新規疼痛治療薬を指向した

コノリジン誘導体の合成研究

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略語表

ACECl	1-chloroethyl chloroformate
AcOEt	ethyl acetate
AcOH	acetic acid
aq.	aqueous
AUC	area under the concentration-time curve
Boc	*tert*-butoxycarbonyl
calcd	calculated
cAMP	cyclic adenosine monophosphate
cat.	catalytic amount
Cbz	benzyloxycarbonyl
CH_2Cl_2	dichloromethane
C_{max}	maximum concentration
conc.	concentrated
DAMGO	(2*S*)-2-({2-[((2*R*)-2-{[(2S)-2-amino-3-(4-hydroxyphenyl)propanoyl]amino}propanoyl)amino]acetyl}-methylamino)-*N*-(2-hydroxyethyl)-3-phenylpropanamide

DIPEA	*N*,*N*-diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulfoxide
EDCI hydrochloride	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
EtI	iodoethane
Et_3N	triethylamine
Et_2O	diethyl ether
EtOH	ethanol
h	hour(s)
HATU	*O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N'*,*N*'-tetramethyluronium hexafluorophospate
HCl	hydrochloric acid
hERG	human Ether-a-go-go Related Gene
HRMS	high-resolution mass spectrometry
H_2SO_4	sulfuric acid

K_2CO_3	potassium carbonate
KO^tBu	potassium *tert*-butoxide
MeI	iodomethane
MeOH	methanol
min	minute(s)
MnO_2	manganese dioxide
MS	mass spectrometry
m/*z*	mass to charge ratio
$NaBH_4$	sodium borohydride
$NaBH(OAc)_3$	sodium triacetoxyborohydride
NaOH	sodium hydroxide
NaH	sodium hydride
NH_4Cl	ammonium chloride
n-BuLi	n-butyllithium
NMR	nuclear magnetic resonance
Pd/C	palladium on carbon
po	oral administration

SAR	structure activity relationship
tert	tertialy
TFA	trifluoroacetic acid
THF	tetrahydrofuran

緒論

第一節　疼痛と疼痛治療薬について

疼痛とは「実際の組織損傷もしくは組織損傷が起こりうる状態に付随する、あるいはそれに似た、感覚かつ情動の不快な体験」と定義される[1)2)]。痛みは不快な感情を伴うため誰もが避けたい現象と考えてしまいがちである。しかし、痛みとは人間にとって必要不可欠なシステムであり、例えば痛みを感じることができるからこそ人体は危険に気付き、身体を守る行動をとることができる。そもそも痛みとは人体の防御システムの一部であり、警告信号としての機能を担っている。しかし、警告信号として機能しているのは急性痛のみであり、慢性痛やがん性の痛みには人体への警告信号としての役割を果たしていない。つまり、長時間にわたって痛みの信号を送り続けることで、むしろ不快な状況を作り出しているのである。そのため、このような警告信号としては機能していない不快な痛みを疼痛治療薬によって取り除く必要がある[3)]。疼痛治療薬には、アセトアミノフェン、非ステロイド抗炎症薬(NSAIDs)、オピオイド治療薬（麻薬性鎮痛薬）、神経障害性疼痛緩和薬など数多くの治療薬が存在する。その中でもμ-オピオイド治療薬が最も強力な鎮痛薬として知られているため、第二節においてはこのμ-オピオイド治療薬について論じることにする。

第二節　μ-オピオイド治療薬の現状と課題

Figure 1 に日米欧の慢性疼痛およびがん性疼痛におけるオピオイド治療薬の市場規模割合を示す[4]。慢性疼痛におけるオピオイド治療薬の使用率は、全体で約3割以上を占めていることがわかる。このデータからも、オピオイド治療薬が疼痛治療において、非常にニーズのある薬剤であることがわかる。

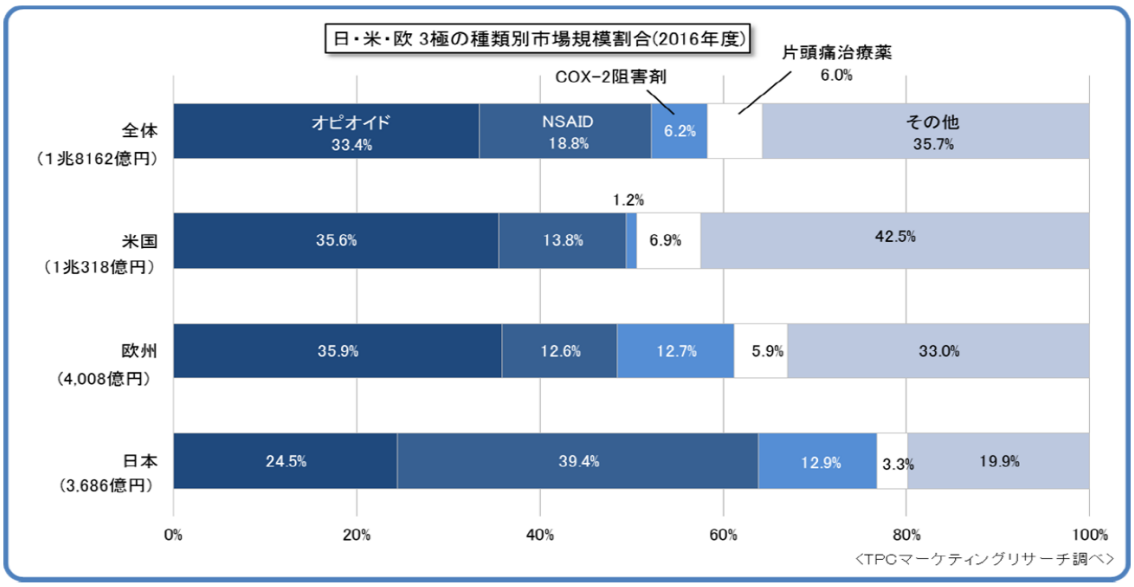


Figure 1　疼痛治療薬のマーケットシェアについて(出典：TPCマーケティングリサーチ　2016年度版)

生理的状況あるいは生体に危機が迫ったときにエンドルフィン、エンケファリン、ダイノルフィン、エンドモルフィン等の内因性のオピオイドペプチドが放出される。これらはオピオイド受容体に特異的に結合することでその効力を発揮することが知られている。このような作用に着目し、臨床で頻繁に使われるμ-オピオイド治療薬としてモルヒネ、フェンタニル、オキシコド

ン、ヒドロモルフォン等がある。これらはμ-オピオイド受容体アゴニスト[5]（Figure 2）とも呼ばれ、脊髄をはじめとして脳、末梢神経などに存在するμ-オピオイド受容体[6]への作用により、非常に強い鎮痛効果を示し、各種がんや慢性疼痛などに対する疼痛緩和などで幅広く使用されている非常に有用な薬剤である。

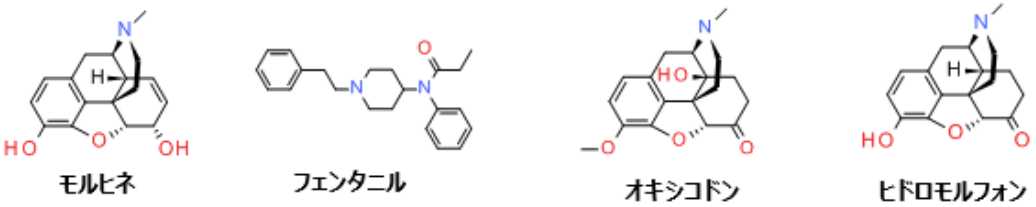


Figure 2 代表的なμ-オピオイド鎮痛薬

その作用機序は、薬剤がμ-オピオイド受容体へ作用することにより、Giタンパク質を介して、アデニル酸シクラーゼ（AC）の機能を抑える。これによりプロテインキナーゼの活性化を促すサイクリックAMP（cAMP）の産生が抑制されることになる。さらにはカリウムチャネルの開口促進、カルシウムチャネルの開口抑制といった応答を引き起こすことが知られている。カリウムチャネルの開口促進は神経細胞の過分極を引き起こし、カルシウムチャネルの開口抑制は主に神経終末から伝達物質遊離抑制を引き起こすことが知られており、それらの作用が合わさって鎮痛作用を示すといわれている[7][8]。しかしながら、μ-オピオイド鎮痛薬には多くの副作用[9]-[11]があることも知られており、悪心・嘔吐、便秘、眠気、せん妄・幻覚、精神依存（乱用）、呼吸抑制、口内乾燥、掻痒感等の深刻な副作用は、患者のＱＯＬの低下や医師

の処方を困難にする要因でもある。このようにオピオイド治療薬は、強力な鎮痛作用を発揮するが、同時に多くの副作用を示すため、臨床での使用が難しい薬剤でもある[12)13)]。

第三節　天然物コノリジンとその鎮痛効果

インド原産のサンユウカと呼ばれるキョウチクトウ科の低木の樹皮は古来より中国やタイで解熱鎮痛薬として使用されてきた（**Figure 3**）[14)]。この植物からは66ものアルカロイド成分が単離されており、その代表的な作用として抗酸化作用、抗感染作用、抗腫瘍作用、鎮痛作用、コリン作動性の増強などが報告されている。中でもコノリジンを含むインドールアルカロイド（ヒドロキシアパリシン、アパリシン、ボアカミン等）には鎮痛作用があることが報告されているが､その各々の明確な作用については未だ不明な点が多い。その一つの有効成分として単離されたコノリジンは､分子量266と非常に小さい分子であるにもかかわらず、インドール環と縮環する1-アザビシクロ[4.2.2]デカン骨格、*E*体のエキソオレフィン、橋頭の15位に*S*配置の不斉炭素を有するなど、非常にユニークな構造的特徴をもつアルカロイドである（**Figure 4**）。

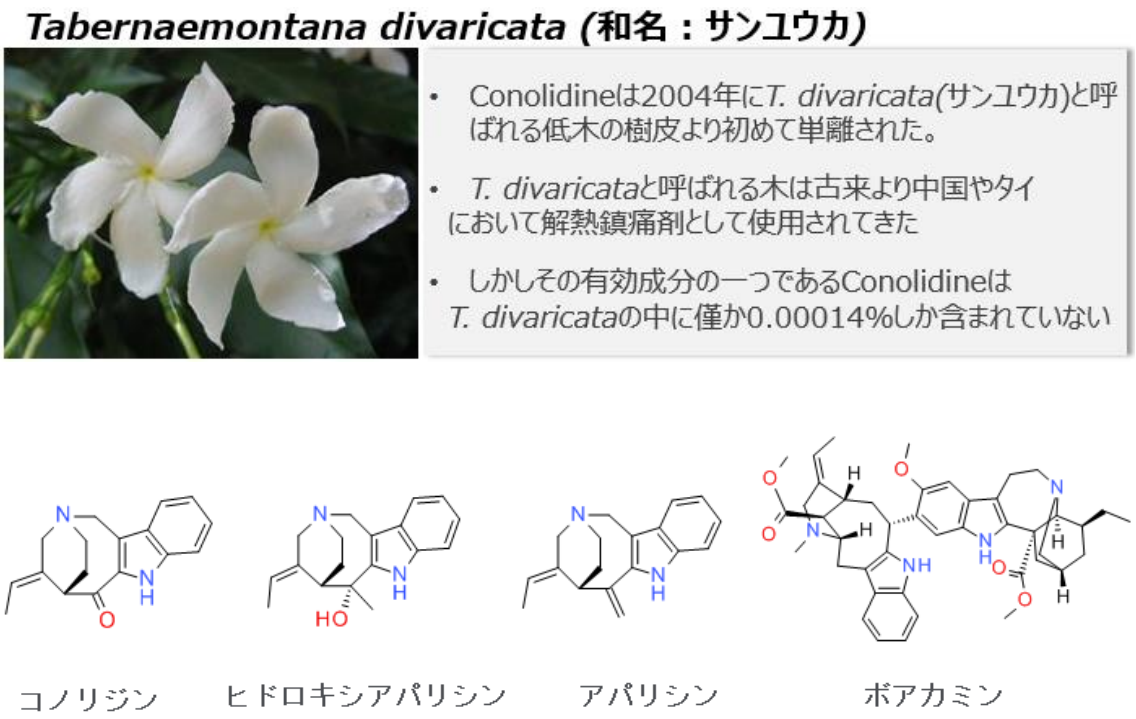


Figure 3 サンユウカ及びサンユウカ由来のインドールアルカロイド類

近年、コノリジンの薬理作用に関して、「強い鎮痛作用を持ちつつも、オピオイド様の副作用は示さない」という報告[15)16)]がなされたことにより、コノリジンは大きく注目を集めることとなった。しかしながら、コノリジンの含有量は0.00014%（樹皮からの単離収率）と極めて僅かであることから本化合物の化学合成には非常に意義があると考えられ、多くの研究者らによってその研究が現在も行われている[17)-23)]。

筆者は、このコノリジンの非常に興味深い薬理作用に着目し、コノリジンをリード化合物として、強力な鎮痛作用を有し、副作用の少ない安全な新規疼痛治療薬の開発を目指せないかと考え、筆者ら合成研究者の提案により創

薬研究を開始した。

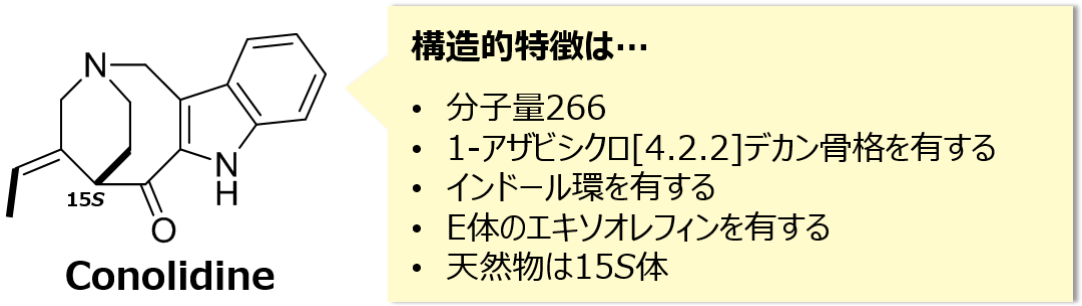


Figure 4 コノリジンの構造的特徴について

第四節　本研究の概要

本研究において、筆者はまず、「μ-オピオイド作動薬に匹敵する鎮痛薬効を有し、 μ-オピオイド作動薬特有の副作用を回避した非麻薬性鎮痛薬の創製」という研究コンセプトを掲げ、研究に取り組むことにした。

また、適応疾患として、従来のμ-オピオイド治療薬で適用されている①慢性疼痛（NSAIDs無効な変形性関節症に伴う疼痛、腰痛など）②術後疼痛③がん疼痛を想定し、現状副作用によって使用が困難とされているμ-オピオイド治療薬に置き換わる薬剤の創出を目標とし、創薬研究を行った。

第一章では、コノリジンからの初期構造活性相関（SAR）研究を通して誘導体展開を実施し、DS39201083を見出した経緯について述べる。コノリジンの鎮痛作用に関するメカニズムは不明であるため、薬効の１次スクリーニングとして*in vivo*試験であるマウス酢酸ライジング試験（詳細については後述）にて化合物の選抜をおこなった。その結果、コノリジンへの種々の置換基の

導入や構造変換の結果より、エキソオレフィンを除去し、C15位にメチル基を導入することで、酢酸ライジング試験においてコノリジンよりも４倍も強い鎮痛活性を有する有望化合物、**DS39201083**を創製することに成功した（**Figure 5**）[24]。

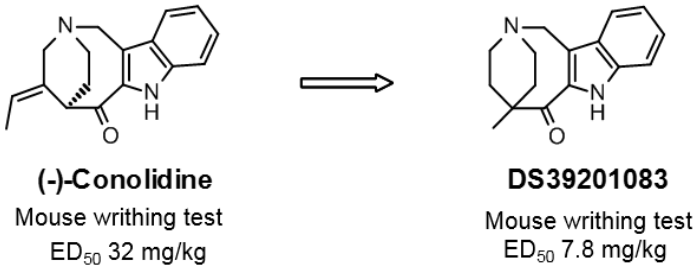


Figure 5　**DS39201083**の獲得

第二章では、**DS39201083**の懸念点であるhERGカリウムイオンチャネル阻害の回避を目的とした研究について述べる。Log $D_{7.4}$を低くすることでhERGカリウムイオンチャネル阻害を低減できるという傾向に着目し、ビシクロ骨格の変換（scaffold hopping）を試みた。その結果、分子内にアミド結合を含み、ピロリジン環とビシクロ骨格を形成した新規の骨格を有する**DS54360155**を創製することに成功した。この**DS54360155**は酢酸ライジング試験における鎮痛薬効は**DS39201083**とほぼ同等でありながら、Log $D_{7.4}$が大幅に低減（Log $D_{7.4}$：0.7）しており、その結果、狙い通りhERGカリウムイオンチャネル阻害の減弱した化合物である（14%inhibition at 10μM）（**Figure 6**）[25]。

Figure 6　DS54360155の獲得

第三章では、鎮静作用を回避し、鎮痛活性のより強い化合物取得のための合成展開を実施し、DS34942424を獲得した経緯について述べる。これまでの共通構造であったインドール環をベンゼン環へと変換することで、安全性マージン（= ロコモーター試験ID_{50}値 / 酢酸ライジング試験ED_{50}値）が拡大する傾向にあることを掴み、更にベンゼン環上の置換基を最適化することでDS34942424を獲得することに成功した。DS34942424はμ-オピオイド受容体（MOR）に作用しないこと、マウスホルマリン試験においても用量依存的に非常に強い薬効（ED_{50}値：16 mg/kg）を示すことを確認した(Figure 7)[26]。

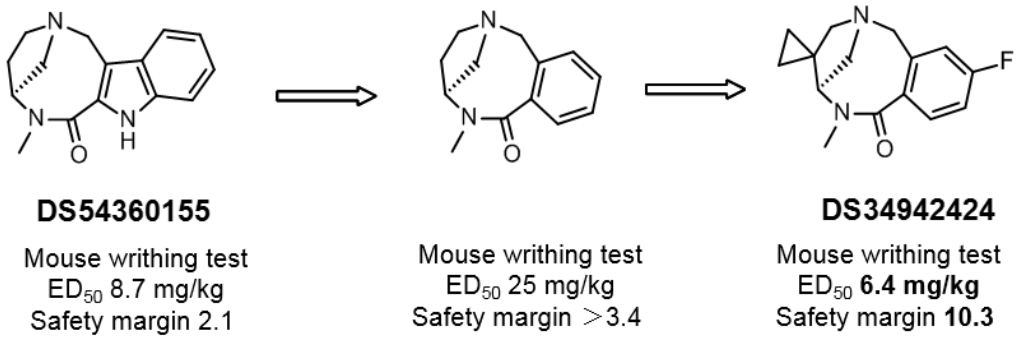


Figure 7　DS34942424の獲得

第一章　コノリジンより活性の向上したDS39201083の獲得

第一節　初期SAR探索のための合成計画

筆者は、まず活性発現に必須な構造を明確にするために、コノリジンの構造活性相関（SAR）研究を行うこととした。Figure 8にコノリジン誘導体の初期合成計画を示す。

まず、コノリジンのインドール環への置換基の導入およびビシクロ環の変換を計画した。これらの誘導体展開から鎮痛活性および物性の変化を確認し、鎮痛薬としてポテンシャルの高いリード化合物の獲得を目指すことにした。

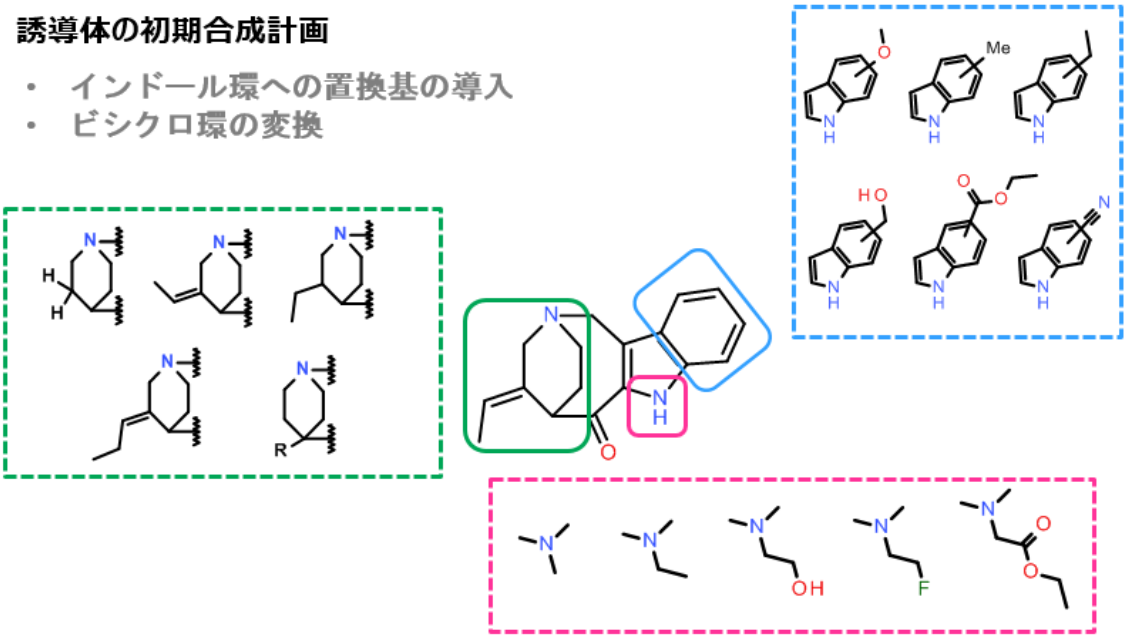


Figure 8　コノリジン誘導体の初期合成計画

第二節　誘導体の合成

合成した化合物2a、2b、3a、3b、3c、3d、4a、4b、4cおよび4dの構造をFigure 9に示し、その合成法についてScheme 1に示した。また、化合物4aは既知の文献報告[27]に従って合成した。化合物2aおよび2bはインドール環のＮＨ基に水酸化ナトリウムと等量のアルキルハライドを用いたアルキル化を経て合成した。インドールのベンゼン環上に置換基（OMe基）を導入した化合物3a-dはMicalizioらによって報告されたアルデヒド5 [15)16)]に対するインドールの求核付加、続くホルムアルデヒドを用いたMannich反応[28)]による環化を鍵反応として合成した。化合物4c、4dは、エキソオレフィン部分を変換したアルデヒド9c, 9dから3a-dと同様の工程にて合成した。4bはコノリジン1へのパラジウムを用いた水素添加反応によりオレフィンを還元することで合成した。

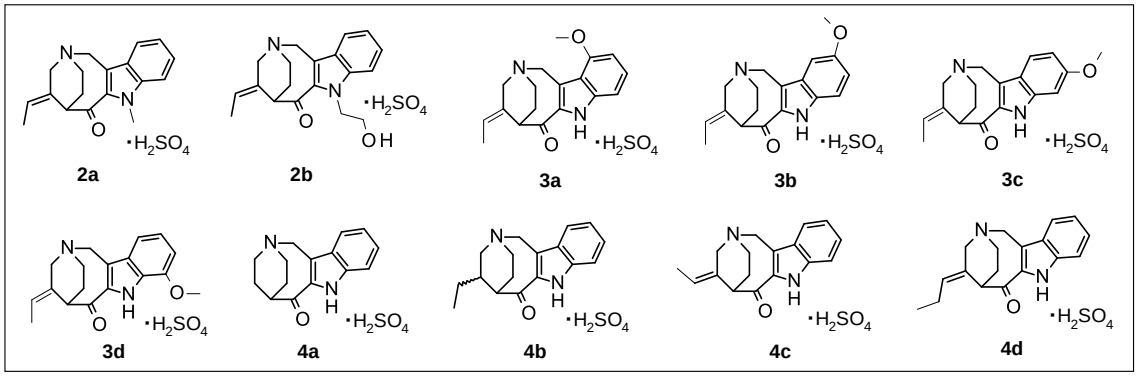
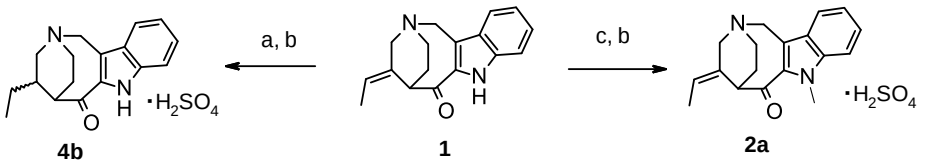
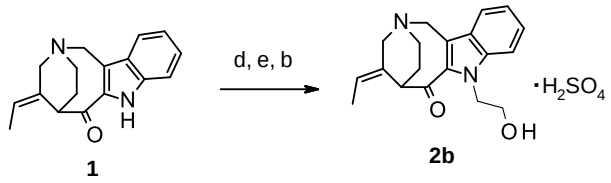
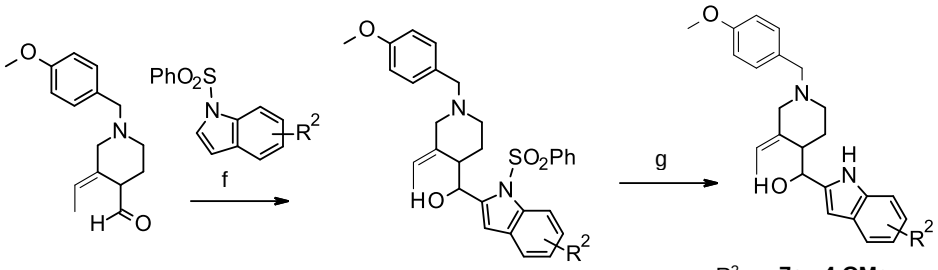
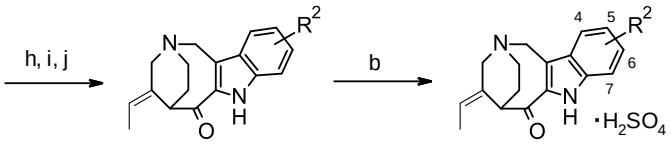


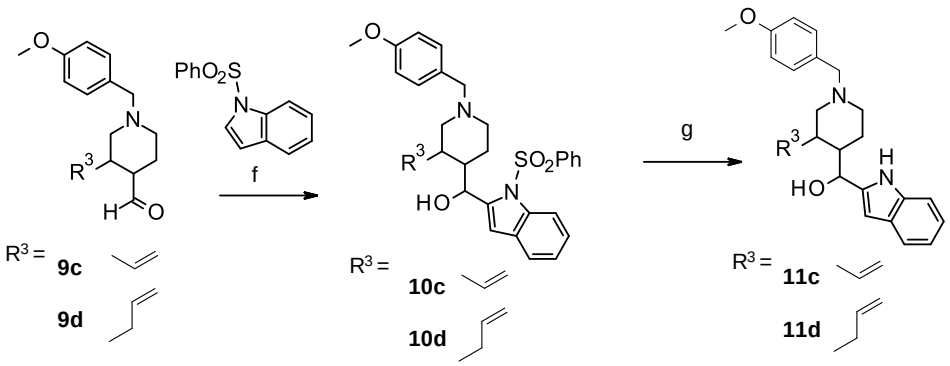
Figure 9　コノリジン誘導体

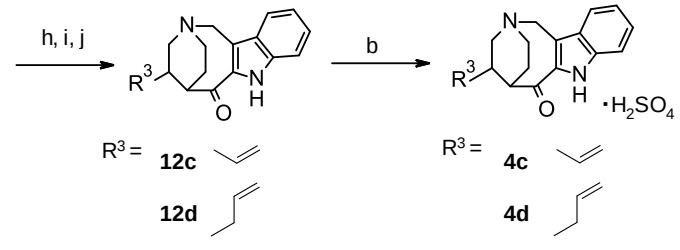

a, b
c, b
·H2SO4
4b
1
2a


d, e, b
·H2SO4
OH
1
2b


PhO2S
f
SO2Ph
g
5
R2 = 6a : 4-OMe
6b : 5-OMe
6c : 6-OMe
6d : 7-OMe
R2 = 7a : 4-OMe
7b : 5-OMe
7c : 6-OMe
7d : 7-OMe

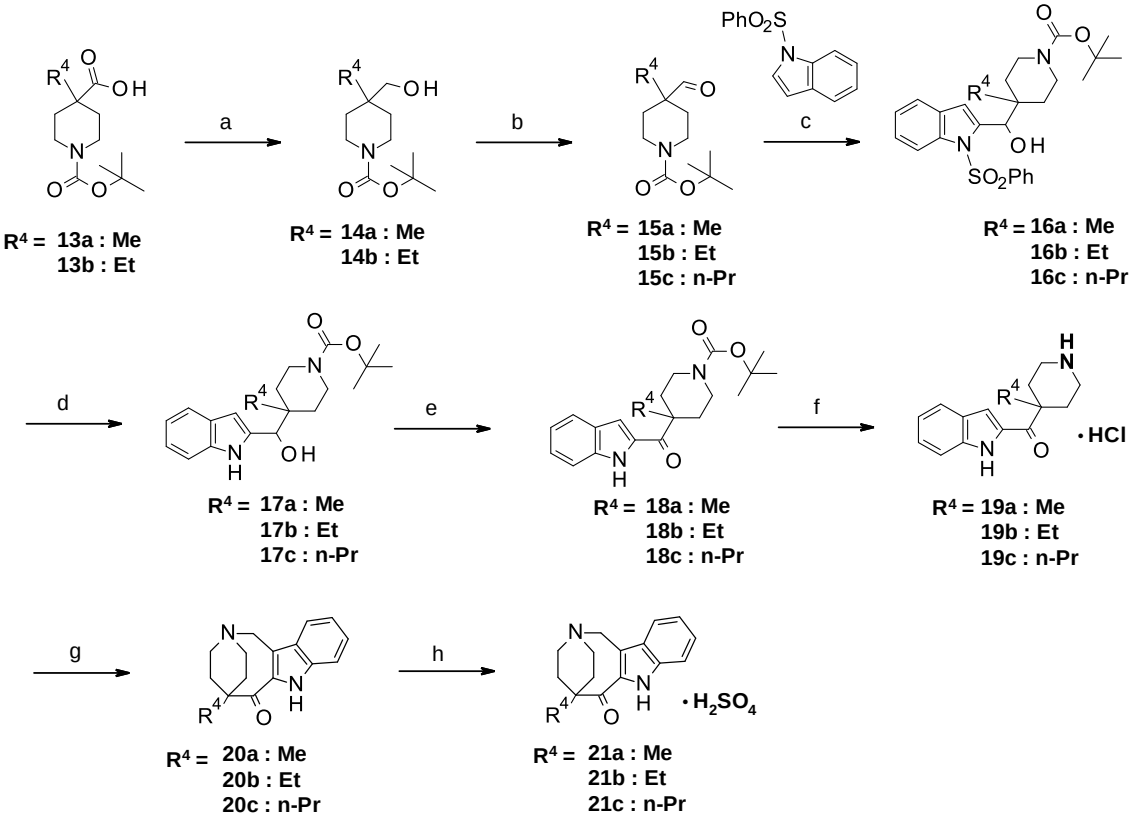

h, i, j
b
·H2SO4
R2 = 8a : 4-OMe
8b : 5-OMe
8c : 6-OMe
8d : 7-OMe
R2 = 3a : 4-OMe
3b : 5-OMe
3c : 6-OMe
3d : 7-OMe


PhO2S
f
SO2Ph
g
R3 = 9c
9d
R3 = 10c
10d
R3 = 11c
11d


h, i, j
b
·H2SO4
R3 = 12c
12d
R3 = 4c
4d

Scheme 1 Reagents and conditions: (a) Pd-C, EtOH, H_2, 97%; (b) H_2SO_4 aq., EtOH, 68-98%; (c) MeI, NaH, DMF, 54%; (d) $BrCH_2CH_2OAc$, NaH, DMF, 58%; (e) NaOH, THF/MeOH, 80%; (f) *n*-BuLi, benzenesulfonyl indole, THF, 62-94%; (g) Cs_2CO_3/MeOH, THF, 82-92%; (h) MnO_2, CH_2Cl_2; (i) ACECl, 1,2-dichloroethane, then MeOH; (j) paraformaldehyde, TFA, 1,2-dichloroethane, 25-38% from **7a-d** or **11c,d**.

