平成 29 年度　博士論文

中枢神経系に作用する有機小分子化合物の合成と評価
―Suvorexant の新規簡便合成および、新規セリンラセマーゼ阻害薬の創製－

Synthesis and evaluations of organic small molecules acting on central nervous system
-Practical synthesis of Suvorexant, and studies on novel serine racemase inhibitors-

富山大学生命融合科学教育部

先端ナノ・バイオ科学専攻

生体機能性分子工学研究室

高原理行

目次

略語

本文中、以下の用語および反応剤は次のように略記した。(アルファベット順)

Ac	Acetyl
aq.	aqua
Arc	Activity-regulated cytoskeleton-associated
Aβ	amyloid-beta
Bn	benzyl
Boc	*tert*-butoxycarbonyl
BW	body weight
Cbz	Benzyloxycarbonyl group
CCD	Colony Collapse Disorder
CDI	carbonyldiimidazole
DMAP	*N*,*N*-dimethyl-4-aminopyridine
DMF	*N*,*N*-dimethylformamide
DMSO	dimethylsulfoxide
DNP	dinitrophenylhydrzine
EDC	1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide
Et	ethyl
Glu	Glutamic acid
His	Histidine
HL	hairless
HOAt	1-Hydroxy-7-azabenzotriazole
HPLC	High Performance Liquid Chromatography
hr	hour
HRMS	high-resolution mass spectrometry
i.p.	intraperitoneal
IR	infrared spectroscopy
KA	kainic acid
KO	knock out
LAH	Lithium aluminium hydride
Luc	Luciferase
Mal	malonate
Me	methyl

min	minute
Mp	melting point
mRNA	messenger ribonucleic acid
MS	mass spectrometry
NMDA	*N*-methyl-D-aspartate
NMDAR	*N*-methyl-D-aspartate receptor
NMR	nuclear magnetic resonance
Ns	2-nitrobenzensulfonyl (nosyl)
OX1R	Orexin receptor type 1
OX2R	Orexin receptor type 2
p.o.	per os
Ph	phenyl
Pr	propyl
Py	Pyridine
r.t.	room temperature
ROI	Regions of interest
s	second
Ser	Serine
SRR	human serine racemase
Srr	rat serine racemase
TFA	trifluoroacetic acid
Tg	transgenic
THF	tetrahydrofuran

序論

平成 29 年現在、日本は、総人口に占める 65 歳以上の割合が 27.7%にものぼる高齢社会である。それに伴いアルツハイマー型認知症やてんかんなどの神経変性疾患の患者数も増加の一途を辿っている。高齢化を迎えている現代社会では、痴呆、記憶障害、情動障害などの原因となる神経変性疾患に対する治療薬を開発する事が、社会保健の観点や高齢者の生活の質を保持する観点から、医学的、社会的に解決すべき重要な課題である。神経変性疾患に対する治療薬は非常に大きな市場ニーズがあり、いくつかの神経変性疾患治療薬が開発されているが、まだ不十分なため新規作用機序に基づく治療薬の開発が望まれている。

本研究では、中枢神経系に作用することが期待される有機小分子化合物のデザインおよび合成とそれらの薬理活性評価を目的としている。

各論

第一章　Suvorexant の新規簡便合成

第一節　ターゲット分子であるオレキシンの概要

オレキシンは、視床下部外側野およびその周辺領域に特異的に発現している神経ペプチドである。その機能は睡眠・覚醒、摂食行動、自発運動量及び自律神経系の調節など多岐にわたり、一見複雑に思われる。しかし、これらはいずれも個体の生存に必要な機能であり、オレキシン神経系は空腹時に活性化して覚醒状態を維持し、食餌を探索する役割を果たすと考えられている。[1] さらに最近、オレキシンがエネルギーの補給だけでなく代謝にも関与することが明らかになってきた。

オレキシンにはオレキシン A と B（ヒポクレチン 1 と 2）の 2 つのアイソフォームが存在する。これらは共通の前駆体プレプロオレキシンから生成されるが、分子内ジスルフィド結合を有するオレキシン A の方が B よりも構造的に安定である。視床下部のオレキシン神経は脳の広範囲に投射し、G タンパク質共役型受容体であるオレキシン 1 受容体（OX1R）またはオレキシン 2 受容体（OX2R）を介して作用する。

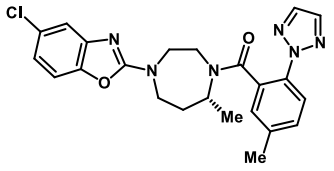
オレキシン神経の活性化やオレキシンの発現量は日内変動しており、覚醒期に増加し、休息時（睡眠時）に減少する。[2] オレキシンは睡眠・覚醒、自発運動量及び熱産生を介してエネルギーバランスを調節している。[3] オレキシンは血糖値が低下する状態において覚醒時間を延長させ、食餌の探索に必要な行動量を増加させる。[1] なお、オレキシンには摂食亢進作用があるが、その作用は体内の栄養状態に依存して変化する。[4, 5] オレキシンの欠損はナルコレプシーを誘発し、ナルコレプシー患者では肥満や 2 型糖尿病のリスクが増大する。[1] またカタプレキシーを伴うナルコプレシー患者では、肥満とは孤立して、インスリン抵抗性などの代謝異常が生じる。[6] オレキシン過剰発現マウスでは、OX2R シグナルを介して高脂肪食負荷による肥満や耐糖能異常が防止される。[5] このようにオレキシンはエネルギー代謝を促進することにより肥満や糖尿病の防止に寄与している。

生体のグルコース恒常性はホルモンの抹消作用だけでなく、視床下部 - 自律神経を介する末梢臓器連関によって維持されている。オレキシンを中枢投与すると交感神経系が活性化し、血圧や心拍数が増加する。[7] 血糖値の制御に関して

も、オレキシンをラット脳室内に投与すると交感神経系を介して肝臓の糖新生が増加し、血糖値が上昇する。また、オレキシンを腹内側核に投与すると交感神経系を介して骨格筋のインスリン感受性や糖取り込み活性が増加する。[1] マウスに低用量のオレキシン A を脳室内投与すると空腹時血糖が低下するため、オレキシンによる糖代謝調節は二相性である可能性がある。[1] 視床下部のオレキシンの発現量は加齢や高血糖の影響で低下する。[3, 4] オレキシン欠損マウスでは、加齢に伴い著明なインスリン抵抗性や耐糖能異常が生じる。[8] したがって、オレキシンはインスリン抵抗性の防御因子であると考えられる。

