物の創出、さらには、臨床開発用原薬の合成方法の構築といった創薬研究における初期段階から後期段階に至るまでの流れを一気通貫で行い、SSRIの治療オンセットの遅さを克服しうる新規抗うつ薬の創出といった課題の解決に対し貢献することができた。本研究で得られた知見が、さらなる新規抗うつ薬の創出に役立つことを願うとともに、私自身も新規治療薬の創出により世の中に貢献できるよう今後も精進を続けていきたいと思う。

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