化合物21a、21bおよび21cはScheme 2に示すように合成した。４級炭素を有するアルデヒド15a、15bは、カルボン酸13a、13bの還元およびParikh-Doering酸化反応[29]にて合成した。このアルデヒド15a、15bと市販のアルデヒド15cからScheme 1に示した合成方法とほぼ同様にして橋頭位にアルキル置換基R^4を有する誘導体21a-cをそれぞれ合成した(Scheme 2)。



Scheme 2. Reagents and conditions: (a) $LiAlH_4$, THF, 72-90%; (b) SO_3/pyridine, DMSO, $(i\text{-Pr})_2EtN$, CH_2Cl_2; (c) *n*-BuLi, benzenesulfonyl indole, THF, 60-89% from **14a**, **b** or **15c**; (d) Cs_2CO_3/MeOH, THF, 69-85%; (e) MnO_2, CH_2Cl_2; (f) HCl, EtOAc; (g) paraformaldehyde, TFA, 1,2-dichloroethane, 29-40% from **17a-c**; (h) H_2SO_4 aq., EtOH, 68-90%.

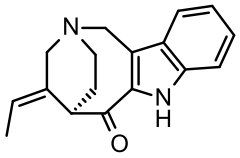
第三節　コノリジン誘導体の薬理評価と考察

コノリジンの鎮痛作用に関するメカニズムは不明であるため、薬効の初期スクリーニングの段階から、*in vivo*の評価系であるマウス酢酸ライジング試験を実施して、化合物の選抜をおこなうことにした。この試験はddYマウス(日本SLC 雄性、4-5週齢）に酢酸を腹腔内投与することで現れる痛みによる特有のライジング（身悶えるような症状）行動の回数を測定することによって鎮痛作用を評価する。本試験は非常に簡便で感度が高いため、初期のスクリーニングとしては非常に優れた評価系と考えられる。ddYマウスはクローズドコロニーとして維持されている非近交系マウスで、繁殖能力が高く、発育も良好であるため、薬効、薬理、毒性などの試験をはじめとして、さまざまな試験研究に広く用いられている[30]。筆者はこの簡便な酢酸ライジング試験にて強い鎮痛薬効を示す化合物を選抜し、最終的にオピオイドと同等の薬効を示すことを評価できる、マウスホルマリン試験を実施することにした。（マウス酢酸ライジング試験、及びマウスホルマリン試験は、第一三共株式会社の動物実験委員会によって承認された方法に準拠し、実施した。）

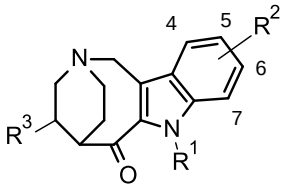
Table 1に示すように、化合物を30 mg/kg経口投与したときのライジング回数の減少を測定することで阻害率（% inhibition）を算出し、鎮痛作用の評価を行った。また同時に、ADMEスクリーニングの中でも特に誘導体の代謝安定性（Metabolic stability）、Log $D_{7.4}$値の測定を行い、化合物の物性的な特徴の評価もおこなった。Log $D_{7.4}$とは分子の脂溶性の指標であり、$pH_{7.4}$のリン酸緩衝溶液（日本薬局方2液）と1-オクタノールの分配係数を測定した値の常用対数である。

その結果、インドール環に置換基を導入した**2a**、**2b**、**3a**、**3b**、**3c**、**3d** はコノリジンと比較して全て活性が減弱する傾向にあることがわかった。また、エキソオレフィン部分の変換では唯一、置換基を除去した**4a**のみがコノリジンと比べて強い薬効（73%阻害）を示した。これらの結果と、物性の値を加味して考えると、脂溶性の向上に起因する代謝安定性の低下が活性の減弱に繋がっていると推察された。またエキソオレフィンは、活性の向上に寄与していないという可能性も示唆された。

Table 1　コノリジン誘導体の薬理評価(1)



(-)-1 Conolidine



Comp.	R^1	R^2	R^3	writhing test[a]	Log $D_{7.4}$[b]	MS (%)[c]
(±)-1	H	H		49	N.T.[d]	N.T.[d]
(−)-1	H	H		49	2.8	78
2a	Me	H		30	3.5	40
2b	CH_2CH_2OH	H		27	2.7	38
3a	H	4-OMe		42	3.4	22
3b	H	5-OMe		3	3.1	45
3c	H	6-OMe		31	3.1	54
3d	H	7-OMe		34	3.3	N.T.[d]
4a	H	H	H	73	1.8	97
4b	H	H		50	N.T.[d]	N.T.[d]
4c[e]	H	H		18	3.3	56
4d	H	H		0	N.T.[d]	N.T.[d]

[a] % inhibition at 30 mg/kg

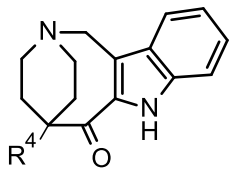
[b] The distribution coefficients (Log $D_{7.4}$) were measured between 1-octanol and phosphate buffered saline (pH $_{7.4}$).

[c] MS (Metabolic stability) : Percentage of the tested compound remaining after 0.5 h of incubation with mouse liver microsome (0.1 mg/mL).

[d] Not tested, [e] Ervaticine [31)]

続いて活性の強かった**4a**の橋頭位（C15位）に置換基（R^4）を導入した化合物の評価を行った(**Table 2**)。その結果、R^4置換基としてMe基を持つ**21a**が最も強い鎮痛活性を示すことがわかった。Me基よりも大きいアルキル置換基(Et基、*sec*-Bu基）の導入は代謝安定性の低下に繋がり、薬効も減弱したものと考えられる。

Table 2　コノリジン誘導体の薬理評価(2)



Compound	R^4	writhing test[a]	Log $D_{7.4}$[b]	MS (%)[c]
4a	H	73	1.8	89
21a	Me	90	2.5	92
21b	Et	48	3.0	43
21c	*sec*-Bu	51	3.7	0

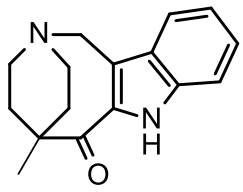
[a] % inhibition at 30 mg/kg

[b] The distribution coefficients (Log $D_{7.4}$) were measured between 1-octanol and phosphate buffered saline (pH $_{7.4}$).

[c] MS (Metabolic stability) : Percentage of the tested compound remaining after 0.5 h of incubation with mouse liver microsome (0.1 mg/mL).

次に化合物**21a**のプロファイルを**Table 3**に示す。**21a**はμ-オピオイド受容体（MOR）に対するBinding試験およびcAMP assayの結果よりμ-オピオイド受容体アゴニスト活性を示さないということがわかった。また、**21a**は経口投与で良好なPKプロファイルを示し、且つ酢酸ライジング試験において強い鎮痛薬効(ED_{50}: 7.8 mg/kg)を示した。コノリジンの酢酸ライジング試験における鎮痛薬効のED_{50}値が32 mg/kgであることから、コノリジンと比較して約4倍程度活性が強くなったことになり、有望なリード化合物**21a**(**DS39201083**)を獲得することができた。

Table 3　化合物21a(DS39201083)のプロファイル



21a（DS39201083）

writhing test ED_{50} (mg/kg)[a]	AUC (μM*h) [b]	C_{max} (μM) [b]	$T_{1/2}$ (h) [b]
7.8	3.21	1.25	2.33
MOR binding test	MOR cAMP assay	MOR cAMP assay	
IC_{50} (μM) [c]	EC_{50} (μM) [d]	E_{max} (%) [e]	
71	>100	12	

[a] The ED_{50} value was calculated from the dose response curve of analgesic activity at 3, 10 and 30mg/kg

[b] Average of three values dosed at 10 mg/kg orally (p.o.) in ddY mice (0.5 w/v% methylcellulose suspension)

[c] Binding affinities (IC_{50}) were obtained by the competitive displacement of radiolabeled [^{3}H] diprenorphinee. Morphine, with an IC_{50} 0.41 μM, was used as a positive control

[d] cAMP assays were carried out using human MOR-expressed CHO cells. DAMGO, with an EC_{50} 0.088μM, was used as a positive control

[e] E_{max} was calculated as the response (in %) obtained with DAMGO

第四節　小括

筆者は天然物コノリジンからの探索的な初期誘導体展開を実施した。まずエキソオレフィン部分を除去することで、コノリジン(49% inhibition at 30 mg/kg, ED_{50}:32 mg/kg)より強い鎮痛薬効を示す化合物**4a** (73% inhibition at 30 mg/kg) を獲得した。続いて**4a**の橋頭位にMe基を導入することで、更に強い鎮痛薬効を示す**21a**(**DS39201083**, 90% inhibition at 30 mg/kg, ED_{50}:7.8 mg/kg)を獲得することに成功した。この化合物はμ-オピオイド受容体アゴニスト活性を示さず、経口投与でコノリジンと比較して約4倍強い鎮痛薬効を示す有望化合物であることがわかった。

第二章　新規骨格を有するDS54360155の獲得

第一節　hERGカリウムイオンチャネル阻害回避のための合成方針

前章では経口薬として優れたPKプロファイルを有し、強い鎮痛薬効を示すDS39201083創製までの経緯を述べた。しかしながら獲得したDS39201083は、その後の化合物プロファイリングにおいて、比較的強いhERGカリウムイオンチャネル阻害活性を有することがわかってきた(49% inhibition at 10 μM)。hERGカリウムイオンチャネルの阻害は、致死性不整脈の原因となるQT延長症候群を引き起こすことが知られている[32)-34)]。

そこで筆者は、hERGカリウムイオンチャネル阻害回避のために、合成してきた化合物群についてのデータ検証を実施することにした。これまでの創薬研究の経験的な知識から、Log $D_{7.4}$とhERGカリウムイオンチャネル阻害には相関がみられるのではないかという仮説をたてた。そこで、**Figure 10**に示すように横軸に化合物のLog $D_{7.4}$の値をとり、縦軸に10 μM におけるhERGカリウムイオンチャネルの阻害率（%）でプロットすると、Log $D_{7.4}$が小さいほどhERGカリウムイオンチャネル阻害も弱くなる傾向があることがわかってきた。また、各化合物のプロットの大きさが大きいほど代謝安定性が高いことを示しており、このグラフよりLog $D_{7.4}$が小さいほど代謝安定性が高くなっていることもわかる。

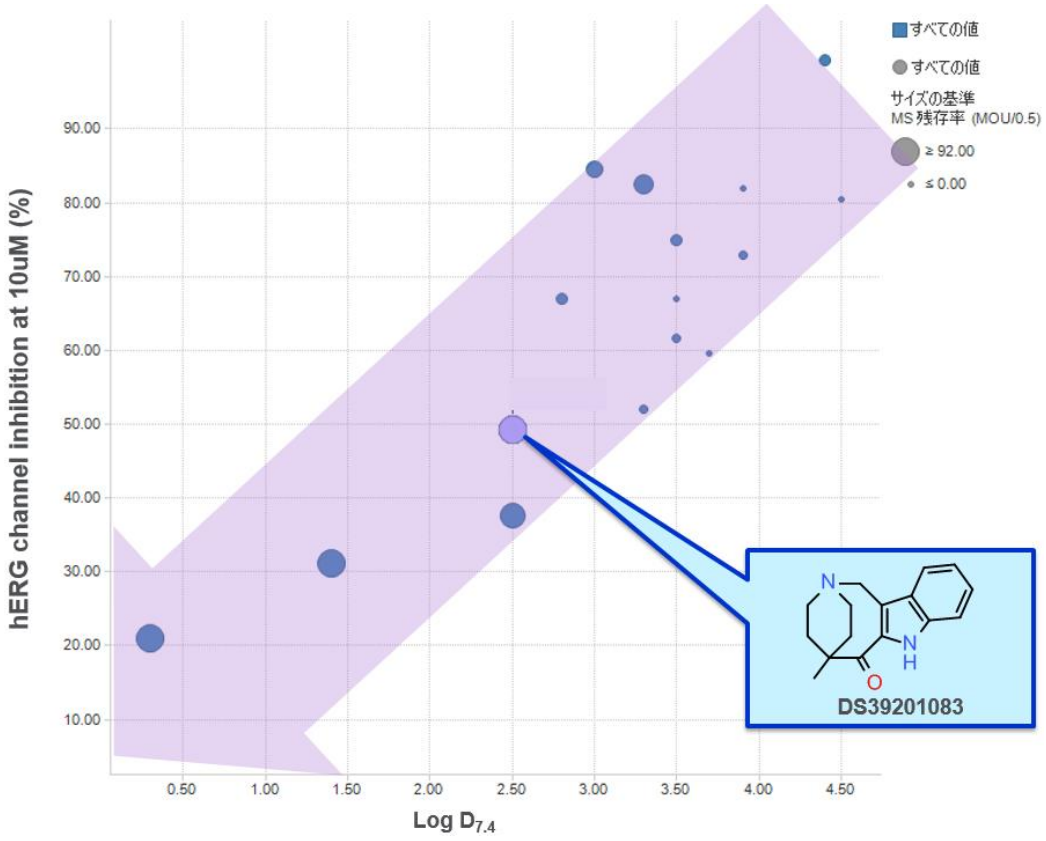


Figure 10　Log $D_{7.4}$とhERGカリウムイオンチャネル阻害の相関関係

この検証結果から、筆者は**DS39201083**からの誘導体合成の方針として、Log $D_{7.4}$を低下させるような合成展開を実施することにした。しかしながら、これまでの合成展開において、インドール環への極性置換基の導入はほとんどが鎮痛活性の低下につながり、良好な結果を得られないことが既にわかっていた。そこで合成の難易度は上がるが、ピペリジン環を含むビシクロ骨格の変換(scaffold hopping)を行うことでLog $D_{7.4}$を下げる試みを実施することにした(**Figure 11**)。その際の誘導体展開によるhERGカリウムイオンチャネル阻害回避の目標値として、経験的な心毒性との関係から「<20% inhibition at

10 μM」と設定した。

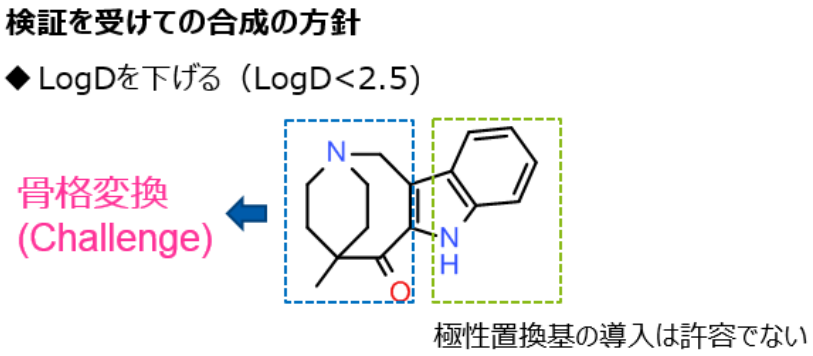
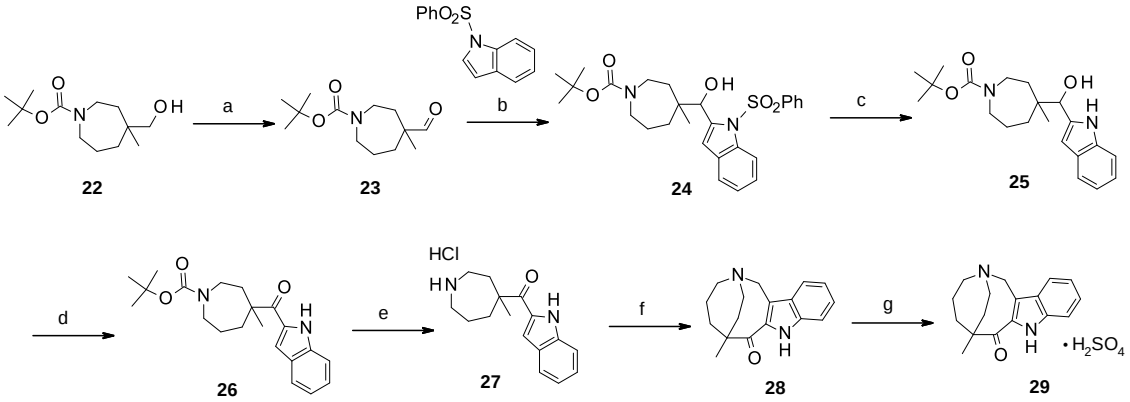


Figure 11　DS39201083からの誘導体合成の方針

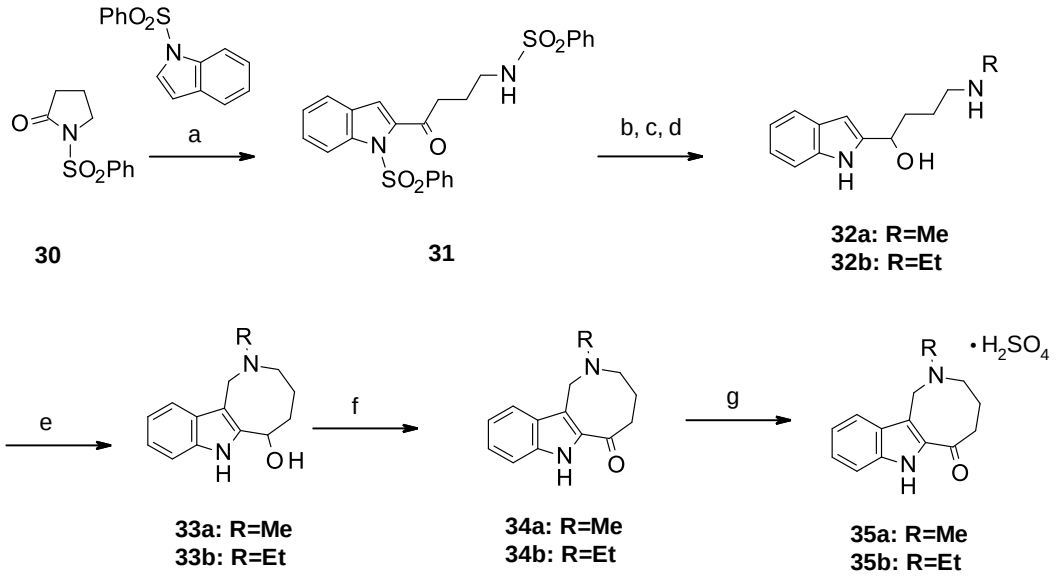
第二節　誘導体の合成

化合物29の合成ルートをScheme 3に示す。市販の化合物であるアルコール22を出発原料として、アルコールの酸化、インドール2位の求核付加反応を経てアルコール24を得た。その後ベシル基の脱保護、アルコールの酸化、続くBoc基の脱保護を経て、環化前駆体のアミン塩酸塩27とした。ホルムアルデヒドを用いた分子内Mannich反応による環化および硫酸塩化により目的化合物29を得ることができた。



Scheme 3. Reagents and conditions: (a) SO_3/pyridine, DMSO, (i-Pr)$_2$EtN, CH_2Cl_2; (b) n-BuLi, THF, 80% from **22**; (c) Cs_2CO_3/MeOH, THF, 85%; (d) MnO_2, CH_2Cl_2; (e) HCl, EtOAc; (f) paraformaldehyde, TFA, 1,2-dichloroethane, 55% from **25**; (g) H_2SO_4 aq., EtOH, 89%.

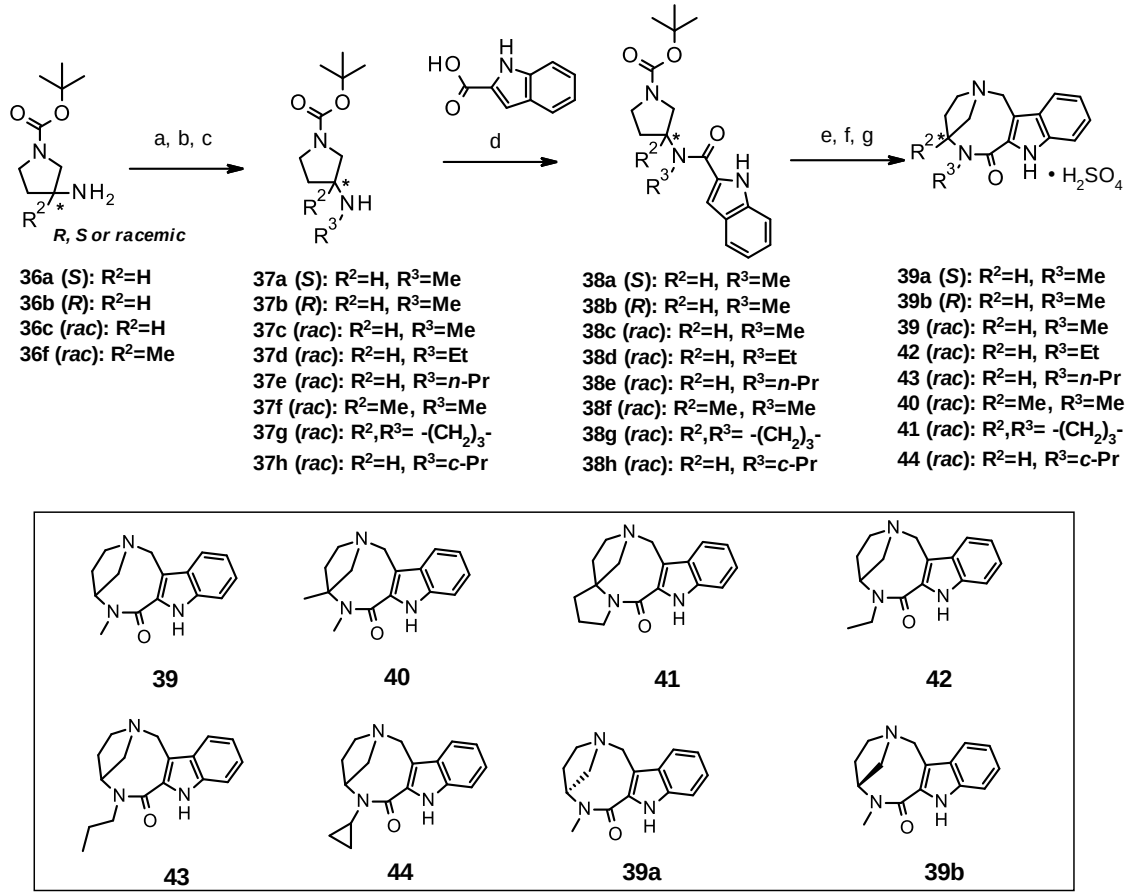
引き続きビシクロ環をもたない**35a**、**35b**の合成ルートを**Scheme 4**に示した。市販化合物のスルホンアミド**30**を出発原料として、インドール2位の求核付加反応によるラクタムの開環反応を得て、ケトン**31**を得た。その後スルホンアミドへのアルキル（Me、Et）化、還元、スルホンアミドの開裂を経て、環化前駆体のアルコール**32a**、**32b**を得た。ホルムアルデヒドを用いたMannich反応による環化反応、続く硫酸塩化により目的化合物**35a**、**35b**を得ることができた。



Scheme 4. Reagents and conditions: (a) *n*-BuLi, THF, 28%; (b) Alkyl halide, K_2CO_3, DMF, 60 °C; (c) $NaBH_4$, EeOH; (d) Mg, NH_4Cl, MeOH, 52-61% from **31**; (e) paraformaldehyde, TFA, 1,2-dichloroethane, 68-73%; (f) MnO_2, CH_2Cl_2 73-81%; (g) H_2SO_4 aq., EtOH, 90-100%.

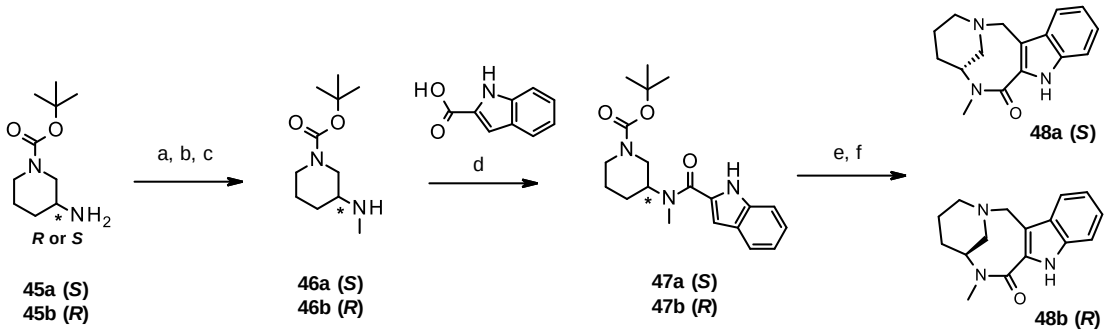
次に化合物**39**、**39a**、**39b**、**40**、**41**、**42**、**43**および**44**の合成法を**Scheme 5**に示した。化合物**39**、**39a**、**39b**、**40**、**42**、**43**は**36a-c**、**36f**を出発原料として、アミンをNosyl基[35]で保護した後に、アルキル（Me、Et、n-Pr）化、Nosyl基の脱保護を経て、アミン中間体**37a-f**を合成した。これらのアミンをインドール-2-カルボン酸とEDCIを用いてアミド化し、**38a-f**を得た。さらにBoc基の脱保護を経て、環化前駆体のアミン塩酸塩とし、ホルムアルデヒドを用いたMannich反応による分子内環化反応､続く硫酸塩化により目的化合物である**39**、**39a**、**39b**、**40**、**42**、**43**を得ることができた。また化合物**41**、**44**も市販のアミン中間体である**37g**、**37h**を出発原料として、同様の合成方法にて合成し

た。



Scheme 5. Reagents and conditions: (a) 2-Nitrobenzenesulfonyl chloride, Et_3N, THF, 95–100%; (b) Alkyl halide, K_2CO_3, DMF, 60 °C, 95–99%; (c) 2-Fluorothiophenol, K_2CO_3, MeCN, 94–100%; (d) EDCI, THF, 88–99%; (e) HCl, 1,4-dioxane/AcOEt; (f) paraformaldehyde, TFA, 1,2-dichloroethane, 4–35% 2 steps from **38a-h**; (g) 1 M H_2SO_4 aq., EtOH, 0 °C, 80–99%.

続いて分子内にピペリジン環を有する化合物**48a**、**48b**の合成法を**Scheme 6**に示した。**45a**、**45b**を出発原料として、アミンをCbz基で保護した後に、メチル化、Cbz基の脱保護を経て、アミン中間体**46a**、**46b**を合成した。これらのアミンをインドール-2-カルボン酸とEDCIを用いてアミド化し、**47a**、**47b**を得た。さらにBoc基の脱保護を経て、環化前駆体のアミン塩酸塩とし、ホルムアルデヒドを用いたMannich反応による分子内環化反応にて目的化合物である**48a**、**48b**を得た。

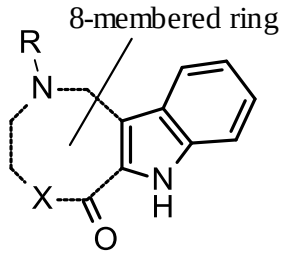


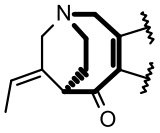
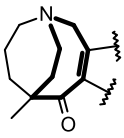
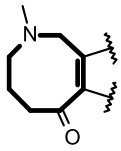
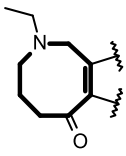
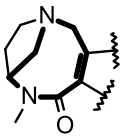
Scheme 6. Reagents and conditions: (a) carbobenzoxy chloride., Et_3N, CH_2Cl_2,; (b) MeI, NaH, THF; (c) Pd/C, H_2, MeOH, 82-88% from **45a**, **45b**; (d) EDCI, THF, 88–92%; (e) HCl, 1,4-dioxane/AcOEt; (f) paraformaldehyde, TFA, 1,2-dichloroethane, 4–8% from **47a**, **47b**.

第三節　新規誘導体の薬理評価と考察

第一節の合成方針で述べたように、まずはビシクロ骨格の骨格変換により脂溶性の低く抑えられた化合物の合成を行い、その評価を行った。**Table 4**に、合成した化合物**29**、**35a**、**35b**、**39**の、鎮痛薬効、物性値（Log $D_{7.4}$、MS）およびhERGカリウムイオンチャネル阻害率について示す。構造を単純化した化合物**35a**、**35b**のLog $D_{7.4}$は狙い通り、1.1および1.3と低く抑えられていたが、酢酸ライジング試験（30 mg/kg）における鎮痛活性が弱くなってしまった（**35a**: 31% inhibition、**35b**: 36% inhibition）。一方、アミド結合を有する化合物**39**はLog $D_{7.4}$の値も0.7と非常に低く、hERGカリウムイオンチャネル阻害も弱い値を示した（**39**:26% inhibition at 10 μM）。しかも30 mg/kgにおける鎮痛活性も強い薬効を示した（83% inhibition）。この化合物は分子内にアミド結合を含み、ピロリジン環とビシクロ環が縮環した、非常にユニークな新規骨格であり、構造的にも興味深い構造であるため、この化合物からの合成展開を実施することにした。

Table 4　新規誘導体の薬理評価(1)



Comp.	8-membered ring (R, X)	writhing test[a]	Log $D_{7.4}$[b]	MS (%)[c]	hERG channel inhibition (%)[d]
(−)-1		49	2.8	34	63
29		21	1.5	91	88
35a		31	1.1	53	50
35b		36	1.3	49	73
39		83	0.7	>100	26

[a] % inhibition at 30 mg/kg

[b] The distribution coefficients (Log $D_{7.4}$) were measured between 1-octanol and phosphate buffered saline (pH $_{7.4}$).

[c] MS (Metabolic stability) : Percentage of the tested compound remaining after

0.5 h of incubation with mouse liver microsome (0.1 mg/mL).