ヒトではポジティブな感情を抱くときや怒った時などに扁桃体のオレキシン濃度が上昇する。[9] また、オレキシン神経はストレスにより急性的に活性化されるが、うつ状態では抑制される。[10] 一方、うつはインスリン抵抗性や 2 型糖尿病の発症と関連していることが報告されている。[11]

現在、新しい不眠治療薬としてオレキシン受容体拮抗薬の開発が複数の製薬企業によって行われている。[12] 睡眠障害はインスリン抵抗性の増悪因子でもあり、オレキシン系の抑制による睡眠の改善は代謝疾患の改善にも役立つ可能性がある。[13] そこで我々は既知のオレキシン受容体拮抗薬である Suvorexant (Figure 1) を用い、インスリン抵抗性の改善がみられるか検討を行いたいと考えた。



Suvorexant

Figure 1: Suvorexant の構造

Suvorexant は入手が困難であり、さらにメルク社独自の合成法しか報告例がない。(Figure 2)現在、鍵中間体である core unit である 7 員環の合成法は 4 例しか報告されていない。光学分割では収率が低く、触媒や酵素は高価なものやメルク社独自のものしかない。そこで我々は、より簡便で大量合成が容易な改良合成法の確立を目指した。

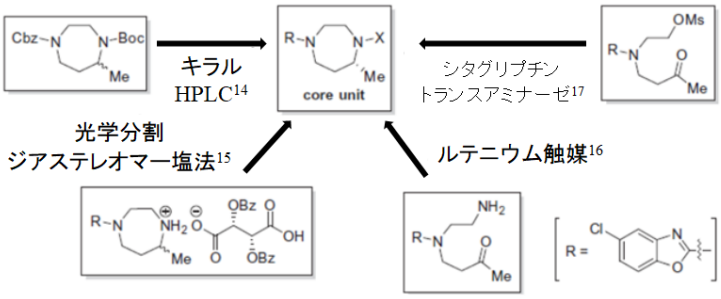
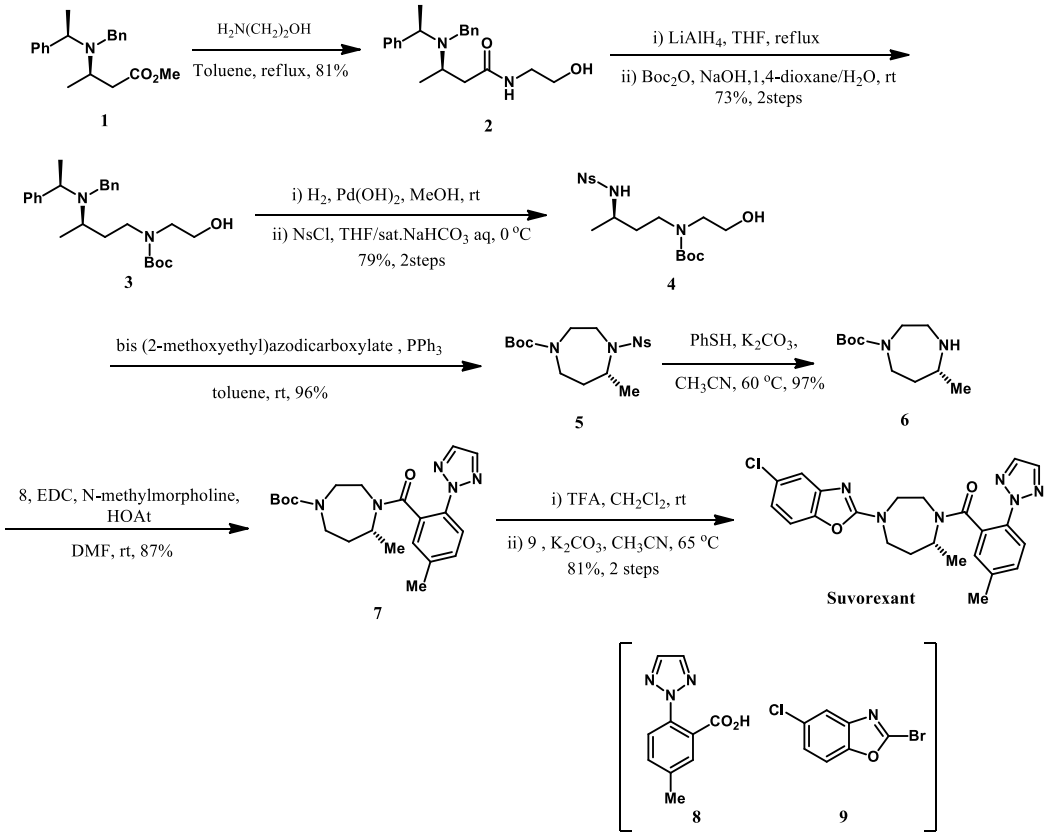


Figure 2: Synthesis strategy of diazepane core unit by Merck.

第二節　オレキシン受容体拮抗薬 Suvorexant の合成

文献既知の手法により、(*R*)-(+)-1-Phenylethylamine から容易に合成可能なβ-アミノエステル **1**[18] を出発原料として用いた。まず、**1** に対して 2-アミノエタノールを用いてアミド **2** を得た。その後 LAH 還元を行い 2 級のアミンとし、Boc 保護を行い Boc 体 **3** を得た。さらに、接触還元を行い Bn 保護基を脱保護し、Ns 化を行い、Ns 体 **4** を得た。この合成の鍵中間体である環化体 **5** は、光延環化反応により首尾よく合成した。[19]

その後、脱 Ns 化を行い、得られた２級アミンに対して文献既知[17]の手法により合成したカルボン酸 **8** を用いた縮合反応によりアミド **7** を得た。最後に脱 Boc 化を行い、文献既知[17]の手法により別途合成したブロモ体 **9** との反応によりアミノエステル **1** から 10 工程、31%の総収率で目的の Suvorexant の簡便合成法の確立に成功した。(原著論文[1])



Scheme 1: Synthetic route of Suvorexant

合成したオレキシン受容体遮断薬である Suvorexant を睡眠期に投与することでオレキシン作用の日内リズムが改善されることが認められた。その結果期待していたインスリン抵抗性の改善が見られた。[20]