[d] % inhibition at 10 μM

Table 5に**39**の左側ビシクロ骨格を変換した化合物**40**、**41**、**42**、**43**、**44**、**39a**、**39b**、**48a**および**48b**の鎮痛活性、物性値（Log $D_{7.4}$、MS）およびhERGカリウムイオンチャネル阻害率について示した。ビシクロ骨格にアルキル置換基を導入、および増炭した化合物**40**、**41**、**42**、**43**、**44**は化合物**39**と比較して鎮痛活性の向上はみられなかった。それに対し、**39**を光学分割した**39a**（*S*体）、**39b**（*R*体）では、*S*体の**39a**でより強い鎮痛活性を示すことがわかった（**39a**: 95% inhibition at 30 mg/kg vs **39b**: 49% inhibition at 30 mg/kg）。加えて化合物**39a**はhERGカリウムイオンチャネル阻害においても弱い値を示した（**39a**:14% inhibition at 10 μM）。また、ピロリジン環をピペリジン環へと変換した**48a**、**48b**も比較的強い鎮痛活性を示しており、やはり*S*体で活性が強い傾向がみられた（**48a**: 77% inhibition at 30 mg/kg vs **48b**: 65% inhibition at 30 mg/kg）。

Table 5　新規誘導体の薬理評価(2)

Compound.	writhing test[a]	Log $D_{7.4}$[b]	MS (%)[c]	hERG channel inhibition (%)[d]
39	83	0.7	>100	26
40	37	0.7	93	36
41	47	1.2	82	13
42	43	1.1	91	14
43	44	1.6	72	43
44	55	1.1	92	19
39a	95	0.7	99	14
39b	49	0.7	>100	23
48a	77	1.0	74	38
48b	65	0.9	82	N.T.

[a] % inhibition at 30 mg/kg

[b] The distribution coefficients (Log $D_{7.4}$) were measured between 1-octanol and phosphate buffered saline (pH $_{7.4}$).

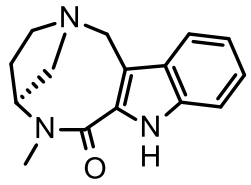
[c] MS (Metabolic stability) : Percentage of the tested compound remaining after 0.5 h of incubation with mouse liver microsome (0.1 mg/mL).

[d] % inhibition at 10 μM

次にTable 5で最も強い鎮痛活性を示した化合物39a(DS54360155)のプロファイルをTable 6に示す。39aはμ-オピオイド受容体（MOR）に対するBinding試験およびcAMP assayの結果よりμ-オピオイド受容体アゴニスト活性を示さないということを確認した。また、39aは脂溶性の大幅な低下（Log $D_{7.4}$：0.7）にもかかわらず、経口投与で良好なPKプロファイルを示し、且つ酢酸ライジング試験においても強い鎮痛薬効(ED_{50}: 8.7 mg/kg)を維持していることもわかった。

39aは*S*配置の不斉中心を有し、分子内アミド結合を含む８員環が、ピロリジン環及びベンゼン環と縮環してビシクロ骨格を形成した、非常にユニークな構造の新規化合物である。hERGカリウムイオンチャネル阻害についても、10 μMの濃度で14%の阻害率と非常に低い値を示し、21a(DS39201083)で懸念されていた安全面での問題点を克服することができた。このように、筆者は21a(DS39201083)からの骨格変換（scaffold hopping）に取り組み、新規の有望なリード化合物39a(DS54360155)を獲得することができた。

Table 6　化合物39a(DS54360155)のプロファイル



39a（DS54360155）

writhing test ED_{50} (mg/kg)[a]	AUC (μM*h) [b]	C_{max} (μM) [b]	$T_{1/2}$ (h) [b]
8.7	5.80	2.15	2.27
MOR binding test	MOR cAMP assay	MOR cAMP assay	
IC_{50} (μM) [c]	EC_{50} (μM) [d]	E_{max} (%) [e]	
>100	>100	19	

[a] The ED_{50} value was calculated from the dose response curve of analgesic activity at 10, 30 and 100 mg/kg

[b] Average of three values dosed at 10 mg/kg orally (p.o.) in ddY mice (0.5 w/v% methylcellulose suspension)

[c] Binding affinities (IC_{50}) were obtained by the competitive displacement of radiolabeled [^{3}H] diprenorphinee. Morphine, with an IC_{50} 0.41 μM, was used as a positive control

[d] cAMP assays were carried out using human MOR-expressed CHO cells. DAMGO, with an EC_{50} 0.088 μM, was used as a positive control

[e] E_{max} was calculated as the response (in %) obtained with DAMGO

第四節　小括

筆者は**21a**(**DS39201083**)で問題となっていたhERGカリウムイオンチャネル阻害(49% inhibition at 10 μM) を克服すべく、**21a**からの誘導体展開を実施した。Log $D_{7.4}$を下げるためにビシクロ骨格の変換 (scaffold hopping) を試みた結果、分子内にアミド結合を含み、ピロリジン環とビシクロ骨格を形成した新規骨格**39a**(**DS54360155**)を獲得することができた。この化合物は**21a**(**DS39201083**)で懸念されていたhERGカリウムイオンチャネル阻害についての問題点を克服し(14% inhibition at 10 μM) 、且つ強い鎮痛薬効(ED_{50}: 8.7 mg/kg)を示す有望化合物であることがわかった。

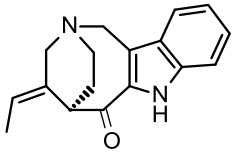
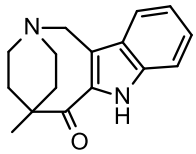
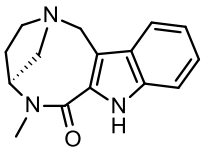
第三章　高活性且つ安全性の高いDS34942424の獲得

第一節　代表化合物の安全性プロファイル

第二章において、経口投与で強い鎮痛作用を示し、且つhERGカリウムイオンチャネル阻害を回避した、新規のビシクロ骨格を有するDS54360155を獲得した。しかしながら、DS54360155を含む、これまで得られてきた有望化合物について、Locomotor activity（ロコモーター）試験を実施したところ、全ての代表化合物で比較的強い鎮静作用があることが明らかになった(Table 7)。ロコモーター試験とは、化合物をマウス（ddYマウス　雄性、4-5週齢）に投与した後に、その総移動距離（自発運動量）を測定することで薬物による行動抑制作用を調べる試験であり、一般に*in vivo*での安全性を評価する代表的な試験系である。

ここでは暫定的にロコモーター試験のID_{50}値 / writhing（酢酸ライジング）試験のED_{50}値＝Safety margin（安全性マージン）と定義することにした。するとTable 7よりコノリジン、DS39201083、およびDS54360155はいずれも安全性マージンが2倍程度しかないことが明らかになった。このような結果より筆者は、より安全域の広がった副作用の少ない化合物の探索が必須と考え、更なる誘導体合成に着手することにした。

Table 7　代表化合物の安全性プロファイル

Compound	writhing test ED_{50} (mg/kg) [a]	Locomotor activity test ID_{50} (mg/kg) [b]	Safety margin [c]
 **(-)-conolidine**	32	69	2.2
 **(DS39201083)**	7.8	16	2.1
 **(DS54360155)**	8.7	19	2.1

[a] The ED_{50} value was calculated from the dose response curve of the analgesic activity of the acetic acid writhing test at 3, 10, 30, and 100 mg/kg.

[b] The ID_{50} value was calculated from the dose response curve of the reduction of the spontaneous activity at 10, 30, and 100 mg/kg.

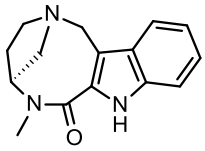
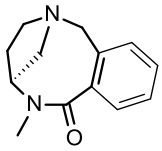
[c] Locomotor activity test ID_{50}/acetic acid writhing test ED_{50}

第二節　安全性マージン拡大のための方策

前掲Table 7の代表化合物の構造について注意深く考察していると、全ての化合物がインドール環を共通骨格として有する点に気がついた。そこでこのインドール環を変換することで、安全性プロファイルが大きく変化するのではないかと考え、インドール環をまずは単純なベンゼン環へと変換することにした。また、このインドール環を変換することで物性の改善も期待でき、誘導体展開の幅が大きく広がるのではないかと考えた。

Table 8に示すように、インドール環をベンゼン環へと変換した化合物49の鎮痛活性はDS54360155と比較して３分の１程度に減弱するものの、安全性マージンは拡大傾向にあることがわかった。そこで、このベンゼン誘導体から鎮痛活性および安全性の向上を目指し、誘導体展開を実施することにした。

Table 8　化合物49の安全性プロファイル

Compound	writhing test ED_{50} (mg/kg) [a]	Locomotor activity test ID_{50} (mg/kg) [b]	Safety margin [c]
 **(DS54360155)**	8.7	19	2.1
 **49**	25	>85	>3.4

[a] The ED_{50} value was calculated from the dose response curve of the analgesic activity of the acetic acid writhing test at 3, 10, 30, and 100 mg/kg.

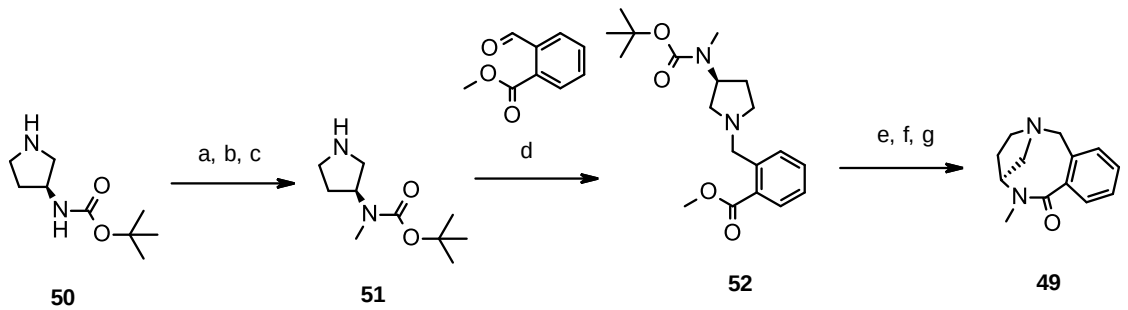
[b] The ID_{50} value was calculated from the dose response curve of the reduction of the spontaneous activity at 10, 30, and 100 mg/kg.

[c] Locomotor activity test ID_{50}/acetic acid writhing test ED_{50}

第三節　誘導体の合成

化合物49は、Scheme 7に示すように分子内アミド化反応を鍵工程として合成した。市販化合物50を出発原料として、ピロリジン環のアミンをCbz基で保護した後に、もう一方の窒素のメチル化、Cbz基の脱保護を経て、ピロリジン中間体51を合成した。この中間体と2-ホルミル安息香酸メチル、$NaBH(OAc)_3$

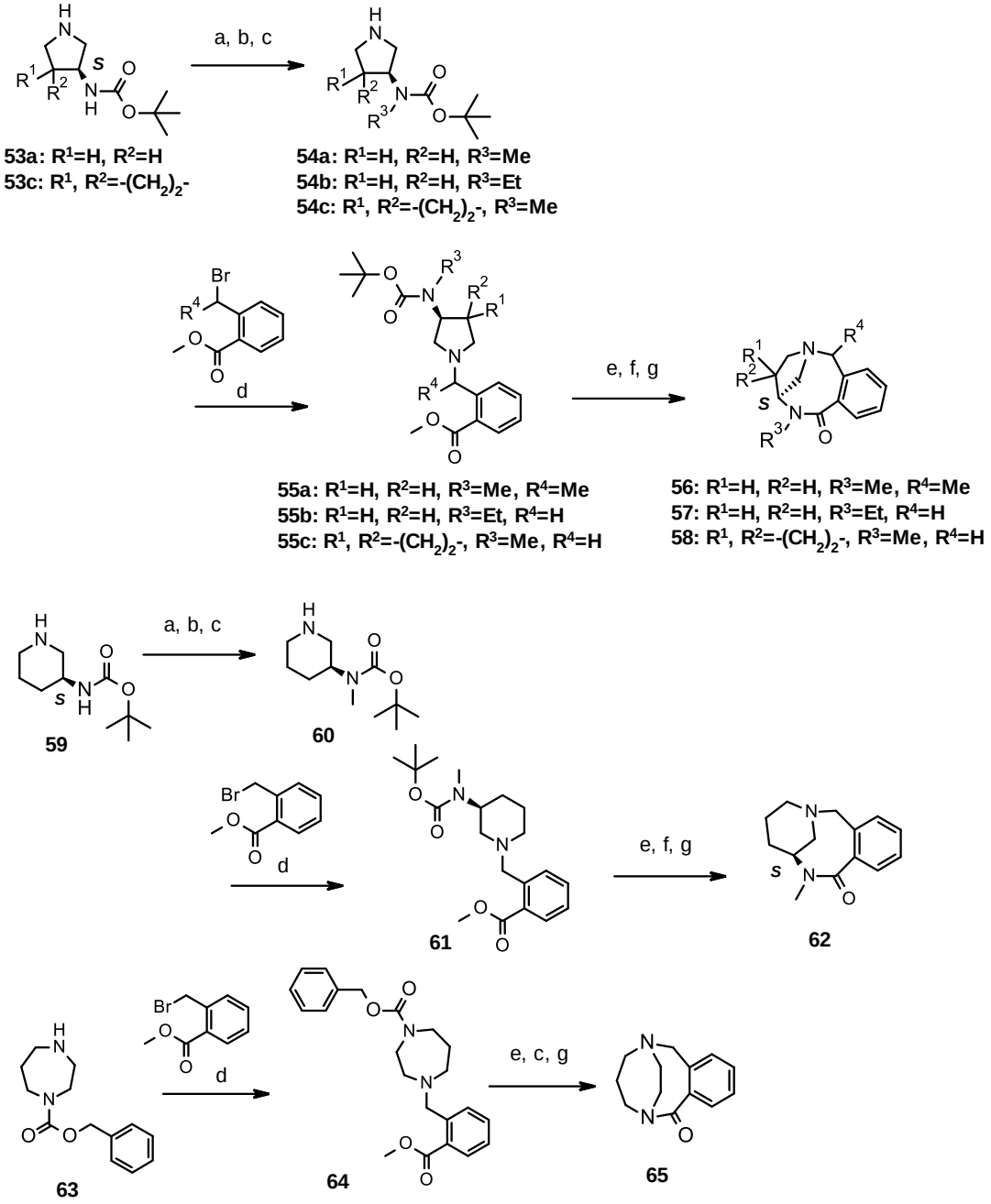
を用いて還元的アミノ化反応を行いメチルエステル**52**を得た。続く加水分解反応、Boc基の脱保護を経て得られた環化前駆体を、分子内アミド化反応によって環化することで目的化合物である**49**を得た。なお、この分子内アミド化反応による環化反応は、分子間反応等の副反応を抑えるために高希釈条件（0.01M～0.05M）を必要とする。しかしこの環化方法を見出したことで、これまで合成出来なかった多くの誘導体を合成することができるようになり、誘導体展開の幅が広がった。



Scheme 7. Reagents and conditions: (a) benzyl chloroformate, Et_3N, THF; (b) MeI, KO^tBu, THF; (c) Pd/C, EtOH, H_2, 59%; (d) $NaBH(OAc)_3$, CH_2Cl_2, 85%; (e) NaOH aq., THF; (f) HCl, 1,4-dioxane/AcOEt; (g) HATU, DIPEA, CH_2Cl_2, 57% three steps.

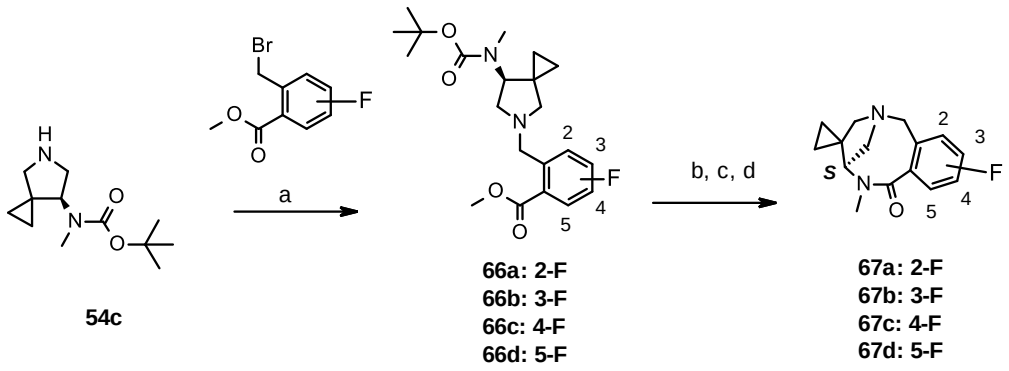
化合物**56**、**57**、**58**、**62**および**65**は**Scheme 8**に示すように合成した。化合物**56**、**57**、**58**、**62**は市販化合物**53a**、**53c**、**59**を出発原料として、**Scheme 7**と同

様にアミンをCbz基で保護した後に、アルキル化、Cbz基の脱保護を経て、ピロリジン中間体**54a-c**、**60**を合成した。この中間体に炭酸カリウムを用いて、対応するベンジルブロミドと反応させることでエステル中間体を**55a-c**、**61**を得た。続く加水分解反応、Boc基の脱保護を経て得られた環化前駆体を、分子内アミド化反応によって環化することで目的化合物である**56**、**57**、**58**、**62**を得た。また化合物**65**は市販化合物**63**より同様の方法にてアルキル化、Cbz基の脱保護、加水分解反応を経て、高希釈条件での分子内アミド化反応によって合成した。



Scheme 8. Reagents and conditions: (a) benzyl chloroformate, Et_3N, THF; (b) MeI or EtI, KO^tBu, THF; (c) Pd/C, EtOH, H_2, 59%-88%; (d) K_2CO_3, CH_2Cl_2, 78%-96%; (e) NaOH aq., THF; (f) HCl, 1,4-dioxane/AcOEt; (g) HATU, DIPEA, CH_2Cl_2, 43%-67% three steps.

Scheme 9にフッ素置換化合物である67a-dの合成方法を示した。Scheme 8で合成した中間体54cを用いて、対応するベンジルブロミドと反応させることでエステル中間体を66a-dを得た。この中間体への加水分解反応、Boc基の脱保護を経て得られた環化前駆体を、分子内アミド化反応によって環化することで目的化合物である67a-dを得た。

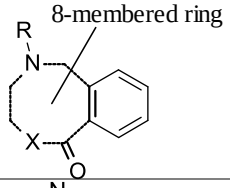
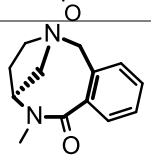
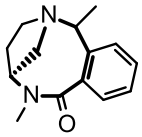
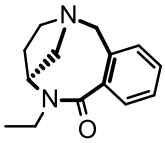
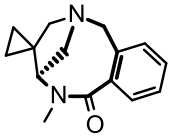
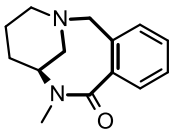
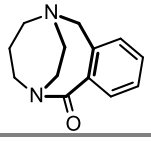


Scheme 9. Reagents and conditions: (a) K_2CO_3, CH_2Cl_2, 76%-96%; (b) NaOH aq., THF; (c) HCl, 1,4-dioxane/AcOEt; (d) HATU, DIPEA, CH_2Cl_2, 55%-70% three steps.

第四節　新規誘導体の薬理評価と考察

ベンゼン誘導体のビシクロ骨格部分を変換した化合物**56**、**57**、**58**、**62**および**65**の評価結果（鎮痛活性、Log $D_{7.4}$、MS）について**Table 9**に示す。まずベンゼン誘導体はインドール誘導体と比べ、全体的にLog $D_{7.4}$が更に下がっており、それに伴って代謝安定性も高い値を示していることがわかる。また、ビシクロ骨格に置換基を導入した化合物**56**、**57**、**58**を比較すると、ピペリジン環にシクロプロピル基を導入した化合物**58**で酢酸ライジング試験において、比較的強い鎮痛薬効を示した（84% inhibition at 30 mg/kg）。また、ビシクロ骨格を変換した化合物**62**、**65**においては、ピロリジン環をピペリジン環へと変換した化合物**62**に比較的強い鎮痛活性がみられた（77% inhibition at 30 mg/kg）。

Table 9　新規誘導体の薬理評価(1)

Compound		writhing test[a]	Log $D_{7.4}$[b]	MS (%)[c]
49		97	0.3	82
56		31	0.3	81
57		45	0.4	80
58		84	0.5	81
62		77	0.3	62
65		24	0.6	78

[a] % inhibition at 30 mg/kg

[b] The distribution coefficients (Log $D_{7.4}$) were measured between 1-octanol and phosphate buffered saline (pH $_{7.4}$).

[c] MS (Metabolic stability) : Percentage of the tested compound remaining after 0.5 h of incubation with mouse liver microsome (0.1 mg/mL).

そこで活性の強かった化合物**58**、**62**について更に詳細な検討（鎮痛活性のED_{50}値、PKプロファイル）を行った(**Table 10**)。その結果、酢酸ライジング試験のED_{50}値において、化合物**58**において最も強い活性を示すことがわかった（ED_{50}値: 15 mg/kg）。またPKプロファイルについても概ね良好な結果であった。そこで次にシクロプロピル誘導体**58**からの誘導体展開を行った。

Compound	writhing test ED_{50} (mg/kg)[a]	AUC (μM*h) [b]	C_{max} (μM) [b]
49	25	7.79	3.74
58	15	5.24	4.13
62	23	1.97	2.82

Table 10　有望化合物の詳細評価

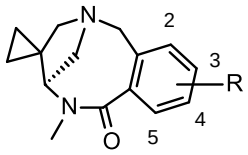
[a] The ED_{50} value was calculated from the dose response curve of analgesic activity at 3, 10 and 30 mg/kg

[b] Average of three values dosed at 10 mg/kg orally (p.o.) in ddY mice (0.5 w/v% methylcellulose suspension)

Table 11にシクロプロピル誘導体**58**のベンゼン環上の置換基を検討した結果を示す。これまでの検討結果より、ベンゼン環上に比較的大きな置換基を導入すると鎮痛活性が減弱してしまう傾向にあったので、比較的小さなフッ

素原子の導入を試みた（**67a-d**）。その結果、酢酸ライジング試験のED_{50}値において、ベンゼン環の3位にフッ素原子を導入した化合物**67b**において最も強い鎮痛活性を示すことがわかった（ED_{50}値: 6.4 mg/kg)。さらに化合物**67b**はhERGカリウムイオンチャネル阻害においても弱い値を示した（2% inhibition at 30 μM)。

Table 11　新規誘導体の薬理評価(2)

Compound	 R =	writhing test ED_{50} (mg/kg)[a]	Log $D_{7.4}$[b]	hERG channel inhibition[d]
58	H	15	0.5	15
67a	2-F	14	0.9	0
67b	3-F	6.4	0.9	2
67c	4-F	36	0.9	14
67d	5-F	35	1.0	0

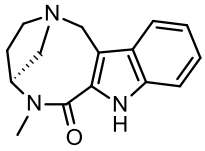
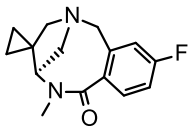
[a] The ED_{50} value was calculated from the dose response curve of analgesic activity at 3, 10 and 30 mg/kg

[b] The distribution coefficients (Log $D_{7.4}$) were measured between 1-octanol and phosphate buffered saline (pH $_{7.4}$).

[d] % inhibition at 30 μM

次にTable 11で最も強い鎮痛活性を示した有望化合物67b(DS34942424)の安全性プロファイルについてTable 12に示した。67b(DS34942424)のロコモーター活性のID_{50}値が66 mg/kgだったことから、安全性マージンは10.3と大きく拡大した。今回の結果より、鎮痛作用（酢酸ライジング試験のED_{50}値）と鎮静作用（ロコモーター試験のID_{50}値）はベンゼン誘導体にすることで大きく乖離する傾向があることが明確になった。従って、鎮静作用を引き起こすメカニズムは鎮痛作用を示すメカニズムとは別であり、その作用はオフターゲットであることが示唆される。

Table 12　化合物67b(DS34942424)の安全性プロファイル

Compound	writhing test ED_{50} (mg/kg) [a]	Locomotor activity test ID_{50} (mg/kg) [b]	Safety margin [c]
 **(DS54360155)**	8.7	19	2.1
 **67b(DS34942424)**	6.4	66	10.3

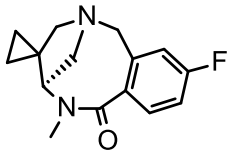
[a] The ED_{50} value was calculated from the dose response curve of the analgesic activity of the acetic acid writhing test at 3, 10, 30, and 100 mg/kg.

[b] The ID_{50} value was calculated from the dose response curve of the reduction of the spontaneous activity at 10, 30, and 100 mg/kg.

[c] Locomotor activity test ID_{50}/acetic acid writhing test ED_{50}

次に有望化合物**67b**(**DS34942424**)の詳細なプロファイルについて精査した結果を**Table 13**に示す。**67b**(**DS34942424**)は10 mg/kgの経口投与で良好なPKプロファイルを示し、また投与1時間後のKp brain値は1.40と、1以上であったことから脳移行性も十分高い化合物であるといえる。また、μ-オピオイド受容体（MOR）に対するBinding試験およびcAMP assayの結果よりμ-オピオイド受容体アゴニスト活性を示さないということも確認できた。

Table 13　化合物67b(DS34942424)のPKプロファイルおよびMOR活性



67b （DS34942424）

AUC (μM*h) [a]	C_{max} (μM) [a]	$T_{1/2}$ (h) [a]	Kp_brain [b]
5.80	2.15	2.27	1.40
MOR binding test	MOR cAMP assay	MOR cAMP assay	
IC_{50} (μM) [c]	EC_{50} (μM) [d]	E_{max} (%) [e]	
>100	>100	19	

[a] Average of three values dosed at 10 mg/kg orally (p.o.) in ddY mice (0.5 w/v% methylcellulose suspension)

[b] Kp brain : brain to plasma concentration ratio at 1h

[c] Binding affinities (IC_{50}) were obtained by the competitive displacement of radiolabeled [^{3}H] diprenorphinee. Morphine, with an IC_{50} 0.41 μM, was used as a positive control

[d] cAMP assays were carried out using human MOR-expressed CHO cells. DAMGO, with an EC_{50} 0.088 μM, was used as a positive control

[e] E_{max} was calculated as the response (in %) obtained with DAMGO

引き続き、**67b(DS34942424)**の鎮痛活性について精査するため、オピオイドと同等の活性かどうかを評価するための試験系である、マウスホルマリン試験[36)37)]を実施した。本試験はddYマウス（日本SLC 雄性、4-5週齢）の後肢足蹠皮下にホルマリンを注入することで生じる Licking/Biting 行動を観察し、その行動時間を測定することで鎮痛作用を評価する。特にinitial phaseと呼ばれるホルマリンの投与から約10分までに起こる知覚神経へのホルマリンの直接的な侵害刺激により生じる疼痛はオピオイド薬のみが効果を示すとされている[38)39)]。**Figure 12**に**67b(DS34942424)**のマウスホルマリン試験の結果を示す。**67b(DS34942424)**は用量依存的に非常に強い薬効を示し、そのED_{50}値は16 mg/kgであった。コノリジンのマウスホルマリン試験におけるED_{50}値は37 mg/kgであることから、**67b(DS34942424)**はコノリジンと比較して、2倍以上も鎮痛薬効が強いことがわかった。このように、筆者は安全性面の問題を解決し（安全性マージン：＞10）、オピオイド治療薬に匹敵する強い鎮痛薬効を有する、新規の有望化合物**67b(DS34942424)**を獲得することができた。

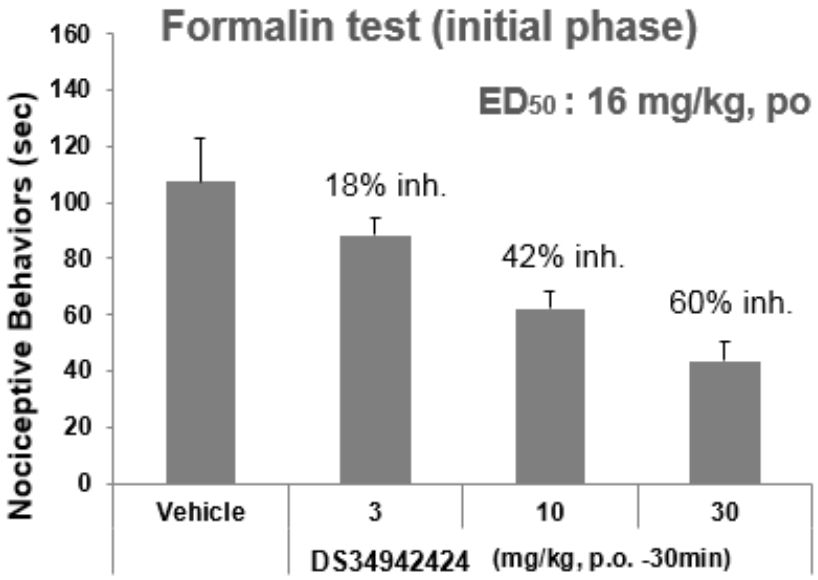


Figure 12 Formalin test (initial phase) of 67b（DS34942424）

The hind paw pad of the ddY mice was injected with 3.5% formalin (20 μL) (n = 8) 30 min after the compound **67b** (**DS34942424**) was administered (p.o.). The reduction in the sum of time spent in paw licking and biting responses was measured for the first 10 min (the initial phase) following the formalin injected.

第五節　小括

筆者は安全性プロファイルの向上を目指し、**39a**(**DS54360155**)のインドール環をベンゼン環へと変換した化合物**49**を合成したところ、安全性マージンが拡大するという傾向を見出した。そこで、化合物**49**から新たに最適化に取り組んだ結果、ビシクロ環にスピロの３員環、ベンゼン環にフッ素を導入した**67b**(**DS34942424**)が酢酸ライジング試験で最も強い薬効を示し（ED_{50}：6.4 mg/kg）、安全性マージンも10倍以上に拡大することを見出した。そして、この化合物はhERGカリウムイオンチャネル阻害においても弱い値を示し（2% inhibition at 30 μM)、マウスホルマリン試験においても用量依存的に非常に強い薬効を示した（ED_{50}値は16 mg/kg）。また、本化合物がコノリジンと同様にμ-オピオイド受容体（MOR）には作用しないことも、MORとのBinding試験、及び、cAMP assayにより確認している。これらの結果より、筆者はMORを介さずに、オピオイドに匹敵する強い鎮痛薬効を有する化合物**67b**(**DS34942424**)を獲得することに成功した。

総括と展望

本研究において、筆者は天然物コノリジンの興味深い薬理作用に着目し、コノリジンを誘導体展開することで、「μ-オピオイド作動薬に匹敵する鎮痛スペクトルを有し、 μ-オピオイド作動薬特有の副作用を回避した非麻薬性鎮痛薬を創製する」という研究コンセプトを掲げ、研究に取り組んだ。

まず天然物コノリジンからの探索的な初期誘導体展開を実施し、エキソオレフィン部分を除去することで、コノリジンより強い鎮痛薬効を示す化合物**4a**を獲得した(73% inhibition at 30 mg/kg) 。続いて**4a**の橋頭位にMe基を導入することで、更に強い鎮痛薬効を示す**21a**(**DS39201083**,ED_{50}: 7.8 mg/kg)を獲得することに成功した。この化合物はμ-オピオイド受容体アゴニスト活性を示さず、経口投与でコノリジンと比較して約4倍強い鎮痛薬効を示す有望化合物であることがわかった。

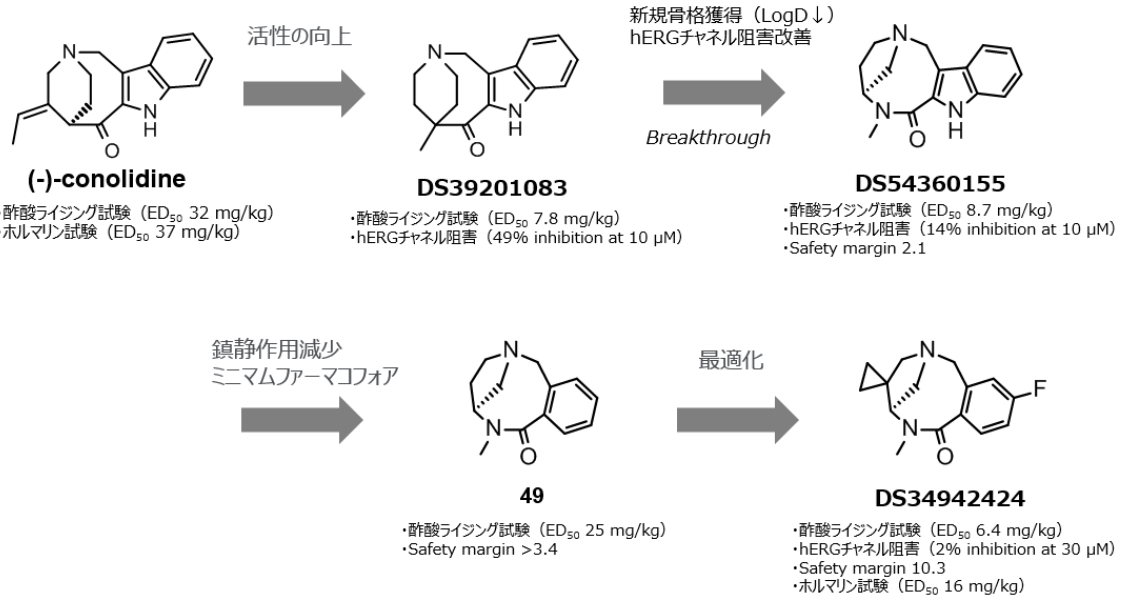
しかしながら**21a**(**DS39201083**)のプロファイリングを行っていく過程で、この化合物に比較的強いhERGカリウムイオンチャネル阻害があることがわかってきた。この問題を克服すべく、合成した種々の化合物データの検証を行った結果、Log $D_{7.4}$を低くすることでhERGカリウムイオンチャネル阻害を低減できるという傾向を掴むことができた。そこで**21a**(**DS39201083**)からLog $D_{7.4}$を下げるべくビシクロ骨格の変換（scaffold hopping）を試みる誘導体展開を実施することにした。その結果、分子内にアミド結合を含み、ピロリジン環とビシクロ骨格を形成した新規骨格**39a**(**DS54360155**)を獲得することがで

きた。この化合物は**21a**(**DS39201083**)で懸念されていたhERGカリウムイオンチャネル阻害についての問題点を克服し(14% inhibition at 10 μM)、且つ強い鎮痛薬効(ED_{50}: 8.7 mg/kg)を示す有望化合物であった。

これまで得られてきた有望化合物について、*in vivo*での安全性試験であるロコモーター試験を実施したところ、全ての代表化合物に比較的強い鎮静作用があることが明らかになった。そこで筆者は構造的な考察から、代表化合物の共通骨格であるインドール環を変換することで、安全性プロファイルが変化するのではないかと考えた。はじめに、**39a**(**DS54360155**)のインドール環をベンゼン環へと変換した化合物**49**を合成したところ、安全性マージンが拡大するという傾向を見出した。この結果を受けて、化合物**49**から新たに最適化に取り組んだ結果、ビシクロ環にスピロの３員環、ベンゼン環にフッ素を導入した**67b**(**DS34942424**)が酢酸ライジング試験で最も強い薬効を示し(ED_{50}:6.4 mg/kg)、安全性マージンも10倍以上に拡大することを見出した。そして、**67b**(**DS34942424**)はhERGカリウムイオンチャネル阻害においても弱い値を示し(2% inhibition at 30 μM)、マウスホルマリン試験においても用量依存的に強い薬効を示した(ED_{50}値は16 mg/kg)。また、本化合物がμ-オピオイド受容体(MOR)には作用しないことも、MORとのバインディング試験、及びcAMP試験により確認している。これらの結果より、筆者はオピオイドを介さずに、オピオイドに匹敵する鎮痛薬効を有する化合物**67b**(**DS34942424**)を創製することに成功した。

今後の展望として、**DS34942424**の作用メカニズムの解析を進めていきたいと考える。一連の誘導体のターゲットは中枢にあるというデータが得られつ

つあることから、ターゲットフィッシング等の手法によってターゲットの同定を試みたいと考えている。



Fizgure 13　本研究の総括

実験の部

合成

General information

Unless otherwise noted, commercial reagents and solvents were obtained from suppliers and used as purchased. Normal-phase column chromatography was performed on silica gel (SiO_2) or amino-silica gel using prepackaged cartridges. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian 400-MHz spectrometer or Bruker AVANCE III 500 spectrometers, and chemical shifts are given in ppm from tetramethylsilane (TMS) as an internal standard. The mass spectra were obtained on an Agilent Technologies system. High-resolution mass (HRMS) spectra were recorded on a JEOL JMS-100LP spectrometer under electron spray ionization conditions (ESI). Elemental analyses were conducted by using a Microcorder JM10 and a Dionex ICS-1500. Optical rotations were recorded on a Rudolph Autopol V plus or a Jasco P-1030 polarimeter. All experimental procedures for the animals were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of Daiichi Sankyo Co., Ltd.

(4*E*)-4-ethylidene-7-methyl-1,4,5,7-tetrahydro-2,5-ethanoazocino[4,3-b]indol-6(3*H*)-one sulfuric acid salt (2a)

To the mixture of conolidine (0.0300g, 0.113mmol) in DMF (1.0 mL), 60% sodium hydride (0.0090g, 0.225 mmol) was added. After the mixture was stirred for 1 h, iodomethane (0.0077 mL, 0.124 mmol) was added and stirred overnight. The mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, and concentrated *in vacuo*. The residue was purified by column chromatography using silica gel to obtain (4*E*)-4-ethylidene-7-methyl-1,4,5,7-tetrahydro-2,5-ethanoazocino[4,3-b]indol-6(3*H*)-one (0.017 g, 54%). This compound was treated with 1 mol/L hydrosulfonic acid (0.062 mL, 0.062 mmol) in ethanol (1 mL) at 0 °C. The resulting solid was collected and dried using a pump to obtain **2a** (0.020 g, 88%) as a solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.83 (1H, br s), 7.70-7.61 (2H, m), 7.49-7.41 (1H, m), 7.26-7.18 (1H, m), 5.90-5.80 (1H, m), 5.06 (1H, d, *J* = 17.0 Hz), 4.62 (1H, d, *J* = 17.0 Hz), 4.23-4.14 (1H, m), 4.04-3.98 (1H, m), 3.60-3.57 (3H, br m), 2.53-2.50 (3H, br m), 2.44-2.30 (2H, m), 1.54 (3H, d, *J* = 5.5 Hz); MS (ESI) *m/z*: 281 $(M+H)^{+}$.

(4*E*)-4-ethylidene-7-(2-hydroxyethyl)-1,4,5,7-tetrahydro-2,5-ethanoazocino[4,3-b]indol-6(3*H*)-one sulfuric acid salt (2b)

To the mixture of conolidine (0.0300g, 0.113mmol) in DMF (1.0 mL), 60% sodium hydride (0.0135g, 0.338 mmol) was added. After the mixture was stirred for 1 h, 2-bromoethyl acetate (0.0248 mL, 0.150 mmol) was added and stirred overnight. The mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, and concentrated *in vacuo*. The residue was purified by column chromatography using silica gel to obtain 2-[(4*E*)-4-ethylidene-6-oxo-3,4,5,6-tetrahydro-2,5-ethanoazocino[4,3-b]indol-7(1*H*)-yl]ethyl acetate (0.021 g, 58%). This compound was treated with 2 mol/L sodium hydroxide (1 mL) in THF / methanol (1 mL / 1 mL) at 0 °C and the mixture was stirred overnight. The mixture was poured into 2 M HCl (1 mL) and extracted with ethyl acetate. The ethyl acetate layer was washed with water and brine, dried over sodium sulfate, and concentrated *in vacuo* to give (4*E*)-4-ethylidene-7-(2-hydroxyethyl)-1,4,5,7-tetrahydro-2,5-ethanoazocino[4,3-b]indol-6(3*H*)-one (0.014 g, 80%). This alcohol was treated with 1 mol/L hydrosulfonic acid (0.045 mL, 0.045 mmol) in ethanol (1 mL) at 0 °C. The resulting solid was collected and dried *in vacuo* to obtain **2b** (0.016 g, 91%) as a solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.18 (1H, br s), 7.70-7.23 (2H, m), 7.40-7.21 (1H, m), 7.24-7.11 (1H, m), 5.67-5.55 (1H, m), 5.01 (1H, d, *J* = 17.2 Hz), 4.32 (1H, d, *J* = 17.2 Hz), 4.31-4.10 (1H, m), 4.00-3.87 (1H, m), 3.74-3.59 (5H, br m), 2.65-2.52 (2H, br m), 2.44-2.30 (2H, m), 1.51 (3H, d, *J* = 5.5 Hz); MS (ESI) *m/z*: 310 $(M+H)^{+}$.

[1-(benzenesulfonyl)-7-methoxy-1*H*-indol-2-yl]-{(3*E*)-3-ethylidene-1-[(4-methoxyphenyl)methyl]-piperidin-4-yl}methanol (6a)

To a solution of lithium diisopropylamide in *n*-hexane-THF (13.3 mL, 14.3 mmol) at −78 °C, 1-(phenylsulfonyl)indole (4.12 g, 14.3 mmol) in THF (40 mL) was added dropwise. After stirring for 1 h, (3*E*)-3-ethylidene-1-[(4-methoxyphenyl)methyl]piperidine-4-carbaldehyde **5** (2.48 g, 9.56 mmol) in THF (20 mL) was added dropwise. The mixture was allowed to warm up to 0 °C and stirred overnight. Then, the mixture was poured into water and extracted with ethyl acetate. The ethyl acetate layer was washed with brine, dried over sodium sulfate, and concentrated *in vacuo*. The residue was passed through silica gel and eluted with 50%–100% ethyl acetate in hexane to give **6a** (5.23 g, 62%) as a solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.71 (2H, d, *J* = 8.0 Hz), 7.50-7.46 (1H, m), 7.39 (2H, t, *J* = 7.8 Hz), 7.26-7.23 (3H, m), 7.12-7.09 (1H, m), 7.04 (1H, d, *J* = 8.0 Hz), 6.90-6.85 (2H, m), 6.63 (1H, d, *J* = 8.4 Hz), 5.55 (1H, d, *J* = 5.4 Hz), 5.35-5.34 (1H, m), 3.81 (3H, s), 3.62 (3H, s), 3.58-3.53 (1H, m), 3.49 (2H, br s), 3.19 (1H, d, *J* = 13.2 Hz), 2.88 (1H, d, *J* = 13.4 Hz), 2.62-2.58 (1H, m), 2.35-2.30 (1H, m), 1.83-1.80 (1H, m), 1.64 (3H, s), 1.27-1.25 (1H, m); MS (ESI) *m/z*: 547 $(M+H)^+$.

{(3*E*)-3-ethylidene-1-[(4-methoxyphenyl)methyl]-piperidin-4-yl}-(7-methoxy-1*H*-indol-2-yl)methanol (7a)

A mixture of **6a** (0.472 g, 0.914 mmol) and cesium carbonate (1.19 g, 3.65 mmol) in THF (8 mL) and methanol (4 mL) was refluxed for 2 h and then poured into brine and extracted with ethyl acetate. The ethyl acetate layer was washed

with water and brine, dried over sodium sulfate, and concentrated *in vacuo*. The residue was passed through silica gel and eluted with 10%–60% ethyl acetate in hexane, to give **7a** (0.282 g, 82%) as a solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.37 (1H, br s), 7.12 (2H, d, *J* = 8.0 Hz), 7.15-7.07 (1H, m), 6.92 (1H, d, *J* = 8.0 Hz), 6.880-6.82 (2H, m), 6.50 (1H, d, *J* = 5.8 Hz), 6.37-6.34 (1H, m), 5.31-5.28 (1H, m), 5.21 (1H, d, *J* = 4.6 Hz), 3.90 (3H, s), 3.78 (3H, s), 3.50-3.43 (2H, m), 3.20 (2H, t, *J* = 10.3 Hz), 2.82 (1H, d, *J* = 136 Hz), 2.70-2.62 (1H, m), 2.36-2.25 (1H, m), 1.99-1.95 (1H, m), 1.68-1.60 (1H, m), 1.51 (3H, d, *J* = 6.6 Hz) ; MS (ESI) *m/z*: 407 $(M+H)^+$.

(4*E*)-4-ethylidene-11-methoxy-1,4,5,7-tetrahydro-2,5-ethanoazocino[4,3-b]indol-6(3H)-one (8a)

A mixture of alcohol **7a** (0.282 g, 0.689 mmol) and manganese oxide (0.898 g, 10.3 mmol) in dichloromethane (10 mL) was stirred at room temperature for 4 h. The insoluble portion was removed, and the filtrate was concentrated *in vacuo* to give

[(3*E*)-3-ethylidene-1-[(4-methoxyphenyl)methyl]-piperidin-4-yl]-(7-methoxy-1*H*-indol-2-yl)methanone as a yellow solid, which was used for the next reaction without further purification. The mixture of ketone and chloroformic acid 1-chloroethyl ester (1.81 mL, 1.78 mmol) in 1,2-dichloroethane (15 mL) was reflux for 1 h and then concentrated in vacuo.The residue was dissolved in methanol(15mL) and heated to 80°C for two hours. The mixture was

concentrated in vacuo and the residue was washed with diisopropyl ether to give [(3*E*)-3-ethylidene-piperidin-4-yl]-(7-methoxy-1*H*-indol-2-yl)methanone hydrochloride as a solid. A mixture of amine HCl salt, paraformaldehyde (0.0386 g, 1.16 mmol), and trifluoroacetic acid (0.0737 mL, 0.963 mmol) in 1,2-dichloroethane (64 mL) was stirred at 80 °C for 6 h. The mixture was poured into saturated sodium bicarbonate solution and extracted with dichloromethane (x3). The dichloromethane layer was washed with brine, dried over sodium sulfate, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel and eluted with 20% methanol in dichloromethane to give **8a** (0.0776 g, 38%) as a foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.94 (1H, s), 7.20 (1H, t, *J* = 8.0 Hz), 6.89 (1H, d, *J* = 8.0 Hz), 6.39 (1H, d, *J* = 8.0 Hz), 5.44-5.43 (1H, m), 5.06 (1H, d, *J* = 19.6 Hz), 4.49 (1H, d, *J* = 19.6 Hz), 3.95-3.93 (1H, m), 3.87 (3H, s), 3.85-3.83 (1H, m), 3.43-3.40 (1H, m), 3.33 (1H, d, *J* = 15.7 Hz), 3.14-3.10 (1H, m), 2.11-2.07 (2H, m), 1.49 (3H, d, *J* = 6.9 Hz): 297 $(M+H)^+$.

(4*E*)-4-ethylidene-11-methoxy-1,4,5,7-tetrahydro-2,5-ethanoazocino[4,3-b]indol-6(3*H*)-one sulfuric acid salt (3a)

Compound **8a** (0.0776 g, 2.62 mmol) was treated with 1 mol/L hydrosulfonic acid (2.4 mL, 2.62 mmol) in ethanol (5 mL) at 0 °C. The resulting solid was collected and dried *in vacuo* to obtain **3a** (0.0721 g, 86%) as a solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.81 (1H, s), 7.23 (1H, t, *J* = 8.0 Hz), 6.92 (1H, d, *J* = 8.0 Hz),

6.30 (1H, d, *J* = 8.0 Hz), 5.49-5.47 (1H, m), 5.00 (1H, d, *J* = 19.7 Hz), 4.43 (1H, d, *J* = 19.7 Hz), 3.94-3.90 (1H, m), 3.79 (3H, s), 3.85-3.84 (1H, m), 3.42-3.39 (1H, m), 3.31 (1H, d, *J* = 15.7 Hz), 3.14-3.11 (1H, m), 2.10-2.02 (2H, m), 1.45 (3H, d, *J* = 6.6 Hz): 297 $(M+H)^+$.

[1-(benzenesulfonyl)-6-methoxy-1*H*-indol-2-yl]-{(3*E*)-3-ethylidene-1-[(4-methoxyphenyl)methyl]-piperidin-4-yl}methanol (6b)

6b was prepared as a solid in a similar manner described for **6a**. 75% yeild: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.61-7.55 (2H, m), 7.40-7.35 (1H, m), 7.34-7.30 (2H, m), 7.26-7.23 (3H, m), 7.15-7.10 (1H, m), 7.14 (1H, d, *J* = 8.2 Hz), 6.91-6.80 (2H, m), 6.61 (1H, d, *J* = 8.2 Hz), 5.65 (1H, d, *J* = 5.2 Hz), 5.35-5.30 (1H, m), 3.82 (3H, s), 3.52 (3H, s), 3.50-3.46 (1H, m), 3.66 (2H, br s), 3.06 (1H, d, *J* = 13.0 Hz), 2.92 (1H, d, *J* = 13.0 Hz), 2.55-2.49 (1H, m), 2.35-2.31 (1H, m), 1.93-1.81 (1H, m), 1.76 (3H, s), 1.27-1.22 (1H, m); MS (ESI) *m/z*: 547 $(M+H)^+$.

{(3*E*)-3-ethylidene-1-[(4-methoxyphenyl)methyl]-piperidin-4-yl}-(6-methoxy-1*H*-indol-2-yl)methanol (7b)

7b was prepared as a solid in a similar manner described for **7a**. 88% yeild: ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.37 (1H, br s), 7.12 (2H, d, *J* = 8.0 Hz), 7.15-7.07 (1H, m), 6.92 (1H, d, *J* = 8.0 Hz), 6.880-6.82 (2H, m), 6.50 (1H, d, *J* = 5.8 Hz), 6.37-6.34 (1H, m), 5.31-5.28 (1H, m), 5.21 (1H, d, *J* = 4.6 Hz), 3.90 (3H, s),

3.78 (3H, s), 3.50-3.43 (2H, m), 3.20 (2H, t, *J* = 10.3 Hz), 2.82 (1H, d, *J* = 136 Hz), 2.70-2.62 (1H, m), 2.36-2.25 (1H, m), 1.99-1.95 (1H, m), 1.68-1.60 (1H, m), 1.51 (3H, d, *J* = 6.6 Hz) ; MS (ESI) *m/z*: 407 $(M+H)^+$.

(4*E*)-4-ethylidene-10-methoxy-1,4,5,7-tetrahydro-2,5-ethanoazocino[4,3-b]indol-6(3H)-one (8b)

8b was prepared as a foam in a similar manner described for **8a**. 37% yeild: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.90 (1H, br s), 7.27-7.25 (1H, m), 7.01 (1H, dd, *J* = 8.8, 2.3 Hz), 6.89 (1H, d, *J* = 2.3 Hz), 5.47-5.45 (1H, m), 4.69 (1H, d, *J* = 18.4 Hz), 4.24 (1H, d, *J* = 18.4 Hz), 3.96 (1H, d, *J* = 5.9 Hz), 3.87-3.86 (1H, m), 3.85 (3H, s), 3.42-3.38 (1H, m), 3.31 (1H, d, *J* = 16.0 Hz), 3.11-3.07 (1H, m), 2.16-2.01 (2H, m), 1.51 (3H, d, *J* = 6.8 Hz); MS (ESI) *m/z*: 297 $(M+H)^+$.

(4*E*)-4-ethylidene-10-methoxy-1,4,5,7-tetrahydro-2,5-ethanoazocino[4,3-b]indol-6(3*H*)-one sulfuric acid salt (3b)

3b was prepared as a solid in a similar manner described for **3a**. 87% yeild: ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.25-7.20 (1H, m), 7.00 (1H, d, *J* = 8.6 Hz), 6.69 (1H, d, *J* = 2.1 Hz), 5.45-5.43 (1H, m), 4.75 (1H, d, *J* = 18.0 Hz), 4.20 (1H, d, *J* = 18.0 Hz), 3.92 (1H, d, *J* = 6.0 Hz), 3.83-3.81 (1H, m), 3.82 (3H, s), 3.45-3.39 (1H, m), 3.30 (1H, d, *J* = 16.4 Hz), 3.13-3.08 (1H, m), 2.16-2.11 (2H, m), 1.43 (3H, d, *J* = 6.4 Hz); MS (ESI) *m/z*: 297 $(M+H)^+$.

[1-(benzenesulfonyl)-5-methoxy-1*H*-indol-2-yl]-{(3*E*)-3-ethylidene-1-[(4-methoxyphenyl)methyl]-piperidin-4-yl}methanol (6c)

6c was prepared as a solid in a similar manner described for **6a**. 64% yeild: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.79 (2H, d, *J* = 7.8 Hz), 7.51-7.40 (1H, m), 7.28-7.24 (2H, m Hz), 7.20-7.14 (3H, m), 7.12-7.06 (1H, m), 7.04 (1H, d, *J* = 8.2 Hz), 6.88-6.80 (2H, m), 6.72 (1H, d, *J* = 8.2 Hz), 5.40 (1H, d, *J* = 5.5 Hz), 5.34-5.29 (1H, m), 3.66 (3H, s), 3.57 (3H, s), 3.55-3.50 (1H, m), 3.44 (2H, br s), 3.24 (1H, d, *J* = 12.8 Hz), 2.98 (1H, d, *J* = 12.8 Hz), 2.71-2.59 (1H, m), 2.40-2.31 (1H, m), 1.80-1.73 (1H, m), 1.66 (3H, s), 1.31-1.28 (1H, m); MS (ESI) *m/z*: 547 $(M+H)^+$.

{(3*E*)-3-ethylidene-1-[(4-methoxyphenyl)methyl]-piperidin-4-yl}-(5-methoxy-1*H*-indol-2-yl)methanol (7c)

7c was prepared as a solid in a similar manner described for **7a**. 85% yeild: ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.60 (1H, br s), 7.21 (2H, d, *J* = 8.2 Hz), 7.04-7.01 (1H, m), 6.90 (1H, d, *J* = 8.2 Hz), 6.94-6.88 (2H, m), 6.58 (1H, d, *J* = 5.8 Hz), 6.35-6.30 (1H, m), 5.38-5.33 (1H, m), 5.02 (1H, d, *J* = 4.8 Hz), 3.92 (3H, s), 3.76 (3H, s), 3.60-3.51 (2H, m), 3.21-2.98 (2H, m), 2.83 (1H, d, *J* = 13.6 Hz), 2.62-2.55 (1H, m), 2.28-2.22 (1H, m), 1.90-1.85 (1H, m), 1.68-1.60 (1H, m), 1.59 (3H, d, *J* = 6.8 Hz) ; MS (ESI) *m/z*: 407 $(M+H)^+$.

(4*E*)-4-ethylidene-9-methoxy-1,4,5,7-tetrahydro-2,5-ethanoazocino[4,3-b]indol-6(3*H*)-one (8c)

8c was prepared as a foam in a similar manner described for **8a**. 37% yeild: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.95 (1H, br s), 7.31-7.26 (1H, m), 7.11 (1H, dd, *J* = 8.4, 2.2 Hz), 6.89 (1H, d, *J* = 2.2 Hz), 5.45-5.41 (1H, m), 4.55 (1H, d, *J* = 18.1 Hz), 4.32 (1H, d, *J* = 18.1 Hz), 3.91 (1H, d, *J* = 5.6 Hz), 3.85-3.83 (1H, m), 3.80 (3H, s), 3.45-3.30 (1H, m), 3.28 (1H, d, *J* = 15.6 Hz), 3.06-3.01 (1H, m), 2.19-2.11 (2H, m), 1.61 (3H, d, *J* = 6.5 Hz); MS (ESI) *m/z*: 297 $(M+H)^+$.

(4*E*)-4-ethylidene-9-methoxy-1,4,5,7-tetrahydro-2,5-ethanoazocino[4,3-b]indol-6(3*H*)-one sulfuric acid salt (3c)

3c was prepared as a solid in a similar manner described for **3a**. 84% yeild: ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.35-7.21 (1H, m), 7.03 (1H, d, *J* = 8.4 Hz), 6.69 (1H, d, *J* = 2.2 Hz), 5.55-5.50 (1H, m), 4.65 (1H, d, *J* = 18.1 Hz), 4.21 (1H, d, *J* = 18.1 Hz), 3.97 (1H, d, *J* = 6.2 Hz), 3.85-3.82 (1H, m), 3.77 (3H, s), 3.41-3.37 (1H, m), 3.21 (1H, d, *J* = 16.8 Hz), 3.15-3.11 (1H, m), 2.21-2.18 (2H, m), 1.43 (3H, d, *J* = 6.0 Hz); MS (ESI) *m/z*: 297 $(M+H)^+$.

[1-(benzenesulfonyl)-4-methoxy-1*H*-indol-2-yl]-{(3*E*)-3-ethylidene-1-[(4-methoxyphenyl)methyl]-piperidin-4-yl}methanol (6d)

6d was prepared as a solid in a similar manner described for **6a**. 64% yeild: ^{1}H

NMR (400 MHz, $CDCl_3$) δ: 7.75 (2H, d, *J* = 8.2 Hz), 7.51-7.46 (1H, m), 7.30 (2H, t, *J* = 8.0 Hz), 7.26-7.22 (3H, m), 7.20-7.15 (1H, m), 7.07 (1H, d, *J* = 8.2 Hz), 6.85-6.79 (2H, m), 6.62 (1H, d, *J* = 8.2 Hz), 5.42 (1H, d, *J* = 5.3 Hz), 5.37-5.32 (1H, m), 3.77 (3H, s), 3.59 (3H, s), 3.50-3.43 (1H, m), 3.33 (2H, br s), 3.14 (1H, d, *J* = 13.0 Hz), 2.76 (1H, d, *J* = 13.0 Hz), 2.50-2.45 (1H, m), 2.38-2.33 (1H, m), 1.94-1.85 (1H, m), 1.68 (3H, s), 1.43-1.33 (1H, m); MS (ESI) *m/z*: 547 $(M+H)^+$.

{(3*E*)-3-ethylidene-1-[(4-methoxyphenyl)methyl]-piperidin-4-yl}-(4-methoxy-1*H*-indol-2-yl)methanol (7d)

7d was prepared as a solid in a similar manner described for **7a**. 92% yeild: ^1H NMR (500 MHz, $CDCl_3$) δ: 8.67 (1H, br s), 7.22 (2H, d, *J* = 8.0 Hz), 7.08-7.03 (1H, m), 6.98 (1H, d, *J* = 8.0 Hz), 6.90-6.88 (2H, m), 6.51 (1H, d, *J* = 5.5 Hz), 6.35-6.34 (1H, m), 5.33-5.31 (1H, m), 5.02 (1H, d, *J* = 4.7 Hz), 3.94 (3H, s), 3.80 (3H, s), 3.55-3.46 (2H, m), 3.22 (2H, t, *J* = 10.6 Hz), 2.87 (1H, d, *J* = 13.7 Hz), 2.72-2.66 (1H, m), 2.34-2.28 (1H, m), 1.99-1.92 (1H, m), 1.68-1.64 (1H, m), 1.53 (3H, d, *J* = 6.7 Hz); MS (ESI) *m/z*: 407 $(M+H)^+$.

(4*E*)-4-ethylidene-8-methoxy-1,4,5,7-tetrahydro-2,5-ethanoazocino[4,3-b]indol-6(3*H*)-one (8d)

8d was prepared as a foam in a similar manner described for **8a**. 38% yeild: ^1H NMR (400 MHz, $CDCl_3$) δ: 8.95 (1H, s), 7.21 (1H, t, *J* = 8.3 Hz), 6.86 (1H, d, *J* =

8.3 Hz), 6.37 (1H, d, *J* = 8.3 Hz), 5.45-5.42 (1H, m), 5.00 (1H, d, *J* = 19.4 Hz), 4.45 (1H, d, *J* = 19.4 Hz), 3.94-3.91 (1H, m), 3.83 (3H, s), 3.82-3.80 (1H, m), 3.46-3.42 (1H, m), 3.32 (1H, d, *J* = 15.4 Hz), 3.14-3.11 (1H, m), 2.22-2.14 (2H, m), 1.41 (3H, d, *J* = 6.7 Hz): 297 $(M+H)^+$.

(4*E*)-4-ethylidene-8-methoxy-1,4,5,7-tetrahydro-2,5-ethanoazocino[4,3-b]indol-6(3*H*)-one sulfuric acid salt (3d)

3d was prepared as a solid in a similar manner described for **3a**. 86% yeild: ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.82 (1H, s), 7.25 (1H, t, *J* = 8.2 Hz), 6.99 (1H, d, *J* = 8.2 Hz), 6.32 (1H, d, *J* = 8.2 Hz), 5.48-5.45 (1H, m), 5.05 (1H, d, *J* = 19.1 Hz), 4.44 (1H, d, *J* = 19.1 Hz), 3.94-3.92 (1H, m), 3.78 (3H, s), 3.85-3.81 (1H, m), 3.49-3.38 (1H, m), 3.33 (1H, d, *J* = 15.6 Hz), 3.14-3.13 (1H, m), 2.12-2.07 (2H, m), 1.44 (3H, d, *J* = 6.5 Hz): 297 $(M+H)^+$.

4-ethyl-1,4,5,7-tetrahydro-2,5-ethanoazocino[4,3-b]indol-6(3*H*)-one sulfuric acid salt (4b)

To the mixture of conolidine (0.200 g, 0.751 mmol) in ethanol (20 mL), 10% palladium carbon (0.200 g) was added and stirred under hydrogen atmosphere for 9 h. The insoluble matter was removed, and then, the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography using silica gel and eluted with 0%-18% methanol in dichloromethane to obtain

4-ethyl-1,4,5,7-tetrahydro-2,5-ethanoazocino[4,3-b]indol-6(3*H*)-one (0.195 g, 97%) as a solid. This compound was treated with 1 mol/L hydrosulfonic acid (0.727 mL, 0.727 mmol) in ethanol (10 mL) at 0 °C. The resulting solid was collected and dried using a pump to obtain **4b** (0.242 g, 91%) as a solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.86 (1H, br s), 10.83 (1H, br s), 7.66 (1H, d, *J* = 8.2 Hz), 7.49 (1H, d, *J* = 8.0 Hz), 7.38-7.36 (1H, m), 7.16 (1H, t, *J* = 8.0 Hz), 5.12 (1H, d, *J* = 17.2 Hz), 4.76 (1H, d, *J* = 17.2 Hz), 3.63-3.56 (2H, m), 3.42-3.40 (1H, m), 3.26-3.21 (1H, m), 2.97-2.94 (1H, m), 2.41-2.35 (3H, m), 1.15-1.11 (2H, m), 0.90 (3H, t, *J* = 7.0 Hz); MS (ESI) *m/z*: 269 $(M+H)^+$

[1-(benzenesulfonyl)indol-2-yl]-{(3*Z*)-3-ethylidene-1-[(4-methoxyphenyl)methyl]-piperidin-4-yl}methanol (10c)

10c was prepared as a solid in a similar manner described for **6a**. 84% yeild: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.11 (1H, d, *J* = 8.6 Hz), 7.71-7.70 (2H, m), 7.47-7.43 (1H, m), 7.40-7.39 (1H, m), 7.35-7.32 (2H, m), 7.24-7.17 (4H, m), 6.89 (2H, d, *J* = 8.4 Hz), 6.79 (1H, s), 5.42 (1H, d, *J* = 5.1 Hz), 5.36-5.35 (1H, m), 3.82 (3H, s), 3.62 (1H, t, *J* = 5.5 Hz), 3.52 (2H, s), 3.24 (1H, d, *J* = 13.3 Hz), 2.86 (1H, d, *J* = 13.3 Hz), 2.67-2.64 (1H, m), 2.29-2.26 (1H, m), 1.76 (2H, d, *J* = 7.4 Hz), 1.61-1.60 (3H, m); MS (ESI) *m/z*: 517 $(M+H)^+$.