第二章　神経細胞の過剰活性を抑制する新規セリンラセマーゼ阻害薬の創製

第一節　新規セリンラセマーゼ阻害薬の創製の概要

セリンラセマーゼ(SRR)はL-セリンをD-セリンへとラセミ化する酵素であり、脳内における遊離 D-セリンの約 90 %の生産に関与していると言われている。(Figure 3)[21, 22] 大脳皮質及び小脳などに多く局在しており、皮質では個体の成長につれて SRR の発現が徐々に増加する。また、D-セリンは内在性の NMDA 受容体コアゴニストとして機能し、NMDA 受容体の活性制御に関わっている。

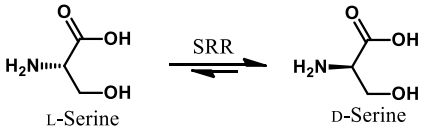


Figure 3: Role of SRR

NMDA 受容体はグルタミン酸受容体チャネルの一種で、グルタミン酸とコアゴニストの結合により活性化され、記憶・学習などの脳の高次機能に大きく関わると共に、病態時興奮性神経毒に関与する。(Figure 4) [23-26]

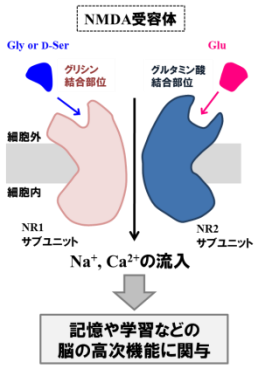


Figure 4: Relation betweeen NMDARs and D-Ser

脳内での D-セリンの機能解析を行うため、SRR 遺伝子ノックアウト (KO) マウスが作製された。その解析の結果、SRR-KO マウスの脳内では、D-セリン濃度がコントロールマウスの 10%程度まで減少した。この SRR-KO マウスでは、NMDAR を介した神経細胞死の減少が示された。[21, 26]

アルツハイマー病では、Aβペプチドが過剰に蓄積することで、SRR mRNA の転写亢進が引き起こされ SRR の発現が増加し、その結果 D-セリンが生産される。[21] また脳梗塞では、虚血により大量のグルタミン酸が放出されることが報告されている。[26] これらの病態では NMDA 受容体の過剰活性化、カルシウム流入、カルシウム依存的酵素群の恒常的活性化、ミトコンドリア膜電位の異常上昇などの一連の反応を伴う興奮毒性が、神経細胞死による神経変性疾患の進展に関わる共通機構と考えられている。

現在、アルツハイマー病に対して日本の臨床現場で使われている薬物は，脳内アセチルコリンの濃度を高めるために、アセチルコリンの分解酵素であるエステラーゼを標的としたドネペジル、ガランタミン、リバスチグミンの 3 種類と、

NMDA 受容体を標的としたメマンチンの合計 4 種類である。コリンエステラーゼ阻害剤は、興奮系の神経障害、胃腸や心臓、尿路、消化器における障害、皮膚炎など様々な副作用を引き起こすと言われており、またメマンチンはアルツハイマー型認知症の症状を抑制することを目的としているため、アルツハイマー型認知症の病態を抑制・改善することは目的としていない。よって新規作用機序におけるアルツハイマー型認知症の病態を抑制・改善する治療薬の開発が必要である。

これまでに報告されている比較的強い SRR 阻害剤として、マロン酸やジペプチド類、またいくつかの化合物が SRR 阻害剤として High-through put beads library screening 法等を用いて検索され報告されている。[28-35] しかしながら、マロン酸やジペプチド類は特異性も低く、毒性もあることが知られており、SRR に対する新規阻害剤の創出が望まれる。

ターゲットタンパクである wild-type SRR は発現量が低く、単離精製が困難であった。近年 SRR を構成するアミノ酸残基の 2 番目と 6 番目のシステイン (C) をアスパラギン酸 (D) に置換し (C2DC6D)、さらに C 末端に His-tag を有するリコンビナント mutant-type SRR とマロン酸の共結晶構造が報告された。[36] この mutant-type SRR は発現量が増加し単離精製が容易になった。そこで我々は、この情報をもとに structure-based の *in silico* スクリーニングを行い、4 種類のヒット化合物を得た。（第二節で詳述）さらに、ヒット化合物の構造を基に合成展開し、それぞれ 20-30 種類程の誘導体を合成した。（第三節で詳述）合成した誘導体を *in vitro* での mutant-type SRR を用いた酵素阻害活性評価を行い、優れた誘導体を見出した。（第四節で詳述）さらに *Arc-Luc* Tg mice を用いた *in vivo* 評価を行うことで、SRR 阻害剤となりうる化合物の探索を行った。（第五節で詳述）その結果、我々は SRR に対する新規阻害剤の開発に成功し、その化合物が持つ構造的特徴について明らかにした。

第二節　mutant-type SRR に対する候補化合物の検討

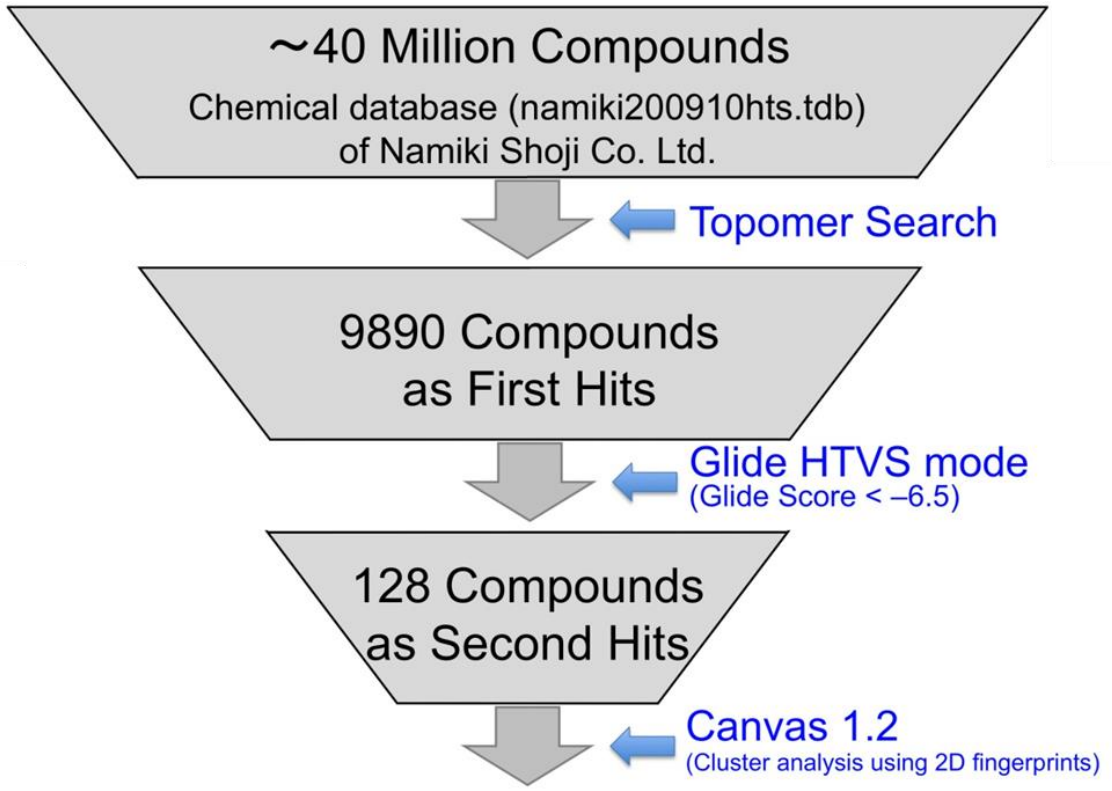


Figure 5: Multi-filter virtual screening protocol for novel mutant-type SRR inhibitor

昭和大学薬学部との共同研究により、Figure 5 に示す mutant-type SRR とマロン酸の共結晶構造情報を基に、structure-based *in silico* スクリーニングを行うことで、mutant-type SRR に対する新規阻害剤となりうる化合物の探索を行った。ナミキ商事の化合物データベースに存在する約 400 万化合物に対してスクリーニング [30, 37-43] を行った結果、19 化合物が候補として選択され、そのうち 18 化合物が購入可能であった。

購入した 18 化合物について、mutant-type SRR を用いて *in vitro* 活性測定による生物学的な評価を行った。その結果、4 つの化合物 (Figure 6) が mutant-type SRR に対して阻害活性を有することが判明した。(Table 1)次いで、この 4 つのヒット化合物に対して合成展開を行い、さらに強力な mutant-type SRR 阻害活性を有するリード化合物の創出を行った。

この中で化合物 **13** は既に報告済み [32] であるため、本研究では残る 3 つの化合物について合成展開、構造活性相関研究を行った。

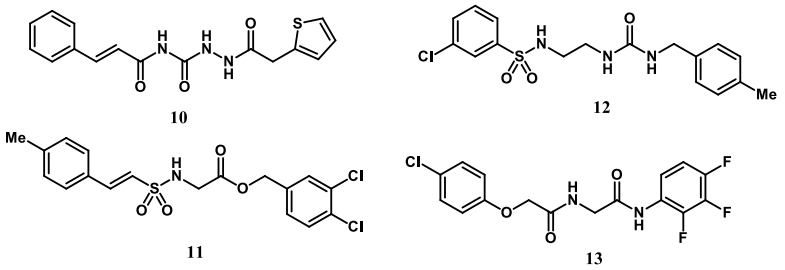


Figure 6: Structure of four small molecules with inhibitory activity against SRR from eighteen virtual hits

Compound	Activity [%]
10	27
11	32
12	36
13	35

Table 1: Results of *in vitro* assay of **10-13** using mutant-type SRR. The remaining activity of SRR with the inhibitors (1 mM) was evaluated with the percentage of the D-serine production compared with that without inhibitors.

もっとも活性の強かった化合物 **10** と mutant-type SRR とのドッキングモデルを作製した。SRR は二量体を形成している。化合物 **10** の中心部位であるアシルヒドラジノチオウレア部位 **14**(Figure 7) は SRR と相互作用し、計 4 つの水素結合を形成した。(Figure 8A) Figure 8B に示すように 4 つの水素結合の外側に利用可能なスペースが見つけられたため置換基を変えた誘導体の合成を行った。

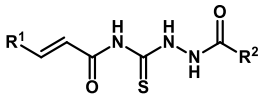


Figure 7: The central structure of acyl hydrazino thiourea moiety of **10**.

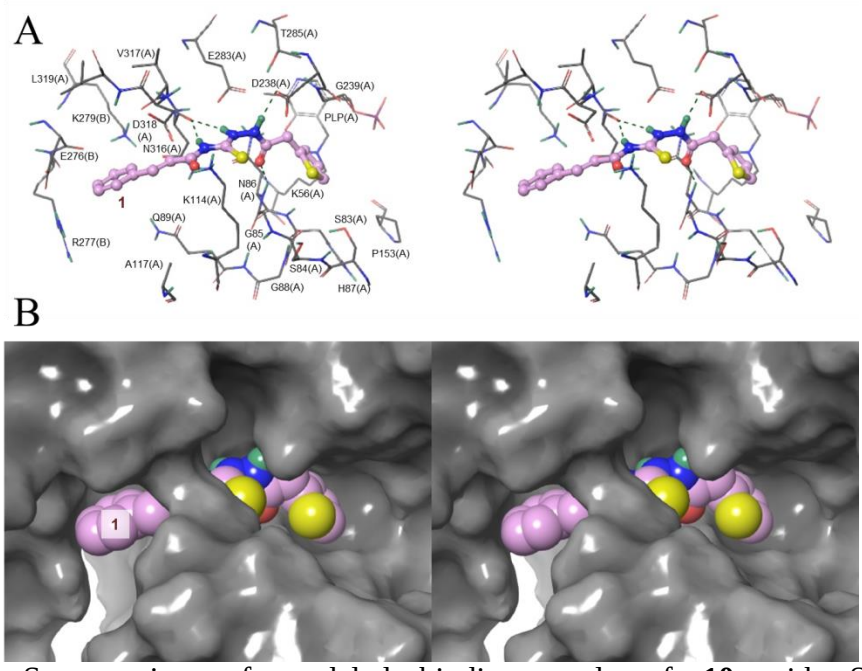


Figure 8: Stereo view of modeled binding mode of **10** with SRR. (A) Hydrogen-bonding interactions are indicated by dashed lines. (B) **10** and SRR are shown in space-filling representation and surface display, respectively.