{(3*Z*)-3-ethylidene-1-[(4-methoxyphenyl)methyl]-piperidin-4-yl}-(1*H*-indol-2-yl)methanol (11c)

11c was prepared as a solid in a similar manner described for **7a**. 88% yeild: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.42 (1H, br s), 7.50 (1H, d, *J* = 8.0 Hz), 7.32 (1H, d, *J* = 8.0 Hz), 7.29-7.24 (2H, m), 7.21-7.17 (1H, m), 7.10-7.05 (1H, m), 6.93-6.90 (2H, m), 6.20 (1H, br s), 5.52 (1H, d, *J* = 5.1 Hz), 5.34-5.32 (1H, m), 3.89 (3H, s), 3.60 (1H, t, *J* = 5.5 Hz), 3.59 (2H, s), 3.22 (1H, d, *J* = 13.6 Hz), 2.88 (1H, d, *J* = 13.6 Hz), 2.67-2.64 (1H, m), 2.29-2.26 (1H, m), 1.76 (2H, d, *J* = 7.4 Hz), 1.61-1.60 (3H, m); MS (ESI) *m/z*: 377 $(M+H)^+$.

(4*Z*)-4-ethylidene-1,4,5,7-tetrahydro-2,5-ethanoazocino[4,3-b]indol-6(3*H*)-one (12c)

12c was prepared as a solid in a similar manner described for **8a**. 35% yeild: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.00 (1H, br s), 7.57 (1H, d, *J* = 8.2 Hz), 7.36-7.33 (2H, m), 7.13-7.09 (1H, m), 5.48-5.46 (1H, m), 4.77 (1H, d, *J* = 14.4 Hz), 4.29 (1H, d, *J* = 14.4 Hz), 3.98-3.97 (1H, m), 3.88-3.84 (1H, m), 3.42-3.39 (1H, m), 3.33-3.29 (1H, m), 3.14-3.05 (1H, m), 2.19-2.01 (2H, m), 1.51 (3H, d, *J* = 7.0 Hz); MS (ESI) *m/z*: 267 $(M+H)^+$.

(4*Z*)-4-ethylidene-1,4,5,7-tetrahydro-2,5-ethanoazocino[4,3-b]indol-6(3*H*)-one sulfuric acid salt (4c)

4c was prepared as a solid in a similar manner described for **3a**. 82% yeild: ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.92 (1H, br s), 7.71 (1H, d, *J* = 8.0 Hz), 7.56-7.42 (2H, m), 7.11-7.06 (1H, m), 5.55-5.48 (1H, m), 4.70 (1H, d, *J* = 14.2 Hz), 4.39 (1H, d, *J* = 14.2 Hz), 3.97-3.95 (1H, m), 3.89-3.80 (1H, m), 3.55-3.42 (1H, m), 3.40-3.27 (1H, m), 3.14-3.06 (1H, m), 2.25-2.11 (2H, m), 1.61 (3H, d, *J* = 7.2 Hz) ; MS (ESI) *m/z*: 267 $(M+H)^+$.

[1-(benzenesulfonyl)indol-2-yl]-{(3*E*)-1-[(4-methoxyphenyl)methyl]-3-propylidene-piperidin-4-yl}methanol (10d)

10d was prepared as a solid in a similar manner described for **6a**. 80% yeild: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.11 (1H, d, *J* = 8.0 Hz), 7.70 (2H, d, *J* = 8.0 Hz), 7.47-7.19 (8H, m), 6.91-6.89 (2H, m), 6.78 (1H, br s), 5.42-5.40 (1H, m), 5.27-5.25 (1H, m), 3.82 (3H, s), 3.57-3.54 (4H, m), 3.28 (1H, d, *J* = 13.0 Hz), 2.87 (1H, d, *J* = 13.0 Hz), 2.68-2.66 (1H, m), 2.27 (3H, s), 1.79-1.67 (1H, m), 0.93 (3H, t, *J* = 8.0 Hz); MS (ESI) *m/z*: 531 $(M+H)^+$.

1H-indol-2-yl-{(3*E*)-1-[(4-methoxyphenyl)methyl]-3-propylidene-piperidin-4-yl}methanol (11d)

11d was prepared as a solid in a similar manner described for **7a**. 92% yeild: ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.62 (1H, br s), 7.54 (1H, d, *J* = 8.0 Hz), 7.35 (1H, d, *J* = 8.0 Hz), 7.26-7.23 (2H, m), 7.13-7.12 (1H, m), 7.07-7.06 (1H, m), 6.90-6.90 (2H, m), 6.25 (1H, br s), 5.25-5.24 (1H, m), 5.03 (1H, d, *J* = 4.4 Hz), 3.82 (3H, d, *J* = 2.0 Hz), 3.56-3.50 (2H, m), 3.29 (1H, d, *J* = 13.4 Hz), 3.21-3.19 (1H, m), 2.89 (1H, d, *J* = 13.4 Hz), 2.74-2.71 (1H, m), 2.31-2.29 (1H, m), 2.09-2.04 (1H, m), 1.97-1.94 (2H, m), 1.69-1.63 (1H, m), 0.88 (3H, t, *J* = 7.4 Hz). ; MS (ESI) *m/z*: 391 $(M+H)^+$.

(4*E*)-4-propylidene-1,4,5,7-tetrahydro-2,5-ethanoazocino[4,3-b]indol-6(3*H*)-one (12d)

12d was prepared as a solid in a similar manner described for **8a**. 32% yeild: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.98 (1H, br s), 7.58 (1H, d, *J* = 8.2 Hz), 7.36-7.33 (2H, m), 7.12-7.10 (1H, m), 5.37-5.35 (1H, m), 4.77 (1H, d, *J* = 18.8 Hz), 4.30 (1H, d, *J* = 18.8 Hz), 3.98-3.96 (1H, m), 3.87 (1H, d, *J* = 16.0 Hz), 3.42-3.39 (1H, m), 3.31 (1H, d, *J* = 16.0 Hz), 3.12-3.06 (1H, m), 2.18-2.10 (1H, m), 2.06-2.01 (2H, m), 1.90-1.83 (1H, m), 0.81 (3H, t, *J* = 7.4 Hz).; MS (ESI) *m/z*: 281 $(M+H)^+$.

(4*E*)-4-propylidene-1,4,5,7-tetrahydro-2,5-ethanoazocino[4,3-b]indol-6(3*H*)-one sulfuric acid salt (4d)

4d was prepared as a solid in a similar manner described for **3a**. 88% yeild: ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.90 (1H, br s), 7.68 (1H, d, *J* = 8.0 Hz), 7.48-7.43 (2H, m), 7.21-7.12 (1H, m), 5.35-5.30 (1H, m), 4.62 (1H, d, *J* = 18.4 Hz), 4.21 (1H, d, *J* = 18.4 Hz), 4.01-3.97 (1H, m), 3.85 (1H, d, *J* = 16.2 Hz), 3.44-3.38 (1H, m), 3.29 (1H, d, *J* = 16.2 Hz), 3.22-3.06 (1H, m), 2.19-2.15 (1H, m), 2.06-2.00 (2H, m), 1.95-1.93 (1H, m), 0.88 (3H, t, *J* = 7.2 Hz).; MS (ESI) *m/z*: 281 $(M+H)^+$.

tert-butyl 4-(hydroxymethyl)-4-methyl piperidine-1-carboxylate (14a)

To a suspension of lithium aluminum hydride (0.702 g, 18.5 mmol) in THF (10 mL) at 0 °C, 1-Boc-4-methyl-piperidine-4-carboxylic acid **13a** (3.60 g, 14.8 mmol) in THF (30 mL) was added dropwise over 30 min. After stirring for 1 h, the reaction was quenched with 700 µL of water, 700 µL of 15% NaOH, and 2.1 mL of water, and then stirred vigorously overnight. The reaction mixture was passed through Celite, and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (100 g) and eluted with 10%–70% ethyl acetate in hexane to give alcohol **14a** (2.45 g, 72%) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.72–3.58 (2H, m), 3.35 (2H, d, *J* = 5.9 Hz), 3.13–3.06 (2H, m), 1.50–1.25 (4H, m), 1.38 (9H, s), 0.99 (3H, s).

tert-butyl 4-{[1-(benzenesulfonyl)-1*H*-indol-2-yl]-hydroxy-methyl}-4-methyl piperidine-1-carboxylate (16a)

A mixture of alcohol **14a** (2.45 g, 18.5 mmol), sulfur trioxide pyridine complex (8.50 g, 53.4 mmol), dimethylsulfoxide (4.55 mL, 64.1 mmol), and *N*,*N*-diisopropylethylamine (18.6 mL, 107 mmol) in dichloromethane (100 mL) was stirred at room temperature for 30 min. The mixture was poured into 1 M HCl and extracted with ethyl acetate. The ethyl acetate layer was washed with water and brine, dried over sodium sulfate, and concentrated *in vacuo* to give *tert*-butyl 4-formyl-4-methyl piperidine-1-carboxylate **15a** as an oil, which was used for the next reaction without further purification. To a solution of lithium diisopropylamide in *n*-hexane-THF (17.0 mL, 19.0 mmol) at −78 °C, 1-(phenylsulfonyl)indole (5.00 g, 19.4 mmol) in THF (20 mL) was added dropwise. After stirring for 1 h, aldehyde **15a** (2.4 g, 11 mmol) in THF (20 mL) was added dropwise. The mixture was allowed to warm up to 0 °C and stirred overnight. Then, the mixture was poured into water and extracted with ethyl acetate. The ethyl acetate layer was washed with brine, dried over sodium sulfate, and concentrated *in vacuo*. The residue was passed through silica gel (100 g) and eluted with 50%–100% ethyl acetate in hexane to give **16a** (4.1 g, 80% from **14a**) as a solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.21 (1H, d, *J* = 8.2 Hz), 7.67 (2H, d, *J* = 9.4 Hz), 7.52–7.44 (2H, m), 7.41–7.22 (4H, m), 6.74 (1H, s), 5.41 (1H, d, *J* = 4.7 Hz), 3.89–3.86 (2H, br m), 2.87–2.85 (2H, br m), 2.06–2.04 (1H, br m), 1.84–1.81 (1H, br m), 1.46 (9H, s), 1.09 (3H, s).

Synthesis of *tert*-butyl 4-[hydroxy(1*H*-indol-2-yl)methyl]-4-methyl piperidine-1-carboxylate (17a)

A mixture of **16a** (4.1 g, 8.5 mmol) and cesium carbonate (14 g, 42 mmol) in THF (60 mL) and methanol (20 mL) was refluxed for 2 h and then poured into

brine and extracted with ethyl acetate. The ethyl acetate layer was washed with water and brine, dried over sodium sulfate, and concentrated *in vacuo*. The residue was passed through silica gel (90 g) and eluted with 10%–60% ethyl acetate in hexane, to give **17a** (2.3 g, 79%) as a solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.38 (1H, s), 7.56 (1H, d, *J* = 7.8 Hz), 7.35 (1H, d, *J* = 4.5 Hz), 7.18–7.14 (1H, m), 7.11–7.07 (1H, m), 6.32 (1H, d, *J* = 1.6 Hz), 4.61 (1H, d, *J* = 3.5 Hz), 3.88–3.85 (2H, br m), 2.97–2.93 (2H, br m), 2.22 (1H, d, *J* = 3.5 Hz), 1.70–1.24 (4H, m), 1.46 (9H, s), 1.00 (3H, s).

5-methyl-1,4,5,7-tetrahydro-2,5-ethanoazocino[4,3-b]indol-6(3*H*)-one (20a)

A mixture of alcohol **17a** (2.3 g, 6.3 mmol) and manganese oxide (23 g, 270 mmol) in dichloromethane (100 mL) was stirred at room temperature for 4 h. The insoluble portion was removed, and the filtrate was concentrated *in vacuo* to give *tert*-butyl 4-(1*H*-indole-2-carbonyl)-4-methyl piperidine-1-carboxylate **18a** as a yellow solid, which was used for the next reaction without further purification. Compound **18a** was dissolved in 4 M HCl/ethyl acetate (28 mL) and stirred at room temperature for 1 h. Then, the reaction mixture was concentrated *in vacuo* to give 1*H*-indol-2-yl-(4-methyl-piperidin-4-yl)methanone hydrochloride **19a** as a solid. A mixture of amine HCl salt **19a**, paraformaldehyde (0.48 g, 16 mmol), and trifluoroacetic acid (1.5 mL, 19 mmol) in 1,2-dichloroethane (420 mL) was stirred at 80 °C for 6 h. The mixture was poured into saturated sodium bicarbonate solution and extracted with dichloromethane (x3). The dichloromethane layer was washed with brine, dried over sodium sulfate, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (50 g) and eluted with 20% methanol in

dichloromethane to give **20a** (510 mg, 30% from **10**) as a foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.05 (1H, br s), 7.59–7.58 (1H, m), 7.38–7.36 (2H, m), 7.17–7.10 (1H, m), 4.52 (2H, s), 3.39–3.33 (2H, m), 3.08–2.97 (2H, m), 2.10–2.03 (2H, m), 1.77–1.67 (2H, m), 1.29 (3H, s).

5-methyl-1,4,5,7-tetrahydro-2,5-ethanoazocino[4,3-b]indol-6(3*H*)-one sulfuric acid salt (21a, DS39201083)

Compound **20a** (510 mg, 2.01 mmol) was treated with 1 mol/L hydrosulfonic acid (2.2 mL, 2.21 mmol) in ethanol (5 mL) at 0 °C. The resulting solid was collected and dried *in vacuo* to obtain **21a** (480 mg, 68%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.58 (1H, br s), 7.65 (1H, d, *J* = 8.2 Hz), 7.48 (1H, d, *J* = 8.2 Hz), 7.34 (1H, t, *J* = 7.6 Hz), 7.12 (1H, t, *J* = 7.4 Hz), 4.72 (2H, s), 3.42–3.37 (2H, m), 3.22–3.19 (2H, m), 2.11–2.04 (2H, m), 1.88–1.86 (2H, m), 1.28 (3H, s). MS (ESI) *m/z*: 255 $(M+H)^+$. Anal. Calcd. for $C_{16}H_{18}N_2O.H_2SO_4$. $1/3C_2H_5OH$: C, 54.43; H, 6.03; N, 7.62; S, 8.72. Found: C, 54.16; H, 5.80; N, 7.89; S, 8.73.

***tert*-butyl 4-{[1-(benzenesulfonyl)-1*H*-indol-2-yl](hydroxy)methyl}-4-ethyl piperidine-1-carboxylate (14b)**

14b was prepared as a solid in a similar manner described for **14a**. 89% yeild: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.51-3.45 (4H, m), 3.32-3.26 (2H, m), 1.45 (9H, s), 1.44-1.41 (4H, m), 1.32-1.22 (2H, m), 0.88-0.82 (3H, m).; MS (ESI) *m/z*: 244 $(M+H)^+$.

***tert*-butyl 4-{[1-(benzenesulfonyl)-1*H*-indol-2-yl](hydroxyl)methyl}-4-ethyl piperidine-1-carboxylate (16b)**

16b was prepared as a solid in a similar manner described for **16a**. 89% yeild: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.19 (1H, d, *J* = 8.2 Hz), 7.66 (2H, d, *J* = 8.2 Hz), 7.52-7.45 (2H, m), 7.31-7.15 (5H, m), 6.76 (1H, s), 5.63 (1H, s), 3.80 (2H, s), 2.87 (2H, t, *J* = 12.3 Hz), 1.95 (1H, s), 1.74-1.70 (4H, m), 1.46 (1H, s), 1.38 (9H, s), 0.96 (3H, s).; MS (ESI) *m/z*: 499 $(M+H)^+$.

***tert*-butyl**
4-ethyl-4-[hydroxy(1*H*-indol-2-yl)methyl]piperidine-1-carboxylatel (17b)

17b was prepared as a solid in a similar manner described for **17a**.85% yeild: ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.36 (1H, br s), 7.57 (1H, d, *J* = 7.8 Hz), 7.37 (1H, d, *J* = 8.2 Hz), 7.18-7.16 (1H, m), 7.11-7.09 (1H, m), 6.33 (1H, s), 4.89 (1H, d, *J* = 3.5 Hz), 3.80-3.77 (2H, m), 3.02-2.99 (2H, m), 1.68-1.61 (4H, m), 1.54-1.46 (2H, m), 1.41 (9H, s), 0.90 (3H, t, *J* = 7.6 Hz); MS (ESI) *m/z*: 359 $(M+H)^+$.

5-ethyl-1,4,5,7-tetrahydro-2,5-ethanoazocino[4,3-b]indol-6(3*H*)-one (20b)

20b was prepared as a solid in a similar manner described for **20a**. 40% yeild: ^{1}H NMR (400 MHz, $CDCl_3$) 10.32 (1H, br s), 7.52 (1H, d, *J* = 8.2 Hz), 7.43 (1H, d, *J* = 8.2 Hz), 7.32-7.30 (1H, m), 7.16-7.10 (1H, m), 4.86 (2H, br s), 3.57-3.49 (2H, m), 3.35-3.31 (2H, m), 2.11-2.03 (4H, m), 1.74-1.70 (2H, m), 0.95 (3H, t, *J* = 7.0

Hz); MS (ESI) *m/z*: 269 $(M+H)^+$.

5-ethyl-1,4,5,7-tetrahydro-2,5-ethanoazocino[4,3-b]indol-6(3*H*)-one sulfuric acid salt (21b)

21b was prepared as a solid in a similar manner described for **21a**. 89% yeild: ^{1}H NMR (400 MHz, DMSO-d_6)δ: 11.77 (1H, br s), 10.68 (1H, br s), 7.63 (1H, d, *J* = 8.2 Hz), 7.48 (1H, d, *J* = 8.2 Hz), 7.36-7.34 (1H, m), 7.15-7.13 (1H, m), 4.91 (2H, br s), 3.57-3.55 (2H, m), 3.38-3.36 (2H, m), 2.08-2.06 (4H, m), 1.74-1.73 (2H, m), 0.88 (3H, t, *J* = 7.2 Hz); MS (ESI) *m/z*: 269 $(M+H)^+$.

***tert*-butyl 4-{[1-(benzenesulfonyl)-1*H*-indol-2-yl](hydroxy)methyl}-4-propylpiperidine-1-carboxylate (16c)**

16b was prepared as a solid in a similar manner described for **16a**. 89% yeild: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.21 (1H, d, *J* = 8.6 Hz), 7.66 (2H, d, *J* = 8.2 Hz), 7.50-7.41 (2H, m), 7.11-7.09 (5H, m), 6.54 (1H, s), 5.21 (1H, s), 3.74 (2H, s), 2.55 (2H, t, *J* = 12.6 Hz), 1.94 (1H, s), 1.79-1.68 (4H, m), 1.40 (1H, s), 1.35 (9H, s), 1.14-1.06 (2H, m), 0.88 (3H, s).; MS (ESI) *m/z*: 513 $(M+H)^+$.

***tert*-butyl 4-[hydroxy(1*H*-indol-2-yl)methyl]-4-propylpiperidine-1-carboxylate (17c)**

17b was prepared as a solid in a similar manner described for **17a**.85% yeild: ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.55 (1H, br s), 7.97 (1H, d, *J* = 7.8 Hz), 7.30 (1H, d, *J* = 8.4 Hz), 7.21-7.19 (1H, m), 7.09-7.04 (1H, m), 6.30 (1H, s), 4.91 (1H, d, *J* = 3.6 Hz), 3.78-3.70 (2H, m), 2.93-2.82 (2H, m), 1.65-1.60 (4H, m), 1.44-1.38 (2H, m), 1.43 (9H, s), 1.14-1.06 (2H, m), 0.84 (3H, t, *J* = 7.6 Hz); MS (ESI) *m/z*: 373 $(M+H)^+$.

5-propyl-1,4,5,7-tetrahydro-2,5-ethanoazocino[4,3-b]indol-6(3*H*)-one (20c)

20b was prepared as a solid in a similar manner described for **20a**. 40% yeild: ^{1}H NMR (400 MHz, $CDCl_3$) 10.43 (1H, br s), 7.76 (1H, d, *J* = 8.4 Hz), 7.32 (1H, d, *J* = 8.4 Hz), 7.21-7.19 (1H, m), 7.14-7.10 (1H, m), 4.80-4.69 (2H, m), 3.59-3.55 (2H, m), 3.39-3.34 (2H, m), 2.17-2.09 (4H, m), 1.75-1.72 (2H, m), 1.15-1.11 (2H, m), 0.85 (3H, t, *J* = 7.2 Hz); MS (ESI) *m/z*: 283 $(M+H)^+$.

5-propyl-1,4,5,7-tetrahydro-2,5-ethanoazocino[4,3-b]indol-6(3*H*)-one sulfuric acid salt (21c)

21b was prepared as a solid in a similar manner described for **21a**. 89% yeild: ^{1}H NMR (400 MHz, DMSO-d_6)δ: 11.85 (1H, br s), 10.45 (1H, br s), 7.43 (1H, d, *J* = 8.1 Hz), 7.49 (1H, d, *J* = 8.2 Hz), 7.37-7.33 (1H, m), 7.17-7.12 (1H, m), 4.91 (2H, br s), 3.59-3.53 (2H, m), 3.44-3.38 (2H, m), 2.13-2.08 (4H, m), 1.68-1.66 (2H, m), 1.14-1.10 (2H, m), 0.97 (3H, t, *J* = 7.2 Hz); MS (ESI) *m/z*: 283 $(M+H)^+$.

tert-butyl

4-{[1-(benzenesulfonyl)-1*H*-indol-2-yl]-hydroxy-methyl}-4-methyl

azepane-1-carboxylate (24)

A mixture of tert-butyl 4-(hydroxymethyl)-4-methylazepane-1-carboxylate **22** (1.8 g, 7.4 mmol), sulfur trioxide pyridine complex (5.9 g, 37 mmol), dimethylsulfoxide (3.2 mL, 44 mmol), and *N*,*N*-diisopropylethylamine (13 mL, 74 mmol) in dichloromethane (40 mL) was stirred at room temperature for 30 min. The mixture was poured into 1 M HCl and extracted with ethyl acetate. The ethyl acetate layer was washed with water and brine, dried over sodium sulfate, and concentrated *in vacuo* to give tert-butyl 4-formyl-4-methyl azepane-1-carboxylate **23** as an oil, which was used for the next reaction without further purification. To a solution of lithium diisopropylamide in *n*-hexane-THF (8.0 mL, 8.7 mmol) at −78 °C, 1-(phenylsulfonyl)indole (2.3 g, 9.1 mmol) in THF (20 mL) was added dropwise. After stirring for 1 h, aldehyde **23** (1.8 g, 7.3 mmol) in THF (20 mL) was added dropwise. The mixture was allowed to warm up to 0 °C and stirred overnight. Then, the mixture was poured into water and extracted with ethyl acetate. The ethyl acetate layer was washed with brine, dried over sodium sulfate, and concentrated *in vacuo*. The residue was passed through silica gel (100 g) and eluted with 50%–100% ethyl acetate in hexane to give **24** (2.9 g, 80% from **22**) as a solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.21 (1H, d, *J* = 8.6 Hz), 7.66 (2H, d, *J* = 8.2 Hz), 7.53-7.41 (2H, m), 7.39-7.22 (5H, m),

6.75 (1H, s), 5.50-5.42 (1H, m), 3.45-3.42 (3H, m), 3.27-3.24 (3H, m), 1.88-1.60 (5H, m), 1.60-1.50 (2H, m), 1.50-1.34 (9H, m); MS (ESI) *m/z*: 499 $(M+H)^+$.

***tert*-butyl**

4-[hydroxy(1*H*-indol-2-yl)methyl]-4-methylazepane-1-carboxylate (25)

A mixture of **24** (2.9 g, 5.8 mmol) and dicesium carbonate (9.5 g, 29 mmol) in THF (40 mL) and methanol (15 mL) was refluxed for 6 h and then poured into brine and extracted with ethyl acetate. The ethyl acetate layer was washed with water and brine, dried over sodium sulfate, and concentrated *in vacuo*. The residue was passed through silica gel (90 g) and eluted with 10%–60% ethyl acetate in hexane, to give **25** (2.1 g, 85%) as a solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.12 (1H, br s), 7.70 (1H, d, *J* = 8.2 Hz), 7.42 (1H, d, *J* = 8.2 Hz), 7.33 (1H, t, *J* = 7.6 Hz), 7.22 (1H, s), 7.15 (1H, t, *J* = 7.6 Hz), 3.77-3.44 (2H, m), 3.36-3.12 (5H, m), 2.55-2.43 (3H, m), 1.90-1.55 (7H, m), 1.47 (9H, s).; MS (ESI) *m/z*: 359 $(M+H)^+$.

6-methyl-1,3,4,5,6,8-hexahydro-7*H*-2,6-ethanoazonino[4,3-b]indol-7-one (28)

A mixture of alcohol **25** (2.1 g, 5.9 mmol) and manganese oxide (20 g, 230 mmol) in dichloromethane (100 mL) was stirred at room temperature for 5 h. The insoluble portion was removed, and the filtrate was concentrated *in vacuo* to give *tert*-butyl 4-(1*H*-indole-2-carbonyl)-4-methylazepane-1-carboxylate **26** as a yellow solid, which was used for the next reaction without further purification. Compound **26** was dissolved in 4 M HCl/ethyl acetate (4.9 mL) and stirred at

room temperature for 1 h. Then, the reaction mixture was concentrated *in vacuo* to give 1*H*-indol-2-yl-(4-methylazepan-4-yl)methanone hydrochloride **27** as a solid. A mixture of **27**, paraformaldehyde (0.31 g, 10 mmol), and trifluoroacetic acid (0.94 mL, 12 mmol) in 1,2-dichloroethane (500 mL) was stirred at 80 °C for 3 h. The mixture was poured into saturated sodium bicarbonate solution and extracted with dichloromethane (x3). The dichloromethane layer was washed with brine, dried over sodium sulfate, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (50 g) and eluted with 20% methanol in dichloromethane to give **28** (510 mg, 55% from **25**) as a foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.15-9.04 (1H, m), 7.66 (1H, d, *J* = 8.2 Hz), 7.39-7.32 (2H, m), 7.12 (1H, t, *J* = 8.0 Hz), 4.77 (1H, d, *J* = 17.4 Hz), 4.43 (1H, d, *J* = 17.4 Hz), 3.43-3.03 (4H, m), 2.78-2.66 (1H, m), 2.14-2.04 (1H, m), 1.89-1.82 (3H, m), 1.75-1.61 (3H, m); MS (ESI) *m/z*: 269 $(M+H)^+$.

6-methyl-1,3,4,5,6,8-hexahydro-7*H*-2,6-ethanoazonino[4,3-b]indol-7-one sulfuric acid (29)

Compound **28** (500 mg, 1.9 mmol) was treated with 3 mol/L hydrosulfonic acid (0.68 mL, 2.1 mmol) in ethanol (9 mL) at 0 °C. The resulting solid was collected and dried using a pump to obtain **29** (610 mg, 89%).^{1}H-NMR (400 MHz, DMSO-d_6) δ: 11.82 (1H, br s), 8.57 (1H, br s), 7.59 (1H, d, *J* = 8.2 Hz), 7.51 (1H, d, *J* = 8.2 Hz), 7.25 (1H, t, *J* = 7.6 Hz), 7.13 (1H, t, *J* = 7.6 Hz), 4.78 (1H, d, *J* = 13.3 Hz), 4.32 (1H, d, *J* = 13.3 Hz), 4.05-3.97 (1H, m), 3.65-3.59 (1H, m), 3.52-3.49 (1H, m), 3.35-3.32 (1H, m), 2.06-2.01 (2H, m), 1.81-1.78 (1H, m), 1.71-1.66 (1H, m), 1.41-1.38 (1H, m), 1.37 (18H, s) ;^{13}C NMR (100 MHz, DMSO-d_6) δ: 141.44, 137.18, 122.99, 121.88, 120.10, 119.55, 113.99, 113.20,

106.53, 58.41, 57.62, 51.81, 43.77, 31.77, 30.13, 25.49, 21.11; HRMS (Positive ESI) m/z 269.1659 (269.1649 calcd for $C_{17}H_{20}N_2O$ + H); EA Anal. calcd for $C_{17}H_{20}N_2O$. 9/8H_2SO_4. H_2O: C, 51.47; H, 6.16; N, 7.06. Found: C, 51.33; H, 6.16; N, 6.80.

_N_-{4-[1-(benzenesulfonyl)-1_H_-indol-2-yl]-4-oxobutyl}benzenesulfonamide (31)

To a solution of *n*-butyllithium, in *n*-hexane (3.8 mL, 49 mmol) at −78 °C, 1-(phenylsulfonyl)indole (12 g, 47 mmol) in THF (500 mL) was added dropwise. After stirring for 1 h, sulfonamide **30** (9.7 g, 43 mmol) in THF (180 mL) was added dropwise. The mixture was allowed to warm up to 0 °C and stirred overnight. Then, the mixture was poured into water and extracted with ethyl acetate. The ethyl acetate layer was washed with brine, dried over sodium sulfate, and concentrated *in vacuo*. The residue was passed through silica gel (100 g) and eluted with 50%–100% ethyl acetate in hexane to give **31** (5.7 g, 28%) as a solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.92-7.84 (4H, m), 7.58-7.42 (8H, m), 7.34-7.27 (4H, m), 7.05 (1H, s), 4.76 (1H, br s), 3.10 (2H, q, *J* = 6.6 Hz), 3.03 (2H, q, *J* = 6.6 Hz), 2.00-1.93 (2H, m).; MS (ESI) *m/z*: 483 $(M+H)^+$.

1-(1_H_-indol-2-yl)-4-(methylamino)butan-1-ol (32a)

To a solution of *N*-{4-[1-(benzenesulfonyl)-1*H*-indol-2-yl]-4-oxobutyl}benzenesulfonamide **31** (2.5 g, 5.2 mmol), potassium carbonate (1.4 g, 10 mmol) in DMF (40 mL), methyl iodide (1.3 mL, 21 mmol) was added. The mixture was stirred at 80 °C

for 2 h. Then, the mixture was poured into water and extracted with ethyl acetate. The ethyl acetate layer was washed with brine, dried over sodium sulfate, and concentrated *in vacuo* to give *N*-{4-[1-(benzenesulfonyl)-1*H*-indol-2-yl]-4-oxobutyl}-*N*-methylbenzenesulfonamide, which was used for the next reaction without further purification. To a solution of *N*-{4-[1-(benzenesulfonyl)-1*H*-indol-2-yl]-4-oxobutyl}-*N*-methylbenzenesulfonamide in EtOH (120 mL) at 0 °C, sodium tetrahydroborate (0.35 g, 9.3 mmol) was added. The mixture was allowed to warm up to room temperature and stirred for 1.5 h. Then, the mixture was poured into saturated sodium bicarbonate solution and extracted with ethyl acetate. The ethyl acetate layer was washed with brine, dried over sodium sulfate, and concentrated *in vacuo* to give *N*-{4-[1-(benzenesulfonyl)-1*H*-indol-2-yl]-4-hydroxybutyl}-*N*-methylbenzenesulfonamide, which was used for the next reaction without further purification. To a solution of *N*-{4-[1-(benzenesulfonyl)-1*H*-indol-2-yl]-4-hydroxybutyl}-*N*-methylbenzenesulfonamide in methanol (170 mL), magnesium (3.4 g, 140 mmol) and ammonium chloride (3.4 g, 64 mmol) were added slowly. The mixture was stirred at room temperature for 2 h. Then, the mixture was poured into water and extracted with ethyl acetate. The ethyl acetate layer was washed with brine, dried over sodium sulfate, and concentrated *in vacuo*. The residue was passed through silica gel (100 g) and eluted with 0%–25% methanol in dichloromethane to give **32a** (0.45 g, 61%) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.65 (1H, br s), 7.55 (1H, d, *J* = 7.4 Hz), 7.34 (1H, d, *J* = 7.4 Hz), 7.16-7.03 (2H, m), 6.22 (1H, s), 4.92-4.90 (1H, m), 3.48 (3H, q, *J* = 7.0 Hz), 2.77-2.68 (1H, m), 2.64-2.55 (1H, m), 2.22-2.12

(1H, m), 2.05-1.95 (1H, m), 1.89-1.78 (1H, m), 1.72-1.62 (1H, m).; MS (ESI) *m/z*: 219 $(M+H)^+$.

2-methyl-2,3,4,5,6,7-hexahydro-1*H*-azocino[4,3-b]indol-6-ol (33a)

The mixture of 1-(1*H*-indol-2-yl)-4-(methylamino)butan-1-ol **32a** (0.46 g, 2.1 mmol), paraformaldehyde (6.4 g, 210 mmol), and acetic acid (5.8 mL, 100 mmol) in 1,2-dichloroethane (720 mL) was stirred at 50 °C for 1.5 h. The mixture was poured into a saturated sodium bicarbonate solution and extracted with dichloromethane. The dichloromethane layer was washed with brine, dried over sodium sulfate salt, and concentrated *in vacuo*. The residue was purified by column chromatography using silica gel (80 g) and eluted with 0–18% methanol in dichloromethane to give the title compound **33a** (0.36 g, 73%) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.42 (1H, br s), 7.37-7.31 (2H, m), 7.20-7.01 (2H, m), 5.43 (2H, dd, *J* = 32.1, 11.7 Hz), 5.09 (1H, br s), 3.79 (1H, d, *J* = 16.0 Hz), 3.61 (1H, d, *J* = 16.0 Hz), 2.73-2.62 (1H, m), 2.47 (3H, s), 2.44-2.35 (1H, m), 1.98-1.74 (2H, m).; MS (ESI) *m/z*: 231 $(M+H)^+$.

2-methyl-1,2,3,4,5,7-hexahydro-6*H*-azocino[4,3-b]indol-6-one (34a)

A mixture of alcohol **33a** (0.36 g, 1.5 mmol) and manganese oxide (4.7 g, 54 mmol) in dichloromethane (120 mL) was stirred at room temperature for 2.5 h. The insoluble portion was removed, and the filtrate was concentrated *in vacuo* The residue was purified by column chromatography using silica gel (80 g) and eluted with 0–18% methanol in dichloromethane to give the title compound (0.36 g, 73%) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.36 (1H, br s), 7.73 (1H,

d, *J* = 8.2 Hz), 7.43 (1H, d, *J* = 8.2 Hz), 7.37-7.35 (1H, m), 7.18 (1H, t, *J* = 7.8 Hz), 4.41 (2H, s), 3.08 (2H, t, *J* = 7.4 Hz), 2.57-2.52 (2H, m), 2.33 (3H, s), 2.11-2.02 (2H, m).; MS (ESI) *m/z*: 229 $(M+H)^+$.

2-methyl-1,2,3,4,5,7-hexahydro-6*H*-azocino[4,3-b]indol-6-one sulfuric acid (35a)

Compound **20** (0.11 g, 0.47 mmol) was treated with 1 mol/L sulfonic acid (0.23 mL, 0.49 mmol) in ethanol (10 mL) at 0 °C. The resulting solid was collected and dried *in vacuo* to obtain the title compound **35a** (0.16 g, 100%). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 12.30 (1H, br s), 9.69 (1H, br s), 7.95 (1H, d, *J* = 8.2 Hz), 7.52 (1H, d, *J* = 8.6 Hz), 7.38 (1H, t, *J* = 8.6 Hz), 7.23 (1H, t, *J* = 8.2 Hz), 5.07 (1H, d, *J* = 14.8 Hz), 4.91 (1H, d, *J* = 14.8 Hz), 3.33-2.99 (6H, m), 2.26-2.18 (1H, m), 2.05-2.01 (1H, m), 1.32 (3H, t, *J* = 7.2 Hz) ; ^{13}C NMR (100 MHz, DMSO-d6) δ: 190.84, 136.39, 128.14, 126.21, 121.16, 120.65, 113.06, 107.54, 56.05, 49.53, 47.06, 41.27, 38.13, 18.55; HRMS (Positive ESI) m/z 229.1350 (229.1336 calcd for $C_{14}H_{16}N_2O$ + H); EA Anal. calcd for $C_{14}H_{16}N_2O$. $5/4H_2SO_4$. $3/20C_4H_{10}O$. $2H_2O$. C, 44.05; H, 6.08; N, 7.04; S, 10.07. Found: C, 43.93; H, 5.92; N, 6.88; S, 10.32.

2-ethyl-1,2,3,4,5,7-hexahydro-6*H*-azocino[4,3-b]indol-6-one sulfuric acid (35b)

The title compound was prepared by a similar method using 2-ethyl-1,2,3,4,5,7-hexahydro-6*H*-azocino[4,3-b]indol-6-one (**34b)**, to produce the desired product in 90% yield as a white solid. ^{1}H-NMR (400 MHz, DMSO-d_6)

δ: 12.29 (1H, br s), 10.01 (1H, br s), 7.97 (1H, d, *J* = 8.2 Hz), 7.52 (1H, d, *J* = 8.2 Hz), 7.38 (1H, t, *J* = 7.6 Hz), 7.22 (1H, t, *J* = 7.6 Hz), 5.10 (1H, d, *J* = 14.0 Hz), 4.88 (1H, d, *J* = 14.0 Hz), 3.27-3.15 (2H, m), 3.06-2.90 (2H, m), 2.82 (3H, s), 2.25-2.12 (1H, m), 2.12-2.00 (1H, m); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 190.87, 136.31, 127.89, 126.19, 121.08, 120.61, 113.05, 107.84, 61.23, 56.02, 49.35, 46.85, 45.69, 38.27, 18.71; MS (ESI) *m/z*: 243 $(M+H)^+$; EA Anal. calcd for $C_{15}H_{18}N_2O$. 11/10H_2SO_4. 1/4$C_4H_{10}O$. 1/2H_2O. C, 50.87; H, 6.32; N, 7.42; S, 9.34. Found: C, 50.58; H, 6.28; N, 7.18; S, 9.60.

tert-butyl (3*S*)-3-[(2-nitrophenyl)sulfonylamino]pyrrolidine-1-carboxylate (37a)

To an ice-cooled solution of *tert*-butyl (3*S*)-3-aminopyrrolidine-1-carboxylate (**36a)** (5.00 g, 26.8 mmol) and triethylamine (5.58 mL, 40.3 mmol) in THF (100 mL), 2-nitrobenzenesulfonyl chloride (7.14 g, 32.2 mmol) was added. The mixture was stirred at room temperature for 6 h and concentrated under reduced pressure. Further, ethyl acetate was added to the residue. The solution was washed with water and brine, dried over anhydrous sodium sulfate salt, and concentrated under reduced pressure. The residue was purified by column chromatography using silica gel (200 g) and eluted with 20–45% ethyl acetate in hexane to obtain *tert*-butyl (3*S*)-3-[(2-nitrophenyl)sulfonylamino]pyrrolidine-1-carboxylate (10.0 g, 100%) as a green amorphous. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.44 (s, 9H), 1.80–2.12 (m, 2H), 3.14–3.44 (m, 4H), 4.02 (br, 1H), 5.48 (d, 1H, J = 7.2 Hz), 7.77 (t, 2H, J = 4.4 Hz), 7.87–7.90 (m, 1H), 8.17–8.19 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6)) δ: 153.25, 147.63, 134.20, 132.81, 132.58, 129.53, 124.26, 78.37, 52.48, 51.68,

50.84, 50.44, 43.59, 43.33, 28.06; HRMS (Negative ESI) m/z 370.1070 (370.1078 calcd for $C_{15}H_{21}N_3O_6S$ - H) ; EA Anal. calcd for $C_{15}H_{21}N_3O_6S$: C, 48.51; H, 5.70; N, 11.31; S, 8.63. Found: C, 48.34; H, 5.82; N, 11.08; S, 8.35; $[\alpha]_D^{20}$: -11.287 (c=1.030, DMSO).

To a suspension of *tert*-butyl (3*S*)-3-[(2-nitrophenyl)sulfonylamino]pyrrolidine-1-carboxylate (5.00 g, 13.5 mmol) and potassium carbonate (3.72 g, 26.9 mmol) in *N*,*N*-dimethylformamide (50 mL), methyl iodide (1.68 mL, 26.9 mmol) was added. The mixture was stirred at 60 °C for 5 h and concentrated under reduced pressure. Ethyl acetate was added to the mixture. The solution was washed with saturated aqueous sodium hydrogen carbonate and brine, dried over anhydrous sodium sulfate salt, and concentrated under reduced pressure. The residue was subjected to column chromatography using silica gel (200 g) and eluted with 0–40% ethyl acetate in hexane to obtain *tert*-butyl (3*S*)-3-[methyl-(2-nitrophenyl)sulfonyl-amino]pyrrolidine-1-carboxylate (5.10 g, 98%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.44 (s, 9H), 1.95 (br, 1H), 2.09 (br, 1H), 2.87 (s, 3H), 3.20−3.31 (m, 2H), 3.53 (br, 2H), 4.58 (br, 1H), 7.65 (br, 1H), 7.71 (br, 2H), 8.04 (br, 1H). MS (ESI) *m/z*: 386 $(M+H)^+$.

To a solution of *tert*-butyl (3*S*)-3-[methyl-(2-nitrophenyl)sulfonyl-amino]pyrrolidine-1-carboxylate (5.10 g, 13.2 mmol) in acetonitrile (120 mL), potassium carbonate (5.49 g, 39.7 mmol) and 2-fluorobenzenethiol (2.83 mL, 26.5 mmol) were added. The mixture was stirred at room temperature for 12 h. Ethyl acetate was added to the mixture. The solution was washed with saturated aqueous sodium hydrogen carbonate, water, and brine, dried over anhydrous sodium sulfate salt, and concentrated under

reduced pressure. The residue was subjected to column chromatography using silica gel (80 g) and eluted with 0–20% ethyl acetate in hexane to obtain the title compound (**37a**) (2.65 g, 100%) as a yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 3.57–3.33 (3H, m), 3.20–3.10 (2H, m), 2.44 (3H, s), 2.09–2.00 (1H, m), 1.73–1.70 (1H, m), 1.44 (9H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 153.58, 77.94, 59.06, 58.21, 51.23, 50.99, 44.17, 43.94, 34.23, 28.15; HRMS (Positive ESI) m/z 201.1594 (201.1598 calcd for $C_{10}H_{20}N_2O_2$ + H); EA Anal. calcd for $C_{10}H_{20}N_2O_2$: C, 57.97; H, 10.07; N, 13.99. Found: C, 57.59; H, 9.94; N, 13.33; $[\alpha]_D^{20}$: -14.45 (c=1.002, $CHCl_3$).

***tert*-butyl**

(3*S*)-3-[1*H*-indole-2-carbonyl(methyl)amino]pyrrolidine-1-carboxylate (38a)

To a solution of *tert*-butyl (3*S*)-3-(methylamino)pyrrolidine-1-carboxylate **(37a)** (2.65 g, 13.2 mmol) in THF (60 mL), indole-2-carboxylic acid (3.20 g, 19.8 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (3.80 g, 19.8 mmol) were added. The mixture was stirred at room temperature for 12 h. Ethyl acetate was added to the mixture. The solution was washed with saturated aqueous sodium hydrogen carbonate, water, and brine, dried over anhydrous sodium sulfate salt, and concentrated under reduced pressure. The residue was subjected to column chromatography using silica gel (80 g) and eluted with 20–60% ethyl acetate in hexane to obtain the title compound **(38a)** (4.48 g, 99%) as an amorphous compound. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.32 (1H, br s), 7.67 (1H, d, *J* = 8.2 Hz), 7.43 (1H, d, *J* = 8.2 Hz), 7.30 (1H, t, *J* = 7.7 Hz), 7.15 (1H, t, *J* = 7.7 Hz), 6.86 (1H, s), 5.39–5.31 (1H, m), 3.75–3.55 (2H, m), 3.48–3.20 (5H, m), 2.23–2.09 (2H, m), 1.48 (9H, s) ;^{13}C NMR (100 MHz,

DMSO-d_6) δ: 163.38, 153.44, 135.85, 130.15, 126.89, 123.27, 121.42, 119.64, 112.01, 104.30, 79.13, 78.46, 54.30, 46.82, 46.68, 44.31, 43.99, 28.11; HRMS (Positive ESI) m/z 344.1972 (344.1969 calcd for $C_{19}H_{25}N_3O_3$ + H); EA Anal. calcd for $C_{19}H_{25}N_3O_3$: C, 66.45; H, 7.34; N, 12.24. Found: C, 66.59; H, 7.35; N, 11.81; $[\alpha]_D^{20}$: -32.54 (c=1.020, $CHCl_3$).

(5*S*)-6-methyl-1,3,4,5,6,8-hexahydro-7*H*-2,5-methano[1,5]diazonino[7,8-b]indol-7-one sulfuric acid salt (39a, DS54360155)

To a solution of **38a** (4.48 g, 13.0 mmol) in 1,4-dioxane (30 mL), 4 M HCl/ethyl acetate (30 mL) was added slowly and stirred at room temperature for 5 h. The reaction mixture was concentrated *in vacuo* to obtain *N*-methyl-*N*-[(3*S*)-pyrrolidin-3-yl]-1*H*-indole-2-carboxamide hydrochloride (3.42 g) as a solid. The mixture of amine HCl salt, paraformaldehyde (1.46 g, 43.8 mmol), and trifluoroacetic acid (2.74 mL, 36.4 mmol) in 1,2-dichloroethane (1200 mL) was stirred at 50 °C for 1.5 h. The mixture was poured into a saturated sodium bicarbonate solution and extracted with dichloromethane. The dichloromethane layer was washed with brine, dried over sodium sulfate salt, and concentrated *in vacuo*. The residue was purified by column chromatography using silica gel (80 g) and eluted with 0–18% methanol in dichloromethane to give

(5*S*)-6-methyl-1,3,4,5,6,8-hexahydro-7*H*-2,5-methano[1,5]diazonino[7,8-b]indol-7-one (0.972 g, 29% from 8) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.75 (1H, br s), 7.69 (1H, d, *J* = 8.2 Hz), 7.42 (1H, d, *J* = 8.2 Hz), 7.28 (1H, t, *J* = 7.6 Hz), 7.15 (1H, t, *J* = 7.6 Hz), 4.61 (1H, d, *J* = 15.2 Hz), 4.09–4.04 (1H, m), 3.86 (1H, d, *J* = 15.2 Hz), 3.31 (3H, s), 3.21 (1H, d, *J* = 13.3 Hz), 3.12 (1H, dd, *J*

= 13.7, 6.6 Hz), 2.93–2.86 (1H, m), 2.71–2.67 (1H, m), 2.00−1.88 (1H, m), 1.70–1.62 (1H, m) ;^{13}C NMR (100 MHz, DMSO-d_6) δ: 161.49, 135.25, 135.24, 130.98, 127.92, 123.15, 119.33, 119.16, 111.85, 62.52, 61.74, 51.42, 48.79, 38.20, 31.74; HRMS (Positive ESI) m/z 256.1448 (256.1445 calcd for $C_{15}H_{17}N_3O$ + H); EA Anal. calcd for $C_{15}H_{17}N_3O$: C, 70.56; H, 6.71; N, 16.46. Found: C, 70.42; H, 6.69; N, 16.43; $[\alpha]_D^{20}$: 66.35 (c=1.033, DMSO).

(5*S*)-6-methyl-1,3,4,5,6,8-hexahydro-7*H*-2,5-methano[1,5]diazonino[7,8-b]indol-7-one (0.405 g, 1.59 mmol) was treated with 1 mol/L sulfonic acid (1.59 mL, 1.59 mmol) in ethanol (20 mL) at 0 °C. The resulting solid was collected and dried using a vacuum pump to obtain the title compound **(39a)** (0.450 g, 80%) as a solid. ^{1}H-NMR (400 MHz, CD_3OD) δ: 11.76 (1H, br s), 7.75 (1H, d, *J* = 8.0 Hz), 7.52 (1H, d, *J* = 8.0 Hz), 7.36−7.32 (1H, m), 7.25−7.21 (1H, m), 4.85 (5H, d, *J* = 14.5 Hz), 4.73 (1H, d, *J* = 14.5 Hz), 4.54−4.49 (1H, m), 3.94 (1H, d, *J* = 13.3 Hz), 3.59 (1H, dd, *J* = 13.3, 7.4 Hz), 3.39−3.37 (2H, m), 3.22 (3H, s), 2.62−2.59 (1H, m), 2.02−1.93(1H, m); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 160.85, 135.39, 132.96, 126.87, 124.11, 120.46, 119.23, 112.39, 103.26, 58.84, 56.92, 51.85, 46.68, 37.46, 29.85; HRMS (Positive ESI) m/z 256.1448 (256.1445 calcd for $C_{15}H_{17}N_3O$ + H); EA Anal. calcd for $C_{15}H_{17}N_3O$. H_2SO_4. 1/2H_2O: C, 49.71; H, 5.56; N, 11.60; S, 8.85. Found: C, 49.81; H, 5.60; N, 11.55; S, 8.55; $[\alpha]_D^{20}$: 65.19 (c=1.022, MeOH).

***tert*-butyl (3*R*)-3-(methylamino)pyrrolidine-1-carboxylate (37b)**

37b was prepared as a solid in a similar manner described for **37a**. 99% yeild:^{1}H-NMR (400 MHz, $CDCl_3$) δ: 3.80−3.25 (3H, m), 3.18−2.95 (2H, m), 2.42 (3H, s), 2.15−2.05 (1H, m), 1.83−1.60 (1H, m), 1.43 (9H, s); ^{13}C NMR (100 MHz,

DMSO-d_6) δ: 153.50, 77.82, 58.80, 58.29, 52.15, 51.01, 44.14, 43.83, 33.65, 27.98; HRMS (Positive ESI) m/z 201.1597 (201.1598 calcd for $C_{10}H_{20}N_2O_2$ + H); EA Anal. calcd for $C_{10}H_{20}N_2O_2$: C, 59.97; H, 10.07; N, 13.99. Found: C, 58.30; H, 10.03; N, 13.50; $[\alpha]_D^{20}$: 15.794 (c=1.020, $CHCl_3$).

***tert*-butyl**

(3*R*)-3-[1*H*-indole-2-carbonyl(methyl)amino]pyrrolidine-1-carboxylate (38b)

38b was prepared as a solid in a similar manner described for **38a**. 95% yeild:^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.36 (1H, br s), 7.66 (1H, d, *J* = 8.0 Hz), 7.40 (1H, d, *J* = 8.0 Hz), 7.30 (1H, t, *J* = 7.6 Hz), 7.15 (1H, t, *J* = 7.5 Hz), 6.86 (1H, s), 5.36−5.31 (1H, m), 3.75−3.55 (2H, m), 3.45−3.22 (5H, m), 2.20−2.06 (2H, m), 1.48 (9H, s) ;^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.10, 153.39, 136.01, 130.02, 126.77, 123.26, 121.33, 119.56, 111.89, 104.35, 79.10, 78.32, 54.21, 46.88, 46.59, 44.30, 44.11, 28.09; HRMS (Positive ESI) m/z 344.1970 (344.1969 calcd for $C_{19}H_{25}N_3O_3$ + H); EA Anal. calcd for $C_{19}H_{25}N_3O_3$: C, 66.45; H, 7.34; N, 12.24. Found: C, 66.54; H, 7.38; N, 11.98; $[\alpha]_D^{20}$: 37.04 (c=1.020, $CHCl_3$).

(5*R*)-6-methyl-1,3,4,5,6,8-hexahydro-7*H*-2,5-methano[1,5]diazonino[7,8-b]indol-7-one sulfuric acid salt (39b)

39b was prepared as a solid in a similar manner described for **39a**. 99% yeild.^{1}H-NMR (400 MHz, CD_3OD) δ: 11.60 (1H, br s), 7.72 (1H, d, *J* = 7.9 Hz), 7.45 (1H, d, *J* = 7.9 Hz), 7.39−7.30 (1H, m), 7.32−7.22 (1H, m), 4.83-4.77 (5H, m), 4.70 (1H, d, *J* = 14.6 Hz), 4.52−4.43 (1H, m), 3.86 (1H, d, *J* = 13.0 Hz), 3.60

(1H, dd, *J* = 13.0, 7.4 Hz), 3.40−3.32 (2H, m), 3.21 (3H, s), 2.70−2.59 (1H, m), 2.11−1.87 (1H, m); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 161.04, 135.28, 132.90, 126.95, 123.97, 120.31, 119.26, 112.44, 103.22, 58.89, 57.01, 51.81, 46.12, 37.47, 29.76; HRMS (Positive ESI) m/z 256.1447 (256.1445 calcd for $C_{15}H_{17}N_3O$ + H). EA Anal. calcd for $C_{15}H_{17}N_3O$. 10/11H_2SO_4. 3/2H_2O: C, 48.49; H, 5.92; N, 11.31; S, 7.85. Found: C, 48.47; H, 5.78; N, 11.32; S, 8.11. $[\alpha]_D^{20}$: -65.16 (c=1.005, DMSO).

6-methyl-1,3,4,5,6,8-hexahydro-7*H*-2,5-methano[1,5]diazonino[7,8-b]indol-7-one sulfuric acid (39)

39 was prepared as a solid in a similar manner described for **39a**. 95% yeild.^{1}H-NMR (400 MHz, CD_3OD) δ: 11.75 (1H, br s), 7.73 (1H, d, *J* = 8.0 Hz), 7.52 (1H, d, *J* = 8.0 Hz), 7.36−7.30 (1H, m), 7.25−7.21 (1H, m), 4.85 (5H, d, *J* = 14.5 Hz), 4.73 (1H, d, *J* = 14.5 Hz), 4.54−4.49 (1H, m), 3.94 (1H, d, *J* = 13.3 Hz), 3.59 (1H, dd, *J* = 13.3, 7.4 Hz), 3.38−3.38 (2H, m), 3.22 (3H, s), 2.62−2.58 (1H, m), 2.02−1.90 (1H, m) ;^{13}C NMR (100 MHz, DMSO-d_6) δ: 160.56, 135.20, 132.45, 127.31, 123.97, 120.31, 119.18, 112.26, 103.77, 59.59, 56.73, 51.37, 47.42, 44.81, 30.22; HRMS (Positive ESI) m/z 256.1466 (256.1445 calcd for $C_{15}H_{17}N_3O$ + H); EA Anal. calcd for $C_{15}H_{17}N_3O$. 21/20H_2SO_4. 3/5H_2O: C, 49.46; H, 5.94; N, 10.82; S, 8.66. Found: C, 49.55; H, 5.94; N, 10.66; S, 8.70.

5,6-dimethyl-1,3,4,5,6,8-hexahydro-7*H*-2,5-methano[1,5]diazonino[7,8-b]indol-7-one sulfuric acid (40)

40 was prepared as a solid in a similar manner described for **39a**. 98% yeild.^{1}H-NMR (400 MHz, CD_3OD) δ: 11.86 (1H, br s), 7.74 (1H, d, *J* = 8.2 Hz), 7.52 (1H, d, *J* = 8.2 Hz), 7.33 (1H, t, *J* = 7.6 Hz), 7.21 (1H, t, *J* = 7.6 Hz), 5.02 (1H, d, *J* = 14.0 Hz), 4.52 (1H, d, *J* = 14.0 Hz), 4.11-4.07 (1H, m), 3.83-3.81 (2H, m), 3.07-2.96 (2H, m), 3.07 (3H, s), 2.42-2.35 (1H, m), 1.64 (3H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 161.34, 136.12, 127.29, 126.78, 122.89, 122.01, 118.11, 115.67, 104.32, 58.70, 55.89, 50.52, 46.22, 43.81, 33.87, 24.01; HRMS (Positive ESI) m/z 270.1619 (270.1601 calcd for $C_{16}H_{19}N_3O$ + H); EA Anal. calcd for $C_{16}H_{19}N_3O$. H_2SO_4. 1/10$C_4H_{10}O$. 9/8H_2O: C, 49.43; H, 6.23; N, 10.42; S, 7.95. Found: C, 49.44; H, 6.01; N, 10.66; S, 7.74.

2,3,4,5,7,12-hexahydro-1*H*,13*H*-3a,6-methanopyrrolo[2',1':4,5][1,5]diazonino[7,8-b]indol-13-one sulfuric acid (41)

41 was prepared as a solid in a similar manner described for **39a**. 99% yeild.^{1}H-NMR (400 MHz, DMSO-d_6) δ: 12.07 (1H, br s), 10.50 (1H, br s), 7.78 (1H, d, *J* = 8.2 Hz), 7.47 (1H, d, *J* = 8.2 Hz), 7.29 (1H, t, *J* = 7.8 Hz), 7.19 (1H, t, *J* = 7.8 Hz), 4.95 (1H, d, *J* = 14.0 Hz), 4.53 (1H, d, *J* = 14.0 Hz), 3.97-3.88 (1H, m), 3.79-3.67 (2H, m), 3.61-3.53 (1H, m), 3.39-3.27 (1H, m), 2.92-2.83 (1H, m), 2.32-2.12 (4H, m), 1.86-1.62 (2H, m); ^{13}C NMR (100 MHz, DMSO-d6) δ: 164.43, 140.34, 127.83, 126.11, 121.58, 120.60, 117.64, 113.11, 104.58, 69.57, 64.37, 51.67, 47.94, 45.38, 36.40, 30.45, 22.63; HRMS (Positive ESI) m/z 282.1623 (282.1601 calcd for $C_{17}H_{19}N_3O$ + H).

6-ethyl-1,3,4,5,6,8-hexahydro-7*H*-2,5-methano[1,5]diazonino[7,8-b]indol-7-one sulfuric acid (42)

42 was prepared as a solid in a similar manner described for **39a**. 90% yeild.^{1}H-NMR (400 MHz, DMSO-d_6) δ: 12.01 (1H, br s), 9.94 (1H, br s), 7.77 (1H, d, *J* = 8.2 Hz), 7.45 (1H, d, *J* = 8.2 Hz), 7.27 (1H, t, *J* = 7.6 Hz), 7.17 (1H, t, *J* = 7.6 Hz), 4.80 (1H, d, *J* = 14.9 Hz), 4.55-4.49 (2H, m), 3.69-3.63 (2H, m), 3.53-3.50 (2H, m), 3.15-3.12 (1H, m), 2.99-2.96 (1H, m), 2.40-2.34 (1H, m), 1.54-1.51 (1H, m), 1.22 (4H, t, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 160.56, 135.20, 132.45, 127.31, 123.97, 120.31, 119.18, 112.26, 103.77, 59.59, 56.73, 51.37, 47.43, 44.81, 30.22, 12.28; HRMS (Positive ESI) m/z 270.1614 (270.1601 calcd for $C_{16}H_{19}N_3O$ + H); EA Anal. calcd for $C_{16}H_{19}N_3O$. 8/9H_2SO_4. 1/10$C_4H_{10}O$. 4/5H_2O: C, 52.06; H, 6.27; N, 11.04; S, 7.49. Found: C, 51.83; H, 6.01; N, 11.26; S, 7.56.

6-propyl-1,3,4,5,6,8-hexahydro-7*H*-2,5-methano[1,5]diazonino[7,8-b]indol-7-one sulfuric acid (43)

43 was prepared as a solid in a similar manner described for **39a**. 98% yeild.^{1}H-NMR (CD_3OD) δ: 11.71 (1H, br s), 7.66 (1H, d, *J* = 8.2 Hz), 7.43 (1H, d, *J* = 8.2 Hz), 7.24 (1H, t, *J* = 7.6 Hz), 7.13 (1H, t, *J* = 7.6 Hz), 4.61 (1H, d, *J* = 15.2 Hz), 4.36-4.34 (1H, m), 3.86 (1H, d, *J* = 15.2 Hz), 3.78-3.75 (1H, m), 3.49-3.40 (1H, m), 3.21-3.17 (2H, m), 2.79-2.70 (1H, m), 2.66-2.57 (1H, m), 2.04-1.93 (1H, m), 1.84-1.72 (2H, m), 1.49-1.38 (1H, m), 1.01 (3H, t, *J* = 7.2 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 160.02, 135.37, 128.29, 127.51, 123.45, 122.35, 118.44, 116.31, 105.06, 58.76, 55.54, 51.02, 46.36, 44.21, 21.45, 13.32; HRMS (Positive ESI) m/z 284.1768 (284.1758 calcd for $C_{17}H_{21}N_3O$ + H); EA Anal. calcd for $C_{17}H_{21}N_3O$. H_2SO_4. 1/10$C_4H_{10}O$. H_2O: C, 51.37; H, 6.52; N, 10.21; S, 7.79. Found: C, 51.34; H, 6.24; N, 10.35; S, 7.89.

6-ethyl-1,3,4,5,6,8-hexahydro-7*H*-2,5-methano[1,5]diazonino[7,8-b]indol-7-one sulfuric acid (44)

44 was prepared as a solid in a similar manner described for **39a**. 94% yeild.^{1}H-NMR (CD_3OD) δ: 11.86 (1H, br s), 7.67 (1H, d, *J* = 8.2 Hz), 7.45 (1H, d, *J* = 8.6 Hz), 7.26 (1H, t, *J* = 8.0 Hz), 7.13 (1H, t, *J* = 8.0 Hz), 4.66 (1H, d, *J* = 15.2 Hz), 4.53-4.48 (1H, m), 3.81 (1H, d, *J* = 15.2 Hz), 3.26-3.23 (1H, m), 3.05-3.01 (2H, m), 2.72-2.52 (2H, m), 2.06-1.96 (1H, m), 1.50-1.38 (1H, m), 1.08-0.98 (1H, m), 0.98-0.84 (2H, m), 0.76-0.66 (1H, m); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 161.12, 134.87, 131.44, 127.56, 122.81, 119.78, 117.18, 110.25, 103.53, 59.06, 55.21, 52.39, 46.81, 44.65, 25.20, 7.01, 6.95; HRMS (Positive ESI) m/z 282.1598 (282.1601 calcd for $C_{17}H_{19}N_3O$ + H); EA Anal. calcd for $C_{17}H_{19}N_3O$: C, 72.57; H, 6.81; N, 14.94. Found: C, 64.44; H, 6.46; N, 12.96.