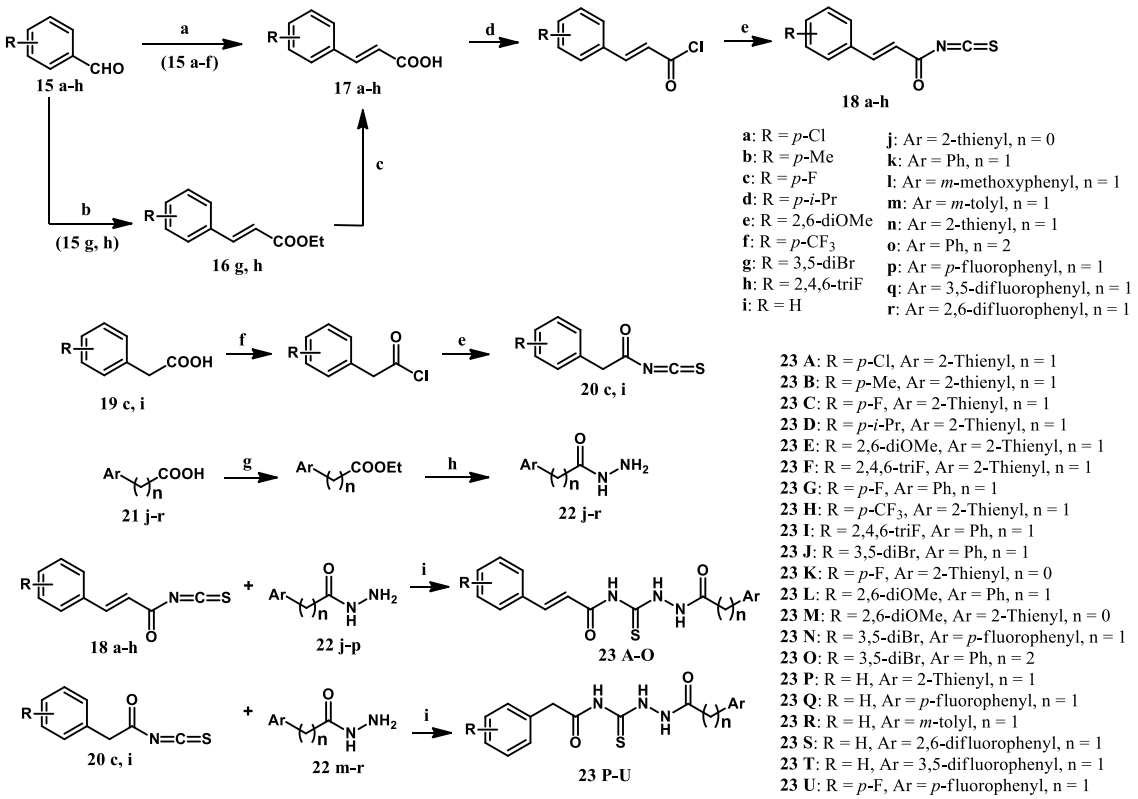
ヒット化合物 **10** は無置換のベンゼン環であったため、このベンゼン環の電子密度による SRR との相互作用の変化を検討する目的で、電子求引性置換基（F, Cl, Br, CF_3）、あるいは電子供与性置換基（OMe）を付与した化合物、アルキル基を付与した化合物の合成に着手した。また、逆側の 2-thienyl 基を同様の置換基を有したベンゼン環に変えた化合物の合成を行った。

両端の芳香環をつなぐリンカー部にも着目し、単結合または炭素数を変更した化合物の合成も同時に行った。

第三節　ヒット化合物の誘導体合成

化合物 **10** と SRR との相互作用の情報を基にアシルヒドラジノチオウレア部位 **14** を基本骨格とした誘導体 **23A-U** の合成を行った。(Scheme 2)

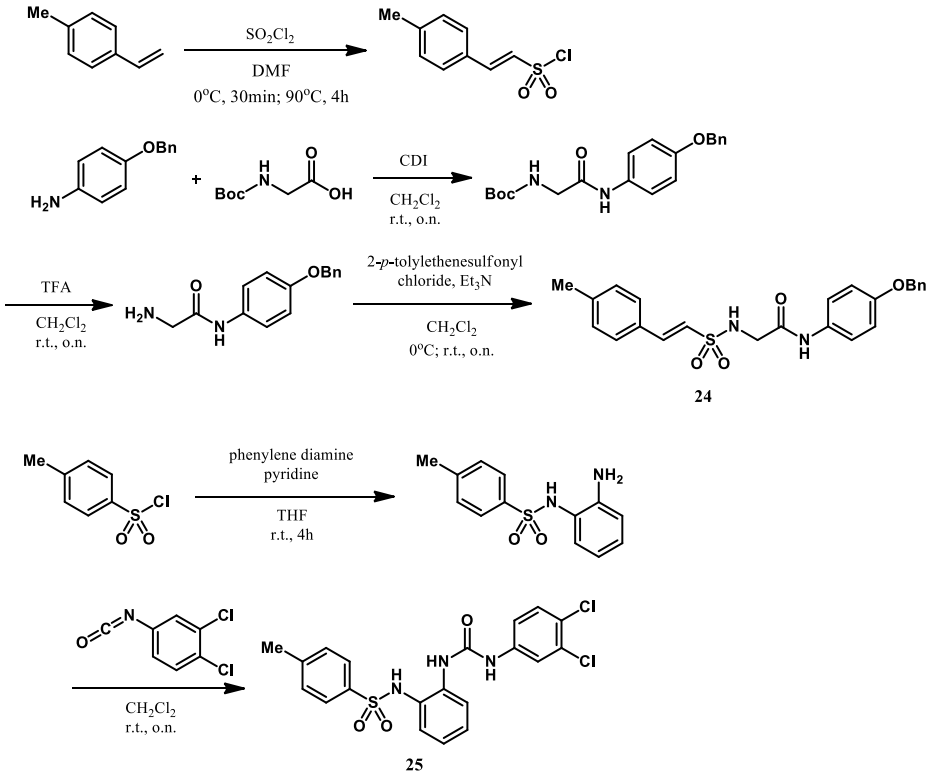
市販のベンズアルデヒド **15a-h** を出発物質とし、Doebner 反応または Horner-Wadsworth-Emmons 反応、加水分解を行い不飽和カルボン酸 **17a-h** を得た。得られたカルボン酸 **17a-h** を酸クロライドに変換後 PEG-400 触媒下、NH_4SCN と反応を行いイソチオシアネート体 **18a-h** を得た。同様に市販のフェニル酢酸 **19c, i** もイソチオシアネート体 **20c, i** とした。またヒドラゾン **22j-r** は市販のカルボン酸からヒドラジンと縮合させて得た。最後にヒドラジド **22j-r** とイソチオシアネート体 **18a-h**, **20c, i** とを縮合させチオウレア体 **23A-U** を得た。



Scheme 2: Synthesis of the derivatives **23A-U**: *Reagents and conditions*: (a) malonic acid, pyridine, aniline (cat.), toluene, reflux, 18 hr; (b) triethyl phosphonoacetate, NaH, THF, 0 ^{o}C, 30 min; r.t., 18 hr; (c) $LiOH•H_2O$, MeOH: H_2O = 3 : 1, reflux, 18 hr; (d) $SOCl_2$, CH_2Cl_2, r.t., 18 hr; (e) NH_4SCN, PEG-400 (cat.), CH_2Cl_2, r.t., 2 hr; (f) $SOCl_2$, benzene, r.t., 18 hr; (g) HCl (cat.), EtOH, reflux, 18 hr; (h) $N_2H_4•H_2O$, EtOH, reflux, 18 hr; (i) CH_2Cl_2, r.t., 18 hr

また候補化合物 **11**, **12** に関してもそれぞれ 35 種、37 種類の誘導体合成を行った。(Scheme 3) 候補化合物 **11**, **12** ともに **10** のように中央に水素結合を有し両端

に芳香環を持った化学構造を有している。従って、候補化合物 **10** と同様の方針に基づき誘導体の合成を行った。その結果、*in vitro* 評価において以下に示す誘導体 **24**, **25** に mutant-type SRR 阻害活性が認められた。合成経路は *p*-メチルスチレンを出発物質としスルホニルクロライドとした。また *p*-ベンジルオキシアニリンと *N*-Boc-グリシンと縮合を行いアミド体とし、TFA を用い酸性条件下で脱保護を行い、先に合成したスルホニルクロライドとの縮合を行い化合物 **24** を得た。また市販の *p*-メチルベンゼンスルホニルクロライドとフェニレンジアミン、次いでイソシアネートと順に縮合を行い化合物 **25** を得た。



Scheme 3: Synthesis of the derivatives **24**, **25**

第四節　mutant-type SRR を用いた *in vitro* 評価

反応の基質として 20 mM L-セリン、また DMSO に溶解した各化合物、及び mutant-type SRR を混合し、37 °C で 8 時間以上反応させた。その後生成された D-セリンを Dsd1[44] を用いて特異的にピルビン酸へと代謝し、さらに 0.05% DNP/HCl と反応させヒドラゾンを得た。そのヒドラゾンに対し 515 nm の吸光度を測定し、D-セリン生成の阻害活性評価を行った[32]。(Figure 9)

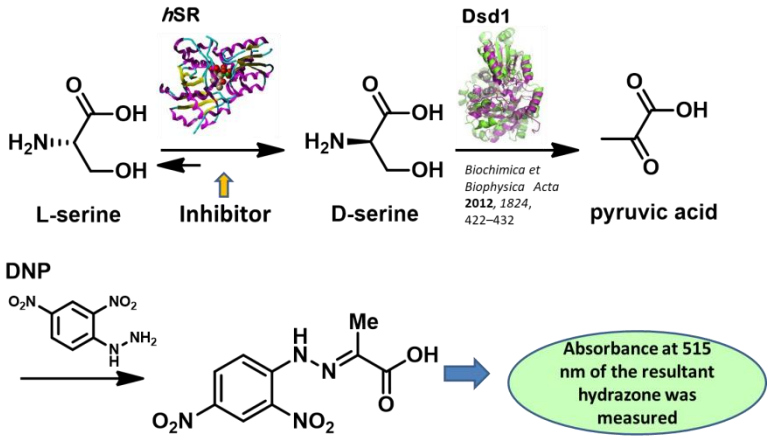


Figure 9: Detection of D-serine produced by mutant-type SRR

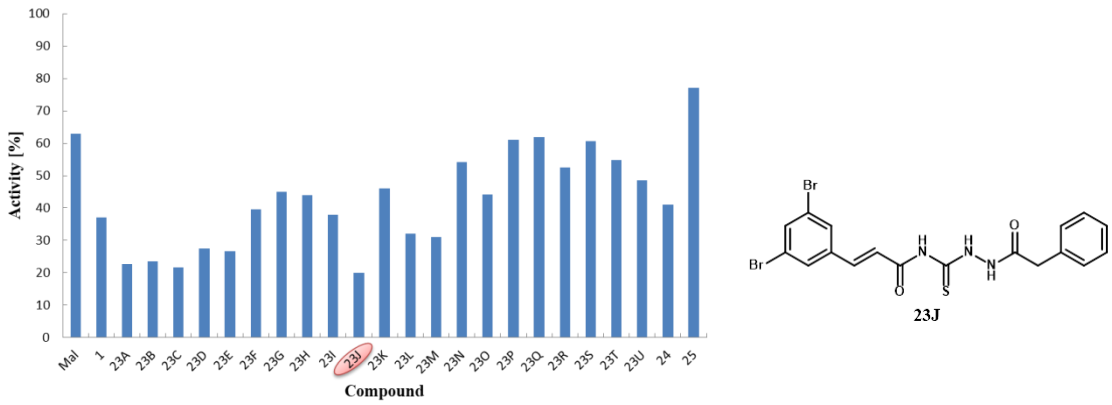
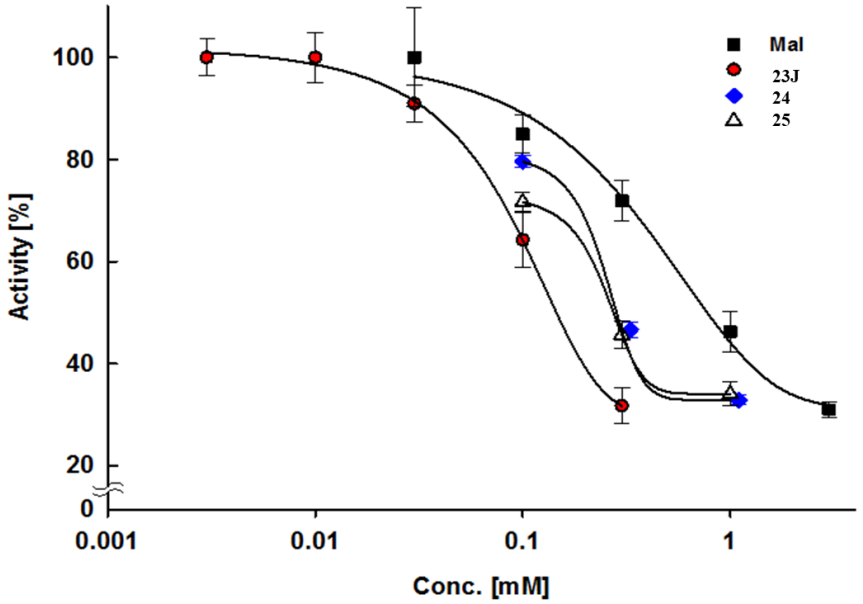