***tert*-butyl (3*S*)-3-(methylamino)piperidine-1-carboxylate (46a)**

To a solution of *tert*-butyl (3*S*)-3-aminopiperidine-1-carboxylate **45a** (2.0 g, 10 mmol) and triethylamine (1.5 mL, 15 mmol) in THF (60 mL), benzyl chloroformate (1.7 mL, 12 mmol) was added. The mixture was stirred at room temperature for 1 h. Then, the mixture was poured into water and extracted with ethyl acetate. The ethyl acetate layer was washed with brine, dried over sodium sulfate, and concentrated *in vacuo* to give tert-butyl (3*S*)-3-{[(benzyloxy)carbonyl]amino}piperidine-1-carboxylate, which was used for the next reaction without further purification. To a solution of tert-butyl (3*S*)-3-{[(benzyloxy)carbonyl]amino}piperidine-1-carboxylate in THF (120 mL) at 0 °C, 63% sodium hydride (0.35 g, 9.3 mmol) and iodomethane (1.5 mL, 15

mmol) were added. The mixture was allowed to warm up to room temperature and stirred for 5 h. Then, the mixture was poured into water and extracted with ethyl acetate. The ethyl acetate layer was washed with brine, dried over sodium sulfate, and concentrated *in vacuo*. The residue was passed through a short pad of silica gel column to give crude *tert*-butyl (3*S*)-3-{[(benzyloxy)carbonyl](methyl)amino}piperidine-1-carboxylate. To a solution of the compound prepared in the previous step in methanol (60 mL) was added 10 percent palladium-activated carbon (2.0 g), and the resulting mixture was stirred at room temperature for 5 hours under a hydrogen atmosphere. After the catalyst in the reaction mixture was filtered off, and the filtrate was concentrated *in vacuo* The residue was purified by column chromatography using silica gel (80 g) and eluted with 0–18% methanol in dichloromethane to give the title compound **46a** (1.88 g, 88%) as an oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 3.50-3.92 (m, 2H) , 2.75-2.90 (m, 1H),. 2.18-2.27 (m, 3H), 1.71-1.90 (m, 2H), 1.38 (s, 9H) ;^{13}C NMR (100 MHz, DMSO-d_6) δ: 153.90, 78.36, 55.39, 48.34, 44.15, 43.44, 33.34, 30.45, 28.03, 23.45, 22.87; HRMS (Positive ESI) m/z 215.1757 (215.1754 calcd for $C_{11}H_{22}N_2O_2$ + H); EA Anal. calcd for $C_{11}H_{22}N_2O_2$. C, 61.65; H, 10.35; N, 13.07. Found: C, 61.09; H, 10.32; N, 12.57; $[\alpha]_D^{20}$: 9.56 (c=1.026, $CHCl_3$).

tert-butyl

(3*S*)-3-[(1*H*-indole-2-carbonyl)(methyl)amino]piperidine-1-carboxylate

(47a)

To a solution of *tert*-butyl (3*S*)-3-(methylamino)piperidine-1-carboxylate **46a** (1.0 g, 4.7 mmol) in THF (30 mL), indole-2-carboxylic acid (1.1 g, 7.0 mmol)

and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.3 g, 7.0 mmol) were added. The mixture was stirred at room temperature for 14 h. Ethyl acetate was added to the mixture. The solution was washed with saturated aqueous sodium hydrogen carbonate, water, and brine, dried over anhydrous sodium sulfate salt, and concentrated under reduced pressure. The residue was subjected to column chromatography using silica gel (60 g) and eluted with 20–60% ethyl acetate in hexane to obtain the title compound **47a** (1.5 g, 92%) as an amorphous compound. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.32 (1H, br s), 7.67 (1H, d, *J* = 8.2 Hz), 7.43 (1H, d, *J* = 8.2 Hz), 7.30 (1H, t, *J* = 7.7 Hz), 7.15 (1H, t, *J* = 7.7 Hz), 6.86 (1H, s), 5.39−5.31 (1H, m), 3.75−3.55 (2H, m), 3.48−3.20 (5H, m), 2.28−2.02 (4H, m), 1.48 (9H, s) ;^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.94, 153.92, 135.79, 130.14, 126.88, 123.23, 121.21, 119.69, 112.05, 104.17, 78.91, 59.69, 43.64, 42.67, 27.97, 27.43, 24.42; HRMS (Positive ESI) m/z 358.2141 (358.2125 calcd for $C_{20}H_{27}N_3O_3$ + H); EA Anal. calcd for $C_{20}H_{27}N_3O_3$. C,67.20; H, 7.61; N, 11.76. Found: C, 67.03; H, 7.69; N, 11.36; $[\alpha]_D^{20}$: -64.15 (c=1.004, $CHCl_3$).

(6*S*)-7-methyl-1,4,5,6,7,9-hexahydro-2,6-methano[1,6]diazecino[3,4-b]indol-8(3*H*)-one (48a)

To a solution of *tert*-butyl (3*S*)-3-[(1*H*-indole-2-carbonyl)(methyl)amino]piperidine-1-carboxylate **47a** (1.5 g, 4.3 mmol) in 1,4-dioxane (30 mL), 4 M HCl/ethyl acetate (30 mL) was added slowly and stirred at room temperature for 5 h. The reaction mixture was concentrated *in vacuo* to obtain *N*-methyl-*N*-[(3*S*)-piperidin-3-yl]-1*H*-indole-2-carboxamide hydrochloride as a

solid. The mixture of amine HCl salt, paraformaldehyde (0.52 g, 16 mmol), and trifluoroacetic acid (1.0 mL, 13 mmol) in 1,2-dichloroethane (500 mL) was stirred at 80 °C for 1 h. The mixture was poured into a saturated sodium bicarbonate solution and extracted with dichloromethane. The dichloromethane layer was washed with brine, dried over sodium sulfate salt, and concentrated *in vacuo*. The residue was purified by column chromatography using silica gel (80 g) and eluted with 0–18% methanol in dichloromethane to give the title compound (29 mg, 4%) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.57 (1H, br s), 7.70 (1H, d, *J* = 8.2 Hz), 7.41 (1H, d, *J* = 8.2 Hz), 7.27 (1H, t, *J* = 7.4 Hz), 7.13 (1H, t, *J* = 7.4 Hz), 4.40 (1H, d, *J* = 12.0 Hz), 4.07 (1H, d, *J* = 12.0 Hz), 3.51-3.46 (1H, m), 3.24-3.20 (2H, m), 3.15 (3H, s), 3.16-3.12 (1H, m), 2.90-2.86 (1H, m), 2.40-2.31 (1H, m), 2.15-2.05 (1H, m), 1.85-1.75(1H, m), 1.29-1.22 (1H, m); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 169.94, 140.86, 132.50, 129.63, 129.32, 128.58, 127.62, 54.89, 54.25, 53.47, 51.81, 47.31, 34.55, 30.09, 13.26; HRMS (Positive ESI) m/z 270.1601 (270.1601 calcd for $C_{16}H_{19}N_3O$ + H). EA Anal. calcd for $C_{16}H_{19}N_3O$. 2/5H_2O: C, 69.49; H, 7.22; N, 15.20. Found: C, 69.57; H, 7.08; N, 15.14. $[\alpha]_D^{20}$: 96.02 (c=0.387, MeOH).

tert-butyl (3*R*)-3-(methylamino)piperidine-1-carboxylate (46b)

46b was prepared as a solid in a similar manner described for **46a**. 82% yeild.^{1}H-NMR (400 MHz, $CDCl_3$) δ: 3.55-3.87 (m, 2H) , 2.77-2.91 (m, 1H),. 2.20-2.15 (m, 3H), 1.77-1.94 (m, 2H), 1.36 (s, 9H) ;^{13}C NMR (100 MHz, DMSO-d_6) δ: 154.21, 78.19, 55.31, 47.98, 44.32, 43.46, 33.50, 30.49, 28.12, 24.10, 22.54; HRMS (Positive ESI) m/z 215.1754 (215.1754 calcd for

$C_{11}H_{22}N_2O_2$ + H); EA Anal. calcd for $C_{11}H_{22}N_2O_2$. C, 61.65; H, 10.35; N, 13.07. Found: C, 61.21; H, 10.62; N, 12.92; $[\alpha]_D^{20}$: -7.77 (c=1.030, $CHCl_3$).

tert-butyl (3*R*)-3-[(1*H*-indole-2-carbonyl)(methyl)amino]piperidine-1-carboxylate (47b)

47b was prepared as a solid in a similar manner described for **47a**. 88% yeild.^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.44 (1H, br s), 7.66 (1H, d, *J* = 8.1 Hz), 7.39 (1H, d, *J* = 8.1 Hz), 7.29 (1H, t, *J* = 7.8 Hz), 7.16 (1H, t, *J* = 7.8 Hz), 6.83 (1H, s), 5.35−5.25 (1H, m), 3.66−3.60 (2H, m), 3.55−3.20 (5H, m), 2.15−2.21 (4H, m), 1.46 (9H, s) ;^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.55, 154.03, 135.71, 130.21, 126.80 122.89, 121.24, 119.76, 112.11, 104.19, 78.86, 59.66, 45.94, 42.69, 27.99, 27.42, 24.41; HRMS (Positive ESI) m/z 358.2128 (358.2125 calcd for $C_{20}H_{27}N_3O_3$ + H); EA Anal. calcd for $C_{20}H_{27}N_3O_3$. C,67.20; H, 7.61; N, 11.76. Found: C, 67.03; H, 7.69; N, 11.36.

(6*R*)-7-methyl-1,4,5,6,7,9-hexahydro-2,6-methano[1,6]diazecino[3,4-b]indol-8(3*H*)-one (**48b**)

48b was prepared as a solid in a similar manner described for **48a**. 8% yeild.^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.69 (1H, br s), 7.69 (1H, d, *J* = 8.1 Hz), 7.48 (1H, d, *J* = 8.1 Hz), 7.23 (1H, t, *J* = 7.2 Hz), 7.09 (1H, t, *J* = 7.2 Hz), 4.38 (1H, d, *J* = 12.6 Hz), 4.15 (1H, d, *J* = 12.1 Hz), 3.56-3.49 (1H, m), 3.33-3.23 (2H, m), 3.15 (3H, s), 3.16-3.11 (1H, m), 2.89-2.86 (1H, m), 2.40-2.31 (1H, m), 2.19-2.08 (1H, m), 1.85-1.72 (1H, m), 1.27-1.21 (1H, m); ^{13}C NMR (100 MHz,

DMSO-d_6) δ: 168.85, 141.99, 132.43, 129.60, 129.12, 129.05, 127.32, 55.01, 54.12, 53.01, 51.92, 47.34, 34.77, 30.07, 13.18; HRMS (Positive ESI) m/z 270.1606 (270.1601 calcd for $C_{16}H_{19}N_3O$ + H). EA Anal. calcd for $C_{16}H_{19}N_3O$. $2H_2O$: C, 58.05; H, 7.07; N, 12.38. Found: C, 57.84; H, 6.87; N, 12.67. $[\alpha]_D^{20}$: -90.51 (c=0.140, MeOH).

第三章

tert-butyl methyl[(3*S*)-pyrrolidin-3-yl]carbamate (51)

To the mixture of *tert*-butyl (3*S*)-pyrrolidin-3-ylcarbamate (**50**, 2.50 g, 13.4 mmol) in THF (50 mL), benzyl chloroformate (2.19 mL, 15.4 mmol) and triethylamine (2.23 mL, 16.1 mmol) were added. The mixture was stirred at room temperature for 30 min. The mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with 1M HCl and brine, dried over sodium sulfate, and concentrated *in vacuo* to give benzyl (3*S*)-3-(*tert*-butoxycarbonylamino)pyrrolidine-1-carboxylate as a solid, which was used for the next reaction without further purification. To the mixture of benzyl (3*S*)-3-(*tert*-butoxycarbonylamino)pyrrolidine-1-carboxylate in THF (100 mL), potassium *tert*-butoxide (1.54 g, 13.7 mmol) was added. After the mixture was stirred for 1 h, iodomethane (2.33 mL, 12.5 mmol) was added and stirred overnight. The mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, and concentrated *in vacuo*. The residue was passed through a short pad of a silica gel column to obtain crude benzyl (3*S*)-3-[*tert*-butoxycarbonyl(methyl)amino]pyrrolidine-1-carboxylate. To the

mixture of crude benzyl (3*S*)-3-[*tert*-butoxycarbonyl(methyl)amino]pyrrolidine-1-carboxylate in ethanol (50 mL), 10% palladium carbon (2.19 mL, 15.4 mmol) was added and stirred under hydrogen atmosphere for 3 h. The insoluble matter was removed, and then, the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography using silica gel and eluted with 0%-18% methanol in dichloromethane to obtain the title compound **(51)** (1.47 g, 59%) as an oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 4.72-4.64 (1H, m), 3.11-3.00 (1H, m), 2.91-2.88 (1H, m), 2.82-2.71 (2H, m), 2.78 (3H, s), 1.74-1.71 (2H, m), 1.45 (9H, s); ^{13}C NMR (500 MHz, DMSO-d_6) δ: 154.74, 78.37, 55.38, 49.11, 45.98, 29.02, 28.81, 27.99; HRMS (Positive ESI) m/z 201.1598 (201.1602 calcd for $C_{10}H_{20}N_2O_2$ + H); EA Anal. calcd for $C_{10}H_{20}N_2O_2$. C, 59.97; H, 10.07; N, 13.99. Found: C, 60.09; H, 9.84; N, 13.95; $[\alpha]_D^{20}$: -10.275 (c=1.004, $CHCl_3$).

methyl 2-({(3*S*)-3-[*tert*-butoxycarbonyl(methyl)amino]pyrrolidin-1-yl}methyl)benzoate (52)

To a solution of *tert*-butyl methyl[(3*S*)-pyrrolidin-3-yl]carbamate **(51)** (3.1 g, 16.0 mmol) in dichloromethane (120 mL), methyl 2-formylbenzoate (2.5 g, 15.0 mmol) and sodium triacetoxyborohydride (4.5 g, 21.0 mmol) were added. The mixture was stirred at room temperature for 12 h. The mixture was poured into a 1N NaOH solution, and the dichloromethane was separated. The dichloromethane was washed with brine, dried over sodium sulfate, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel by eluting with 0%-20% methanol in dichloromethane to obtain the title compound **(52)** (4.52 g,

85%) as an oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.77-7.72 (1H, m), 7.48-7.40 (2H, m), 7.33-7.27 (1H, m), 3.95-3.89 (2H, m), 3.89-3.76 (3H, m), 2.80-2.76 (1H, m), 2.79 (3H, s), 2.63-2.56 (1H, m), 2.50-2.43 (1H, m), 2.30-2.23 (1H, m), 2.13-2.04 (1H, m), 1.75-1.65 (1H, m), 1.61-1.54 (1H, m), 1.43 (9H, s).; HRMS (Positive ESI) m/z 349.2124 (349.2127 calcd for $C_{19}H_{28}N_2O_4$ + H).

(5*S*)-6-methyl-3,4,5,6-tetrahydro-2,5-methano-2,6-benzodiazonin-7(1*H*)-one (49)

To a solution of methyl 2-({(3*S*)-3-[*tert*-butoxycarbonyl(methyl)amino]pyrrolidin-1-yl}methyl)benzoate **(52)** (4.48 g, 13.0 mmol) in THF (20 mL) and methanol (20 mL), a 1M LiOH solution (20 mL) was added and stirred for 14 h. The resulting mixture was concentrated, and the residue was partitioned between ethyl acetate and the 1M HCl solution. The organic layer was separated, washed with brine, dried over sodium sulfate, and concentrated *in vacuo* to obtain 2-({(3*S*)-3-[(*tert*-butoxycarbonyl)(methyl)amino]pyrrolidin-1-yl}methyl)benzoi c acid as a foam, which was used for the next reaction without further purification. To the mixture of 2-({(3*S*)-3-[(*tert*-butoxycarbonyl)(methyl)amino]pyrrolidin-1-yl}methyl)benzoi c acid in 1,4-dioxane (30 mL), 4M HCl/ethyl acetate (30 mL) was added slowly and the resulting mixture was stirred at room temperature for 5 h. The reaction mixture was concentrated *in vacuo* to obtain 2-{[(3*S*)-3-(methylamino)pyrrolidin-1-yl]methyl}benzoic acid hydrochloride as a solid. To a solution of 2-{[(3*S*)-3-(methylamino)pyrrolidin-1-yl]methyl}benzoic acid hydrochloride in

dichloromethane (30 mL), O-(benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (11.0 g, 30.0 mmol) and *N*,*N*-diisopropylethylamine (7.90 mL, 45.0 mmol) were added and the resulting mixture was stirred for 12 h. The mixture was poured into water and extracted with dichloromethane. The organic layer was washed with brine, dried over sodium sulfate, and concentrated *in vacuo*. The residue was purified by column chromatography using silica gel and eluted with 0%-18% methanol in dichloromethane to obtain the title compound (1.47 g, 57%) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$): 7.69-7.68 (1H, m), 7.49-7.36 (2H, m), 7.26-7.24 (1H, m), 4.18 (1H, d, *J* = 14.5 Hz), 4.03-3.88 (1H, m), 3.84-3.81 (2H, m), 3.56 (1H, d, *J* = 14.5 Hz), 3.27 (3H, s), 3.08-2.80 (2H, m), 2.51-2.18 (2H, m); ^{13}C NMR (500 MHz, DMSO-d_6) δ: 168.71, 138.32., 135.39, 130.06, 129.78, 128.93, 127.76, 63.47, 62.85, 59.12, 54.98, 51.24, 37.91; HRMS (Positive ESI) m/z 217.1338 (217.1341 calcd for $C_{13}H_{16}N_2O$ + H); EA Anal. calcd for $C_{13}H_{16}N_2O$: C, 72.19; H, 7.46; N, 12.95. Found: C, 72.22; H, 7.60; N, 12.58; $[\alpha]_D^{20}$: 76.487 (c=1.008, MeOH).

tert-butyl _N_-[(7_S_)-5-azaspiro[2.4]heptan-7-yl]-_N_-methylcarbamate (54c)

To the mixture of *tert*-butyl *N*-[(7*S*)-5-azaspiro[2.4]heptan-7-yl]carbamate **(53c)** (8, 4.18 g, 19.7 mmol) in THF (80 mL), benzyl chloroformate (3.22 mL, 22.6 mmol) and triethylamine (3.28 mL, 23.6 mmol) were added. The mixture was stirred at room temperature for 30 min. The mixture was poured into water and extracted with ethyl acetate. The ethyl acetate was washed with 1M HCl and brine, dried over sodium sulfate, and concentrated *in vacuo* to obtain benzyl (7*S*)-7-(*tert*-butoxycarbonylamino)-5-azaspiro[2.4]heptane-5-carboxylate as a solid, which was used for the next reaction without further purification. To the

mixture of benzyl (7*S*)-7-(*tert*-butoxycarbonylamino)-5-azaspiro[2.4]heptane-5-carboxylate in THF (100 mL), potassium *tert*-butoxide (2.40 g, 21.0 mmol) was added. After the mixture was stirred for 1 h, iodomethane (3.60 mL, 58.0 mmol) was added and stirred overnight. The mixture was poured into water and extracted with ethyl acetate. The ethyl acetate was washed with brine, dried over sodium sulfate, and concentrated *in vacuo*. The residue was passed through a short pad of a silica gel column to obtain crude benzyl (7*S*)-7-[*tert*-butoxycarbonyl(methyl)amino]-5-azaspiro[2.4]heptane-5-carboxylate. To the mixture of crude benzyl (7*S*)-7-[*tert*-butoxycarbonyl(methyl)amino]-5-azaspiro[2.4]heptane-5-carboxylate in ethanol (60 mL), 10% palladium carbon (1.0 g) was added and stirred under hydrogen atmosphere for 3 h. The insoluble matter was removed, and then, the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography using silica gel and eluted with 0%-18% methanol in dichloromethane to obtain the title compound (3.77 g, 85%) as an oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 4.52-4.31 (1H, m), 3.38-3.35 (1H, m), 3.01-2.99 (1H, m), 3.00 (1H, d, *J* = 10.9 Hz), 2.84 (3H, s), 2.72 (1H, d, *J* = 10.9 Hz), 1.43 (9H, s), 0.62-0.56 (4H, m) ; ^{13}C NMR (500 MHz, DMSO-d_6) δ: 155.21, 78.35, 61.45, 55.67, 50.70, 30.05, 27.97, 25.43, 14.26, 8.49; HRMS (Positive ESI) m/z 227.1772 (227.1759 calcd for $C_{12}H_{22}N_2O_2$ + H); EA Anal. calcd for $C_{12}H_{22}N_2O_2$. C, 63.68; H, 9.80; N, 12.38. Found: C, 63.72; H, 9.74; N, 12.00; $[\alpha]_D^{20}$: 51.919 (c=1.017, MeOH).

methyl 2-({(7*S*)-7-[*tert*-butoxycarbonyl(methyl)amino]-5-azaspiro[2.4]heptan-5-yl}methyl)benzoate (55c)

To a solution of *tert*-butyl *N*-[(7*S*)-5-azaspiro[2.4]heptan-7-yl]-*N*-methylcarbamate **(54c)** (2.10 g, 9.30 mmol) in dichloromethane (20 mL), methyl 2-(bromomethyl)benzoate (1.80 g, 11.0 mmol) and potassium carbonate (2.34 g, 16.9 mmol) were added and stirred for 14 h. The mixture was poured into water, and the dichloromethane was separated. The dichloromethane was washed with brine, dried over sodium sulfate, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel by eluting with 0%-20% methanol in dichloromethane to obtain the title compound (3.10 g, 96%) as an oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.76 (1H, t, *J* = 8.0 Hz), 7.46-7.44 (2H, m), 7.31-7.29 (1H, m), 4.56-4.42 (1H, m), 3.90-3.86 (2H, m), 3.88 (3H, s), 2.87 (3H, d, *J* = 9.8 Hz), 2.80-2.78 (2H, m), 2.63-2.61 (1H, m), 2.41-2.39 (1H, m), 1.40 (9H, d, *J* = 6.3 Hz), 0.69-0.52 (4H, m); HRMS (Positive ESI) m/z 375.2280 (375.2284 calcd for $C_{21}H_{30}N_2O_4$ + H).

(5'*S*)-6'-methyl-5',6'-dihydro-3'*H*-spiro[cyclopropane-1,4'-[2,6]diaza[2,5]methano[2,6]benzodiazonin]-7'(1'*H*)-one (58)

To a solution of methyl 2-({(7*S*)-7-[*tert*-butoxycarbonyl(methyl)amino]-5-azaspiro[2.4]heptan-5-yl}methyl)benzoate (3.10 g, 8.30 mmol) in THF (30 mL)/methanol (15 mL), a 1M LiOH solution (15 mL) was added and stirred for 12 h. The mixture was concentrated, and the residue was partitioned between ethyl acetate and the 1M HCl solution.

The ethyl acetate was separated, washed with brine, dried over sodium sulfate, and concentrated *in vacuo* to obtain 2-({(7*S*)-7-[*tert*-butoxycarbonyl(methyl)amino]-5-azaspiro[2.4]heptan-5-yl}methyl}benzoic acid as a foam, which was used for the next reaction without further purification. To the mixture of 2-({(7*S*)-7-[*tert*-butoxycarbonyl(methyl)amino]-5-azaspiro[2.4]heptan-5-yl}methyl}benzoic acid in 1,4-dioxane (20 mL), 4M HCl/ethyl acetate (20 mL) was added slowly and stirred at room temperature for 5 h. The reaction mixture was concentrated *in vacuo* to obtain 2-{[(7*S*)-7-(methylamino)-5-azaspiro[2.4]heptan-5-yl]methyl}benzoic acid hydrochloride as a solid. To a solution of 2-{[(7*S*)-7-(methylamino)-5-azaspiro[2.4]heptan-5-yl]methyl}benzoic acid hydrochloride in dichloromethane (20 mL), *O*-(benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (6.10 g, 16.0 mmol) and *N*,*N*-diisopropylethylamine (6.30 mL, 36.0 mmol) were added and stirred for 12 h. The mixture was poured into water and extracted with dichloromethane. The organic layer was washed with brine, dried over sodium sulfate, and concentrated *in vacuo*. The residue was purified by column chromatography using silica gel and eluted with 0%-18% methanol in dichloromethane to obtain the title compound (1.30 g, 67%) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.58 (1H, d, *J* = 7.4 Hz), 7.37-7.31 (2H, m), 7.15 (1H, d, *J* = 7.0 Hz), 4.20 (1H, d, *J* = 14.5 Hz), 3.89 (1H, d, *J* = 14.5 Hz), 3.56 (1H, d, *J* = 5.1 Hz), 3.46-3.42 (1H, m), 3.17 (3H, s), 3.15-3.12 (1H, m), 2.72 (1H, d, *J* = 12.5 Hz), 2.33 (1H, d, *J* = 12.1 Hz), 0.77-0.76 (1H, m), 0.42-0.34 (2H, m), 0.48-0.50 (1H, m); ^{13}C NMR (500 MHz, DMSO-d_6) δ: 169.60, 138.63, 134.22, 130.64, 129.82, 128.36, 127.04, 70.45, 63.95, 59.43, 58.62, 37.73, 24.71, 16.96,

14.70; HRMS (Positive ESI) m/z 243.1500 (243.1497 calcd for $C_{15}H_{18}N_2O$ + H); EA Anal. calcd for $C_{15}H_{18}N_2O$: C, 74.35; H, 7.49; N, 11.56. Found: C, 74.19; H, 7.35; N, 11.46; $[\alpha]_D^{20}$: -218.319 (c=1.044, MeOH).

methyl 2-({(3*S*)-3-[(*tert*-butoxycarbonyl)(methyl)amino]pyrrolidin-1-yl}methyl)benzoate (55a)

Compound **55a** was prepared as a solid using a method similar to that used for **55c**. Yield: 78%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.74-7.68 (2H, m), 7.48-7.46 (1H, m), 7.36-7.31 (1H, m), 4.94-4.70 (1H, m), 3.90 (3H, s), 2.95-2.89 (1H, m), 2.89 (3H, s), 2.76-2.67 (1H, m), 2.61-2.48 (1H, m), 2.45-2.38 (1H, m), 2.30-2.18 (1H, m), 2.18-2.01 (1H, m), 1.82-1.61 (1H, m), 1.58-1.54 (3H, m) , 1.43 (9H, s); HRMS (Positive ESI) m/z 372.9203 (372.9206 calcd for $C_{20}H_{30}N_2O_4$ + H).

(5*S*)-1,6-dimethyl-3,4,5,6-tetrahydro-2,5-methano-2,6-benzodiazonin-7(1*H*)-one (56)

Compound **56** was prepared as a solid using a method similar to that used for **58**. Yield: 43%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.43-7.40 (3H, m), 7.35-7.31 (1H, m), 4.02-3.99 (1H, m), 3.93-3.91 (1H, m), 3.26 (3H, s), 3.19 (1H, d, *J* = 14.1 Hz), 2.93 (1H, dd, *J* = 12.9, 5.1 Hz), 2.78-2.71 (1H, m), 2.41-2.37 (1H, m), 1.59-1.49 (1H, m), 1.52 (3H, d, *J* = 5.1 Hz), 1.27-1.19 (1H, m); ^{13}C NMR (500 MHz, DMSO-d_6) δ: 168.97, 138.78, 137.97, 128.48, 128.26, 127.13, 125.91, 62.90, 62.45, 59.22, 49.19, 37.60, 29.00, 22.85; HRMS (Positive ESI) m/z 231.1485 (231.1497 calcd for $C_{14}H_{18}N_2O$ + H); EA Anal. calcd for $C_{14}H_{18}N_2O$. C, 73.01; H, 7.88; N, 12.16. Found: C, 72.39; H, 7.88; N, 11.98.

***tert*-butyl ethyl[(3*S*)-pyrrolidin-3-yl]carbamate (54b)**

Compound **54b** was prepared as a solid using a method similar to that used for **51**. Yield: 65%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 4.70-4.62 (1H, m), 4.15-4.08 (2H, m), 3.10-3.02 (1H, m), 2.95-2.92(1H, m), 2.82-2.70 (2H, m), 1.78-1.73 (2H, m), 1.44 (9H, s), 1.36 (3H, t, *J* = 7.2 Hz); HRMS (Positive ESI) m/z 215.1760 (215.1762 calcd for $C_{11}H_{22}N_2O_2$ + H).

methyl 2-({(3*S*)-3-[(*tert*-butoxycarbonyl)(ethyl)amino]pyrrolidin-1-yl}methyl)benzoate (55b)

Compound **55b** was prepared as a solid using a method similar to that used for **55c**. Yield: 85%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.75 (1H, d, *J* = 7.4 Hz), 7.46-7.43 (1H, m), 7.32-7.28 (1H, m), 4.92-4.55 (1H, m), 4.21-4.10 (2H, m), 3.94-3.81 (1H, m), 3.87 (3H, s), 2.79 (3H, s), 2.60-2.58 (1H, m), 2.47 (1H, t, *J* = 9.2 Hz), 2.27-2.25 (1H, m), 2.10-2.05 (1H, m), 1.71-1.70 (1H, m), 1.43 (9H, s), 1.35 (3H, m); HRMS (Positive ESI) m/z 363.2280 (363.2284 calcd for $C_{20}H_{30}N_2O_4$ + H).

(5*S*)-6-ethyl-3,4,5,6-tetrahydro-2,5-methano-2,6-benzodiazonin-7(1*H*)-one (57)

Compound **57** was prepared as a solid using a method similar to that used for **58**. Yield: 52%. ^{1}H-NMR (400 MHz, $CDCl_3$): 7.69-7.68 (1H, m), 7.49-7.36 (2H, m), 7.24-7.20 (1H, m), 4.25 (1H, d, *J* = 14.0 Hz), 4.12-4.01 (2H, m), 4.03-3.90 (1H,

m), 3.89-3.81 (2H, m), 3,51 (1H, d, *J* = 14.0 Hz), 3.08-2.80 (2H, m), 2.42-2.16 (2H, m), 1.31 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (500 MHz, DMSO-d_6) δ: 170.32, 138.67, 133.08, 130.65, 130.12, 129.39, 127.96, 57.52, 56.22, 55.90, 50.30, 41.23, 29.45, 13.95; HRMS (Positive ESI) m/z 231.1497 (231.1497 calcd for $C_{14}H_{18}N_2O$ + H); EA Anal. calcd for $C_{14}H_{18}N_2O$. C, 73.01; H, 7.88; N, 12.16. Found: C, 72.33; H, 8.05; N, 11.94; $[\alpha]_D^{20}$: 15.209 (c=1.009, MeOH).

tert-butyl _N_-methyl-_N_-[(3_S_)-piperidin-3-yl]carbamate (60)

To the mixture of *tert*-butyl *N*-[(3*S*)-piperidin-3-yl]carbamate **(59)** (2.50 g, 12.5 mmol) in THF (60 mL), benzyl chloroformate (2.13 mL, 16.0 mmol) and triethylamine (2.60 mL, 18.7 mmol) were added. The mixture was stirred at room temperature for 30 min. The mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with 1M HCl and brine, dried over sodium sulfate, and concentrated *in vacuo* to obtain benzyl (3*S*)-3-(*tert*-butoxycarbonylamino)piperidine-1-carboxylate as a solid, which was used for the next reaction without further purification. To the mixture of benzyl (3*S*)-3-(*tert*-butoxycarbonylamino)piperidine-1-carboxylate in THF (80 mL), potassium *tert*-butoxide (1.53 g, 13.7 mmol) was added. After the mixture was stirred for 1 h, iodomethane (2.32 mL, 37.2 mmol) was added, and the resulting mixture was stirred overnight. The mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, and concentrated *in vacuo*. The residue was passed through a short pad of a silica gel column to obtain crude benzyl (3*S*)-3-[*tert*-butoxycarbonyl(methyl)amino]piperidine-1-carboxylate. To the mixture of crude benzyl

(3*S*)-3-[*tert*-butoxycarbonyl(methyl)amino]piperidine-1-carboxylate in ethanol (60 mL), 10% palladium carbon (1.50 g) was added and the resulting mixture was stirred under hydrogen atmosphere for 2 h. The insoluble matter was removed by filtration, and then, the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography using silica gel and eluted with 0%-18% methanol in dichloromethane to obtain the title compound (2.36 g, 88%) as an oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 3.87 (1H, br s), 2.98 (2H, d, *J* = 12.1 Hz), 2.73 (3H, s), 2.60 (1H, t, *J* = 11.5 Hz), 2.47-2.41 (1H, m), 1.82-1.75 (2H, m), 1.69-1.66 (2H, m), 1.62-1.43 (1H, m), 1.45 (9H, s) ; ^{13}C NMR (500 MHz, DMSO-d_6) δ: 154.55, 78.34, 54.28, 52.22, 48.51, 45.18, 28.74, 28.03, 26.00; HRMS (Positive ESI) m/z 215.1771 (215.1759 calcd for $C_{11}H_{22}N_2O_2$ + H); EA Anal. calcd for $C_{11}H_{22}N_2O_2$. C, 61.65; H, 10.35; N, 13.07. Found: C, 62.09; H, 10.16; N, 12.37; $[\alpha]_D^{20}$: -13.666 (c=1.001, $CHCl_3$).

methyl 2-({(3*S*)-3-[*tert*-butoxycarbonyl(methyl)amino]-1-piperidyl}methyl)benzoate (61)

To a solution of *tert*-butyl *N*-methyl-*N*-[(3*S*)-piperidin-3-yl]carbamate **(60)** (0.300 g, 1.38 mmol) in dichloromethane (3 mL), methyl 2-(bromomethyl)benzoate (0.295 g, 1.38 mmol) and potassium carbonate (0.543 g, 3.93 mmol) were added and the resulting mixture was stirred for 14 h. The mixture was poured into water, and the organic layer was separated. The organic layer was washed with brine, dried over sodium sulfate, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel by eluting with 0%-20% methanol in dichloromethane to obtain the title compound

(0.435 g, 92%) as an oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.69 (1H, d, *J* = 7.3 Hz), 7.42-7.40 (2H, m), 7.29-7.27 (1H, m), 3.89 (3H, s), 3.74 (2H, br s), 2.74-2.72 (2H, m), 2.73 (3H, s), 2.04-1.99 (1H, m), 1.87-1.85 (1H, m), 1.71-1.67 (2H, m), 1.58-1.55 (1H, m), 1.41 (9H, s), 1.41-1.39 (2H, m).^{13}C NMR (500 MHz, DMSO-d_6) δ: 168.58, 154.52, 138.71, 131.81, 130.71, 129.66, 128.89, 127.03, 78.44, 59.93, 55.66, 54.20, 52.48, 51.73, 28.58, 27.95, 24.38; HRMS (Positive ESI) m/z 363.2279 (363.2284 calcd for $C_{20}H_{30}N_2O_4$ + H); EA Anal. calcd for $C_{20}H_{30}N_2O_4$. C, 66.27; H, 8.34; N, 7.73. Found: C, 65.82; H, 8.01; N, 7.55; $[\alpha]_D^{20}$: 11.347 (c=1.001, MeOH).

(6*S*)-7-methyl-4,5,6,7-tetrahydro-1*H*-2,6-methano-2,7-benzodiazecin-8(3*H*)-one (62)

To a solution of methyl 2-({(3*S*)-3-[*tert*-butoxycarbonyl(methyl)amino]-1-piperidyl}methyl)benzoate **(61)** (3.40 g, 8.30 mmol) in THF (20 mL) and methanol (20 mL), a 1M LiOH solution (40 mL) was added and the resulting mixture was stirred for 12 h. The mixture was concentrated, and the residue was partitioned between ethyl acetate and the 1M HCl solution. The organic layer was separated, washed with brine, dried over sodium sulfate, and concentrated *in vacuo* to obtain 2-({(3*S*)-3-[(*tert*-butoxycarbonyl)(methyl)amino]piperidin-1-yl}methyl)benzoic acid as a foam, which was used for the next reaction without further purification. To the mixture of 2-({(3*S*)-3-[(*tert*-butoxycarbonyl)(methyl)amino]piperidin-1-yl}methyl)benzoic acid in 1,4-dioxane (20 mL), 4M HCl/ethyl acetate (20 mL) was added slowly and stirred at room temperature for 5 h. The reaction mixture was concentrated

in vacuo to obtain 2-{[(3*S*)-3-(methylamino)-1-piperidyl]methyl}benzoic acid hydrochloride as a solid. To a solution of 2-{[(3*S*)-3-(methylamino)-1-piperidyl]methyl}benzoic acid hydrochloride in dichloromethane (40 mL), *O*-(benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (8.15 g, 21.4 mmol) and *N*,*N*-diisopropylethylamine (5.68 mL, 32.6 mmol) were added and stirred for 12 h. The mixture was poured into water and extracted with dichloromethane. The dichloromethane layer was washed with brine, dried over sodium sulfate, and concentrated *in vacuo*. The residue was purified by column chromatography using silica gel and eluted with 0%-18% methanol in dichloromethane to obtain the title compound (0.860 g, 44%) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.50-7.48 (1H, m), 7.37-7.33 (2H, m), 7.22-7.21 (1H, m), 4.21 (1H, d, *J* = 9.8 Hz), 3.58 (1H, d, *J* = 10.2 Hz), 3.25-3.24 (1H, m), 3.17-3.12 (2H, m), 3.14 (3H, s), 2.98 (1H, d, *J* = 16.0 Hz), 2.58 (1H, dd, *J* = 16.0, 2.5 Hz), 2.54-2.50 (1H, m), 2.19-2.07 (1H, m), 1.69-1.65 (1H, m), 1.27-1.22 (1H, m); ^{13}C NMR (500 MHz, DMSO-d_6) δ: 169.95, 140.86, 132.48, 129.64, 129.33, 128.59, 127.63, 54.89, 53.47, 51.81, 47.30, 34.55, 30.08, 13.27; HRMS (Positive ESI) m/z 231.1481 (231.1497 calcd for $C_{14}H_{18}N_2O$ + H); EA Anal. calcd for $C_{14}H_{18}N_2O$: C, 73.01; H, 7.88; N, 12.16. Found: C, 73.28; H, 8.03; N, 11.28; $[\alpha]_D^{20}$: -91.334 (c=1.019, MeOH).

benzyl 4-[(2-methoxycarbonylphenyl)methyl]-1,4-diazepane-1-carboxylate (64)

To a solution of benzyl 1-homopiperazine carboxylate **(63)** (3.07 g, 13.1 mmol) in THF (50 mL), methyl 2-(bromomethyl)benzoate (1.50 g, 6.55 mmol) was added and the resulting mixture was stirred at 50°C for 4 h. The mixture was

poured into water, and the dichloromethane was separated. The organic layer was washed with brine, dried over sodium sulfate, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel by eluting with 0%-10% methanol in dichloromethane to obtain the title compound (2.35 g, 94%) as an oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.70-7.68 (1H, m), 7.45-7.26 (8H, m), 5.22-5.13 (2H, m), 3.94 (2H, s), 3.86 (3H, s), 3.43-3.38 (2H, m), 2.85-2.83 (2H, m), 2.77-2.75 (2H, m), 2.68-2.61 (2H, m), 1.64-1.61 (2H, m); HRMS (Positive ESI) m/z 383.1973 (383.1970 calcd for $C_{22}H_{26}N_2O_4$ + H).

4,5-dihydro-3*H*-2,6-ethano-2,6-benzodiazonin-1(7*H*)-one (65)

To a solution of benzyl 4-[(2-methoxycarbonylphenyl)methyl]-1,4-diazepane-1-carboxylate **(64)** (2.35 g, 6.14 mmol) in THF (20 mL) and methanol (20 mL), a 1M LiOH solution (40 mL) was added and the resulting mixture was stirred for 12 h. The mixture was concentrated, and the residue was partitioned between ethyl acetate and the 1M HCl solution. The organic layer was separated, washed with brine, dried over sodium sulfate, and concentrated *in vacuo* to give 2-[(4-benzyloxycarbonyl-1,4-diazepan-1-yl)methyl]benzoic acid as a foam, which was used for the next reaction without further purification. To the mixture of 2-[(4-benzyloxycarbonyl-1,4-diazepan-1-yl)methyl]benzoic acid in ethanol (60 mL), 10% palladium carbon (1.10 g) was added and the resulting mixture was stirred under hydrogen atmosphere for 3 h. The insoluble matter was removed by filtration, and then, the filtrate was concentrated *in vacuo* to obtain crude 2-(1,4-diazepan-1-ylmethyl)benzoic acid as a solid. To a solution of 2-(1,4-diazepan-1-ylmethyl)benzoic acid in dichloromethane (996 mL),

O-(benzotriazol-1-yl)-*N*,*N*,*N'*,*N'*-tetramethyluronium hexafluorophosphate (5.68 g, 14.9 mmol) and *N*,*N*-diisopropylethylamine (3.96 mL, 22.7 mmol) were added and the resulting mixture was stirred for 12 h. The mixture was poured into water and extracted with dichloromethane. The dichloromethane layer was washed with brine, dried over sodium sulfate, and concentrated *in vacuo*. The residue was purified by column chromatography using Silica gel and eluted with 0%-18% methanol in dichloromethane to obtain the title compound (0.355 g, 28%) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.34-7.28 (3H, m), 7.22-7.21 (1H, m), 4.69-4.66 (1H, m), 4.16 (2H, q, *J* = 14.5 Hz), 3.36-3.33 (2H, m), 3.07 (1H, q, *J* = 15.7 Hz), 3.00-2.92 (2H, m), 2.86-2.82 (2H, m), 2.36-2.25 (1H, m), 1.52-1.47 (1H, m); ^{13}C NMR (500 MHz, DMSO-d_6) δ: 173.00, 138.36, 137.13, 129.50, 128.78, 127.62, 124.96, 55.38, 54.40, 49.74, 47.36, 46.98, 21.23, 21.09; HRMS (Positive ESI) m/z 217.1341 (217.1341 calcd for $C_{13}H_{16}N_2O$ + H); EA Anal. calcd for $C_{13}H_{16}N_2O$: C, 72.19; H, 7.46; N, 12.95. Found: C, 72.01; H, 7.52; N, 12.90.

methyl 2-({(7*S*)-7-[(*tert*-butoxycarbonyl)(methyl)amino]-5-azaspiro[2.4]heptan-5-yl}methyl)-4-fluorobenzoate (66b)

To a solution of *tert*-butyl (7*S*)-5-azaspiro[2.4]heptan-7-yl(methyl)carbamate **54c** (1.01 g, 4.06 mmol) in dichloromethane (40 mL), methyl 2-(bromomethyl)-4-fluoro benzoate (1.00 g, 4.00 mmol) and potassium carbonate (0.73 g, 5.30 mmol) were added and the resulting mixture was stirred for 14 h. The mixture was poured into water, and the dichloromethane was separated. The organic layer was washed with brine, dried over sodium sulfate,

and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel by eluting with 0%-20% methanol in dichloromethane to obtain the title compound (1.24 g, 78%) as an oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.86-7.82 (1H, m), 7.30 (1H, d, *J* = 9.8 Hz), 6.98 (1H, t, *J* = 9.8 Hz), 4.59-4.45 (1H, m), 3.96-3.90 (2H, m), 3.88 (3H, s), 2.91 (3H, s), 2.87-2.78 (2H, m), 2.69-2.65 (1H, m), 2.43 (1H, d, *J* = 9.0 Hz), 1.41 (9H, s), 0.83-0.44 (4H, m) ; HRMS (Positive ESI) m/z 393.2184 (393.2189 calcd for $C_{21}H_{29}FN_2O_4$ + H).

(5'*S*)-10'-fluoro-6'-methyl-5',6'-dihydro-3'*H*-spiro[cyclopropane-1,4'-[2,6]diaza[2,5]methano[2,6]benzodiazonin]-7'(1'*H*)-one (67b, DS34942424)

To a solution of methyl 2-({(7*S*)-7-[(*tert*-butoxycarbonyl)(methyl)amino]-5-azaspiro[2.4]heptan-5-yl}methyl)-4-fluorobenzoate **(66b)** (1.24 g, 3.16 mmol) in THF (10 mL) and methanol (10 mL), a 1M LiOH solution (6 mL) was added and the resulting mixture was stirred for 12 h. The mixture was concentreated, and the residue was partitioned between ethyl acetate and the 1M HCl solution. The organic layer was separated, washed with brine, dried over sodium sulfate, and concentrated *in vacuo* to obtain 2-({(7*S*)-7-[(*tert*-butoxycarbonyl)(methyl)amino]-5-azaspiro[2.4]heptan-5-yl}methyl)-4-fluorobenzoic acid as a foam, which was used for the next reaction without further purification. To the 2-({(7*S*)-7-[(*tert*-butoxycarbonyl)(methyl)amino]-5-azaspiro[2.4]heptan-5-yl}methyl)-4-fluorobenzoic acid in 1,4-dioxane (10 mL), 4M HCl/ethyl acetate (10 mL) was added slowly and the resulting mixture was stirred at room temperature for 5 h. The reaction mixture was concentrated *in vacuo* to obtain

4-fluoro-2-{[(7*S*)-7-(methylamino)-5-azaspiro[2.4]heptan-5-yl]methyl}benzoic acid hydrochloride as a solid. To a solution of 4-fluoro-2-{[(7*S*)-7-(methylamino)-5-azaspiro[2.4]heptan-5-yl]methyl}benzoic acid hydrochloride in dichloromethane (500 mL), O-(benzotriazol-1-yl)-*N*,*N*,*N'*,*N'*-tetramethyluronium hexafluorophosphate (2.54 g, 6.69 mmol) and *N*,*N*-diisopropylethylamine (1.77 mL, 10.2 mmol) were added and stirred for 12 h. The mixture was poured into water and extracted with dichloromethane. The organic layer was washed with brine, dried over sodium sulfate, and concentrated *in vacuo*. The residue was purified by column chromatography using the Silica gel and eluted with 0%-18% methanol in dichloromethane to obtain the title compound (0.491 g, 60%) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.60-7.58 (1H, m), 7.07-7.05 (1H, m), 6.88-6.86 (1H, m), 4.15 (1H, d, *J* = 14.5 Hz), 3.88 (1H, d, *J* = 14.5 Hz), 3.56 (1H, d, *J* = 5.5 Hz), 3.46-3.43 (1H, m), 3.17 (3H, s), 3.15-3.10 (1H, m), 2.75 (1H, d, *J* = 12.5 Hz), 2.37-2.34 (1H, m), 0.81-0.78 (1H, m), 0.44-0.36 (2H, m), 0.39-0.36 (1H, m); ^{13}C NMR (500 MHz, DMSO-d_6) δ: 168.58, 162.56 (d, J_{CF} = 195 Hz), 137.21 (d, J_{CF} = 5 Hz), 135.37 (d, J_{CF} = 3 Hz), 132.34 (d, J_{CF} = 7 Hz), 116.91 (d, J_{CF} = 16 Hz), 114.18 (d, J_{CF} = 17 Hz), 70.38, 63.89, 58.85, 58.56, 37.83, 24.75, 17.05, 14.73; HRMS (Positive ESI) m/z 261.1397 (261.1403 calcd for $C_{15}H_{17}FN_2O$ + H); EA Anal. calcd for $C_{15}H_{17}FN_2O$: C, 69.21; H, 6.58; N, 10.76; F, 7.30. Found: C, 69.38; H, 6.67; N, 10.73; F, 7.17; $[\alpha]_D^{20}$: -203.877 (c=1.002, MeOH).

methyl

2-({(7*S*)-7-[(*tert*-butoxycarbonyl)(methyl)amino]-5-azaspiro[2.4]heptan-5-yl}methyl)-3-fluorobenzoate (66a)

Compound **66a** was prepared as a solid using a method similar to that used for **66b**. Yield: 96%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.51-7.47 (1H, m), 7.31-7.25 (1H, m), 7.17-7.14 (1H, m), 4.53-4.37 (1H, m), 3.96-3.91 (2H, m), 3.88 (3H, s), 2.79 (3H, s), 2.79-2.77 (2H, m), 2.59-2.57 (1H, m), 2.42 (1H, d, *J* = 8.6 Hz), 1.40 (9H, s), 0.69-0.48 (4H, m) ; HRMS (Positive ESI) m/z 393.2182 (393.2189 calcd for $C_{21}H_{29}FN_2O_4$ + H).

(5'*S*)-11'-fluoro-6'-methyl-5',6'-dihydro-3'*H*-spiro[cyclopropane-1,4'-[2,6]diaza[2,5]methano[2,6]benzodiazonin]-7'(1'*H*)-one (67a)

Compound **67a** was prepared as a solid using a method similar to that used for **67b**. Yield: 55%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.38 (1H, d, *J* = 7.8 Hz), 7.31-7.29 (1H, m), 7.12-7.10 (1H, m), 4.73 (1H, d, *J* = 14.5 Hz), 3.57 (1H, d, *J* = 5.1 Hz), 3.47-3.44 (2H, m), 3.17 (3H, s), 3.13 (1H, dd, *J* = 12.9, 3.1 Hz), 2.79 (1H, d, *J* = 12.9 Hz), 2.28-2.25 (1H, m), 0.80-0.75 (1H, m), 0.40-0.38 (2H, m), -0.43--0.46 (1H, m) ; ^{13}C NMR (500 MHz, DMSO-d_6) δ: 166.45, 160.61 (d, J_{CF} = 197 Hz), 141.26, 131.68 (d, J_{CF} = 7 Hz), 126.59 (d, J_{CF} = 3 Hz), 116.48 (d, J_{CF} = 18 Hz), 114.56 (d, J_{CF} = 12 Hz), 66.26, 60.21, 56.40, 46.51, 37.81, 22.87, 18.33, 14.14; HRMS (Positive ESI) m/z 261.1406 (261.1403 calcd for $C_{15}H_{17}FN_2O$ + H); EA Anal. calcd for $C_{15}H_{17}FN_2O$: C, 69.21; H, 6.58; N, 10.76; F, 7.30. Found: C, 69.09; H, 6.09; N, 10.31; F, 7.39; $[\alpha]_D^{20}$: -108.967 (c=1.016, DMSO).

methyl

2-({(7*S*)-7-[(*tert*-butoxycarbonyl)(methyl)amino]-5-azaspiro[2.4]heptan-5-yl}methyl)-5-fluorobenzoate (66c)

Compound **66c** was prepared as a solid using a method similar to that used for **66b**. Yield: 79%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.48-7.43 (2H, m), 7.14-7.13 (1H, m), 4.57-4.42 (1H, m), 3.88 (3H, s), 3.82 (2H, s), 2.86 (3H, d, *J* = 10.6 Hz), 2.80-2.75 (2H, m), 2.62-2.59 (1H, m), 2.39-2.38 (1H, m), 1.41 (9H, s), 0.81-0.51 (4H, m) ; HRMS (Positive ESI) m/z 393.2189 (393.2189 calcd for $C_{21}H_{29}FN_2O_4$ + H).

(5'*S*)-9'-fluoro-6'-methyl-5',6'-dihydro-3'*H*-spiro[cyclopropane-1,4'-[2,6]diaza[2,5]methano[2,6]benzodiazonin]-7'(1'*H*)-one (67c)

Compound **67c** was prepared as a solid using a method similar to that used for **67a**. Yield: 65%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.31-7.29 (1H, m), 7.14-7.12 (1H, m), 7.05-7.02 (1H, m), 4.20 (1H, d, *J* = 14.5 Hz), 3.85 (1H, d, *J* = 14.5 Hz), 3.56 (1H, d, *J* = 5.5 Hz), 3.44 (1H, dd, *J* = 12.9, 5.5 Hz), 3.17 (3H, s), 3.12 (1H, dd, *J* = 12.9, 2.7 Hz), 2.74 (1H, d, *J* = 12.5 Hz), 2.30 (1H, dd, *J* = 12.5, 2.7 Hz), 0.82-0.78 (1H, m), 0.45-0.40 (2H, m), -0.35--0.37 (1H, m) ; ^{13}C NMR (500 MHz, DMSO-d6) δ: 168.18, 162.08 (d, J_{CF} = 193 Hz), 140.49 (d, J_{CF} = 6 Hz), 132.91 (d, J_{CF} = 6 Hz), 130.84 (d, J_{CF} = 3 Hz), 116.17 (d, J_{CF} = 18 Hz), 115.44 (d, J_{CF} = 17 Hz), 70.46, 63.87, 58.59, 58.41, 37.75, 24.77, 16.98, 14.74; HRMS (Positive ESI) m/z 261.1391 (261.1403 calcd for $C_{15}H_{17}FN_2O$ + H); EA Anal. calcd for $C_{15}H_{17}FN_2O$: C, 69.21; H, 6.58; N, 10.76; F, 7.30. Found: C, 68.78; H, 6.56; N, 10.78; F, 7.23; $[\alpha]_D^{20}$: -189.732 (c=1.006, MeOH).

methyl

2-({(7*S*)-7-[(*tert*-butoxycarbonyl)(methyl)amino]-5-azaspiro[2.4]heptan-5-yl}methyl)-6-fluorobenzoate (66d)

Compound **66d** was prepared as a solid using a method similar to that used for **66b**. Yield: 76%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.37-7.28 (1H, m), 7.14-7.08 (1H, m), 7.06-6.98 (1H, m), 4.62-4.41 (1H, m), 3.90 (3H, s), 3.75-3.60 (2H, m), 2.83 (3H, s), 2.80-2.65 (2H, m), 2.61-2.53 (1H, m), 2.37 (1H, d, *J* = 8.4 Hz), 1.41 (9H, s), 0.79-0.39 (4H, m) ;HRMS (Positive ESI) m/z 393.2182 (393.2189 calcd for $C_{21}H_{29}FN_2O_4$ + H).

(5'*S*)-8'-fluoro-6'-methyl-5',6'-dihydro-3'*H*-spiro[cyclopropane-1,4'-[2,6]diaza[2,5]methano[2,6]benzodiazonin]-7'(1'*H*)-one (67d)

Compound **67d** was prepared as a solid using a method similar to that used for **67b**. Yield: 70%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.29-7.27 (2H, m), 7.09-7.06 (1H, m), 6.97 (1H, d, *J* = 7.4 Hz), 4.22 (1H, d, *J* = 14.5 Hz), 3.88 (1H, d, *J* = 14.5 Hz), 3.55 (1H, d, *J* = 5.5 Hz), 3.49-3.45 (1H, m), 3.17 (3H, s), 3.11 (1H, dd, *J* = 13.1, 2.9 Hz), 2.75 (1H, d, *J* = 12.5 Hz), 2.25 (1H, dd, *J* = 12.5, 2.9 Hz), 0.83-0.78 (1H, m), 0.43-0.38 (1H, m), -0.36--0.39 (1H, m); ^{13}C NMR (500 MHz, DMSO-d_6) δ: 165.28, 160.56 (d, J_{CF} = 196 Hz), 137.29 (d, J_{CF} = 1 Hz), 129.40 (d, J_{CF} = 7 Hz), 126.77 (d, J_{CF} = 13 Hz), 126.51 (d, J_{CF} = 2 Hz), 114.67 (d, J_{CF} = 18 Hz), 70.69, 64.07, 59.00, 58.60, 37.17, 25.00, 16.20, 13.40; HRMS (Positive ESI) m/z 261.1398 (261.1403 calcd for $C_{15}H_{17}FN_2O$ + H); EA Anal. calcd for $C_{15}H_{17}FN_2O$: C, 69.21; H, 6.58; N, 10.76; F, 7.30. Found: C, 68.80; H, 6.61; N, 10.78; F, 6.93; $[\alpha]_D^{20}$: -244.850 (c=1.006, MeOH).

生物学的評価

Acetic acid writhing test

This test was used to evaluate the peripheral antinociceptive activity. The ddY mice were administered with 1% acetic acid (0.1 mL) intraperitoneally (i.p) 30 min after the administration of compounds (p.o.). The stretching movements (arching of the back, development of tension in the abdominal muscles, elongation of the body, and extension of forelimbs) were assessed for 25 min.

Formalin test (initial phase)

The hind paw pad of the ddY mice was injected with 3.5% formalin (20 μL) (n = 8) 30 min after the compound was administered (30 mg/kg, p.o.). The reduction in the sum of time spent in paw licking and biting responses was measured for the first 10 min (the initial phase) following the formalin injected.

Spontaneous locomotor activity test

After the oral administration of the test compound or vehicle (control), the spontaneous locomotor activity was measured for 1 h by using the SUPERMEX system (model SM-32; Muromachi Kikai Co., Ltd., Tokyo, Japan).

MOR binding assay

This assay was based on the [^{3}H] diprenorphine binding competition to MOR. The competitive binding of compounds to the human MOR was measured using

the tritium-labelled diprenorphine. The commercial MOR membrane was incubated with the test compound and 1 nM [^{3}H] diprenorphine for 1 h at room temperature. The membrane was washed, and the remaining [^{3}H] diprenorphine bound to the MOR was detected and quantified. Morphine was used as the relative control.

MOR cAMP assay

The CHOK1 cell line, which stably expresses human MOR, was used to measure the MOR agonist activity. The MOR agonist activity of the compounds was detected by measuring the inhibition of cAMP production induced by forskolin. cAMP was measured using a commercial Forster resonance energy transfer (FRET) detection system. E_{max} was calculated relative to the E_{max} value of 10μM DAMGO as the control.

hERG inhibition assay

hERG-transfected human embryo kidney 293 (HEK293) cells were perfused with 10, 30 and 100 μmol/L each compound. Cells were perfused (perfusion speed: approximately 4 mL/ min) with the external solution (contents: NaCl, 137; KCl, 4; HEPES, 10; $CaCl_2$, 1.8; $MgCl_2$, 1; glucose, 10 mmol/L; adjusted to pH 7.32 and 7.37 with NaOH) in a bath chamber. Glass pipettes filled with the internal solution (contents: KCl, 130; $MgCl_2$, 1; EGTA, 5; HEPES, 10; ATP, 5 mmol/L; adjusted to pH 7.21 with KOH) with a resistance of 2.1 to 3.6 MΩ were used to record the hERG current. The cell membrane voltage was held at −80 mV via a patch clamp amplifier (EPC8, HEKA) using patch clamp software (Clampex

9.0 [pCLAMP 9], Axon Instruments, Molecular Devices). The test pulse was applied as follows: step from −80 to +20 mV for 1.5 s, step to −40 mV for 1.5 s, then step to a holding potential of −80 mV. This voltage protocol was applied continuously to cells once every 15 s. After the peak current for each voltage protocol was stable at 500 pA or higher for at least 1 min, the cell was perfused with the external solution containing the test article. In the kinetics assay, the voltage pulses were run continuously for 20 min (10 min for perfusion of the test article, and 10 min following the washout).

Pharmacokinetic evaluation in mice

Male ddY mice (5 weeks old) were purchased from Charles River Laboratories Japan, Inc. (Kanagawa, Japan). The mice were allowed to acclimatize in stainless steel cages for 7-11 days under controlled conditions. The test compounds were suspended in a 0.5% (w/v) methyl cellulose 400 solution (Wako Pure Chemical Industries) for oral and intraperitoneal administration. The dosing formulations (3 mg/mL) were administered to male ddY mice at 10 mL/kg after overnight fasting. An approximately 0.2 mL blood sample was collected from the jugular vein by using a heparinized syringe. The blood was centrifuged at 14,000 rpm for 3 min at 4°C (Himac CR15D, Hitachi Koki Co., Ltd.; rotor: RT15A2) to obtain the plasma. The plasma was stored at −20°C until use. The plasma concentration was determined by liquid chromatography tandem mass spectrometry (LC-MS/MS) using API 4000QTRAP (Applied Biosystems/MDS SCIEX). The PK parameters were calculated with a non-compartmental model using Winnonlin (version 4.0.1, Pharsight Corp.).

Microsome metabolic stability assay (MS)

The pooled CD1 mouse liver microsomes were purchased from Xenotech, LLC. β-nicotinamide adenine dinucleotide phosphate (β-NADP), *D*-glucose-6-phosphate (G-6-P), and glucose-6-phosphate dehydrogenase (G-6-PDH) were purchased from Oriental Yeast Co., Ltd. The reaction mixture was prepared using 100 µL of microsomes (final concentration: 0.5 mg protein/mL), 30 mM of G-6-P, 3 U/mL of G-6-PDH, and the substrate (final concentration: 1 µM). The metabolic reaction was initiated by the addition of 3 mM β-NADP to the reaction mixture. After 0min and 30min incubation at 37°C, a 90 µL aliquot of the reaction was drawn and added to 410 µL of the mixture (acetonitrile:methanol; 75:25 (v/v)) containing 15 ng/mL of niflumic acid (as the internal standard) to terminate the reaction. Each sample was centrifuged at 2,400 *g* for 12 min at 4°C. The supernatant was subjected to the LC-MS/MS analysis. The metabolic stability (%) was calculated using the peak area ratio (PAR) of the test substance to the internal standard from Equation 1 given below:

$$\text{Metabolic stability (\%)} = \frac{PAR\ at\ 30\ min}{PAR\ at\ 0\ min} \times 100$$

Measurement of Log $D_{7.4}$

Equal amounts of PBS and 1-octanol were shaken and left overnight. The upper layer (1-octanol) and lower layer (PBS) were collected separately. Each test compound was dissolved in 1-octanol or PBS (200 mM). The same amount of either PBS or 1-octanol was added and the mixture was shaken vigorously for 30

min at room temperature followed by centrifugation at 2100 g for 5 min at room temperature. Subsequently, both phases were separated and assayed by HPLC and LC-MS. Log $D_{7.4}$ was calculated by the follwing equation:

Log $D_{7.4}$ = log (peak area of compound in 1-octanol / peak area of compound in PBS)

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