Figure 10: Results of *in vitro* assay of **23A-U**, **24**, and **25** using SRR. The remaining activity of SRR with the inhibitors (1 mM) was evaluated with the percentage of the D-serine production compared to that without inhibitors

阻害活性は、化合物濃度が 1 mM の際の残存 SRR 活性を、既知の SRR に対する阻害剤として報告されているマロン酸と比較して示す。(Figure 10)
また強い阻害活性を示した化合物 **23J**, **24**, **25** について濃度依存性の評価も行った。(Figure 11)
阻害活性は溶媒の DMSO のみを加え、SRR により産生された D-セリン由来の吸

光度を 100%とし、化合物によって阻害された SRR により産生される D-セリン由来の吸光度を示している。



化合物	IC_{50} [mM]
Mal	0.77
23J	0.14
24	0.28
25	0.27

Figure 11: Dose-response curves of compounds inhibition against the SRR activity. Curves of malonate (■), **24** (◆), **25** (△), and **23J** (●) are indicated. Data are presented as mean ± SD (n = 5-15 in each concentration). The IC_{50} values of malonate, **24**, **25**, and **23J** are 0.77 mM, 0.27 mM, 0.28 mM, and 0.14 mM, respectively. The IC_{50} value of **23J** is significantly lower than that of malonate ($P = 6.97 \times 10^{-7}$).

第五節　*Arc-Luc* mice を用いた *in vivo*　評価

このようにして見出した **23J** について、*Arc-Luc* Tg hairless (HL) mice を用いた発光イメージング[45] により *in vivo* での薬効評価を行った。この *Arc-Luc* Tg HL mice は、NMDAR の活性依存的に遺伝子発現する Activity-regulated cytoskeleton-associated protein (Arc タンパク質) 遺伝子のプロモーター配列の下流にホタル発光たんぱく質 Luciferase (Luc) をコードする遺伝子配列を導入したマウスで、NMDAR の活性に伴う Arc 遺伝子の発現を、Luc の基質 Luciferin の酸化による発光として検出できる。この発光量を CCD カメラを用いて検出、評価することにより、リアルタイムで Arc 遺伝子の発現をモニタリングできるマウスである。またこのマウスは、発育過程で無毛となるマウス (Hairless (HL) mice, Hoshino laboratory Animals, inc.より購入) と交配させ体毛による吸光を減らし、簡便に化合物の脳内への移行性及び NMDAR の活性化状態を観測できる。その評価方法について Figure 12A に示す。

カイニン酸 (KA) は脳内に存在する KA 型グルタミン酸受容体のアゴニストである。この KA 受容体の活性化が起こると、海馬を中心としてグルタミン酸の過剰放出が引き起こされ、それに伴って NMDAR も活性化される。今回見出した **23J** が NMDAR の内在性コアゴニストである D-セリン濃度を低下させている場合、KA 投与によって間接的に引き起こされる NMDAR の過剰活性化を抑制すると考えられた。そこで **23J** 投与による NMDAR の活性依存的な Arc 遺伝子の発現上昇に与える作用を評価した。**23J** の溶媒 (Vehicle) は PEG-400 を用いた。

まず KA 投与 3 時間前に PEG-400 を用いて 15 mg/kg に調整した **23J**、または Vehicle を経口投与 (p.o.) した。3 時間後、Luc の基質となる Luciferin を 150 mg/kg 腹腔内投与 (i.p.) し、マウスの頭部における発光量を 1.5% Isoflurane による麻酔下にて *in vivo* imaging system を用いた CCD カメラで 1 分間測定した。その後、生理食塩水を用いて 20 mg/kg に調整した KA を腹腔内投与し、各タイムポイントでの発光量について上記と同様の方法を用いて測定を行った。Figure 12B に各タイムポイントにおけるイメージングの画像例を示す。

解析する ROI (Regions of interest) はマウスの目尻から耳の下端までの範囲とし、その面積と光線量の積を発光量とした。**23J** 投与群の発光量が抑制されていることが観察されたため各タイムポイントでの発光量変化について比較を行った。(Figure 12C)

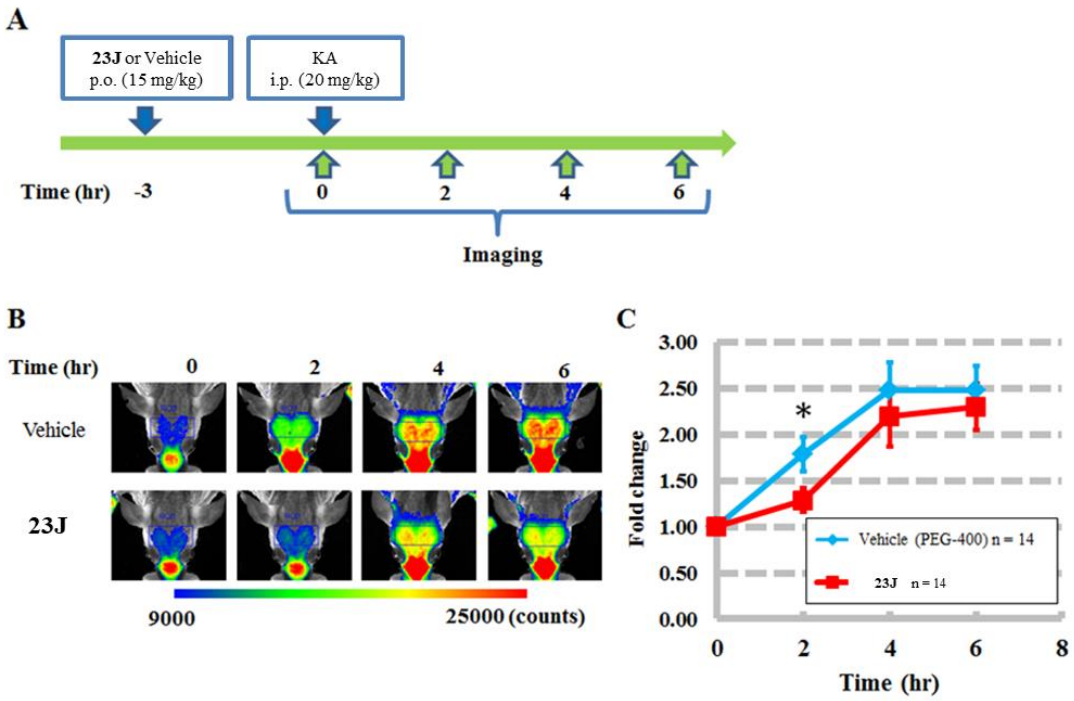


Figure 12: Effect of **23J** treatment on changes in bioluminescence signal intensity in the brain of *Arc-Luc* Tg HL mice after KA injection. (A) The protocol for *in vivo* imaging using *Arc-Luc* Tg HL mice. (B) Representative bioluminescence images (pseudocolored, 9,000-25,000 counts) show the regions of interest (ROIs) of vehicle (PEG-400) administered mice (upper) and **23J** administered mice (lower). (C) Fold change of bioluminescence signal intensity in vehicle and **23J** administered mice after KA injection. The fold changes of the bioluminescence signal intensity are calculated from the measured photon intensity of ROIs at 0 hr and each time point. The data are represented as mean ± SD. *$p < 0.05$; two-tailed Student's t test.

Figure 12C に示すように、Vehicle 群 (n = 14) と **23J** 投与群 (n = 14) において、KA 投与 2 時間後の時点で、**23J** の投与により Arc タンパク質由来の発光を有意に低下させた (t 検定 (two-tailed student t test) $p = 0.04$) 。この結果から、**23J** が消化管から体内へと取りこまれ、マウス脳内に移行し SRR を阻害することで D-セリン濃度を低下させ、NMDAR の機能を抑制し神経細胞の過剰活性化を抑制する可能性が示唆された。(原著論文 [2])

第三章　wild-type SRR に対する新規阻害薬の創製

第一節　wild-type SRR に対する候補化合物の検討

最近、本学薬学部の水口教授が wild-type SRR の単離精製を達成し wild-type SRR の結晶構造(Figure 13)が明らかになった。mutant-type SRR の阻害薬として我々が既に報告している化合物 **49**[32] を基に wild-type SRR 阻害活性を検討したところ、mutant-type SRR に対して IC_{50} = 0.52 mM であったが、wild-type SRR に対して IC_{50} = 1.603 mM と阻害活性に差が生じることが認められた。(第三節で詳述) そこで我々は wild-type SRR の構造を基に mutant-type SRR と同様に、再度 *in silico* スクリーニングを行った。

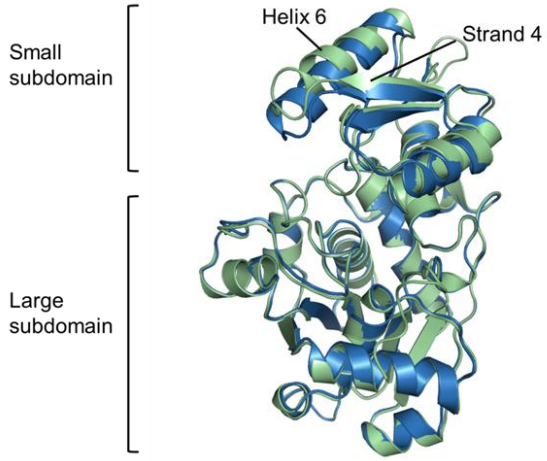


Figure 13: Comparison of the structure of human wild-type SSR (PDB code 5X2L) and the homology-modeled structure of ligand-free form of human SRR with C2D and C6D mutations. The homology-modeled structure is derived from the X-ray crystal structure of ligand-free rat SSR (PDB code 3HMK). The wild-type SRR and the homology-modeled structure are colored in blue and green, respectively.

その結果約 400 万化合物の中から 9 種類の SRR 阻害候補化合物を得た。購入した 9 化合物について、wild-type SRR を用いて *in vitro* 活性測定による生物学的な評価を行った。その結果、2 つの化合物 (**26**, **27**, Figure 14) が wild-type SRR に対して阻害活性を有することが判明した。(Table 2) 本研究では、より阻害作用が強力な化合物 **26** について、有機合成化学を用いた構造変換を行うことにより、さらに強力な wild-type SRR 阻害活性を有するリード化合物の創出を行った。

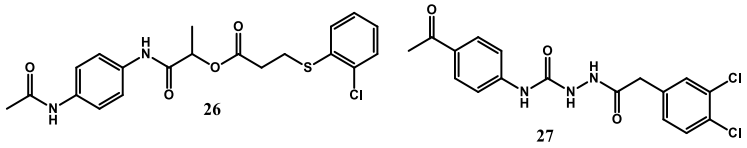


Figure 14: Structure of two hit compounds for wild-type SRR

Compound	Activity [%]
26	49
27	56

Table 2: Results of *in vitro* assay using wild-type SRR. The activity of compounds (1 mM) was evaluated with the percentage of the D-serine production and compared with vehicle (DMSO).

ヒット化合物 **26** と wild-type SRR との相互作用モデルを以下に示した。(Figure 15)

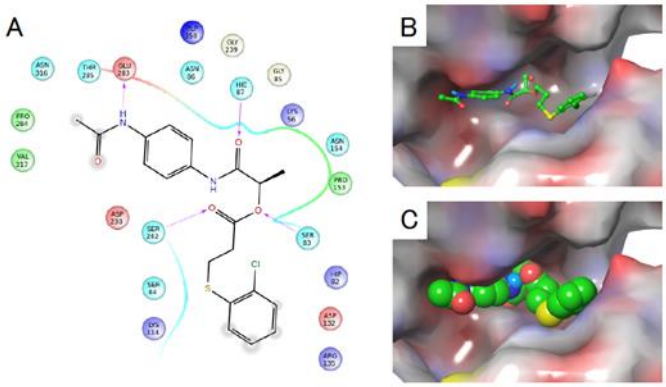
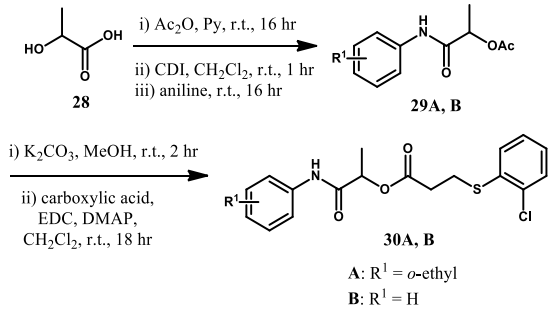


Figure 15: Interaction model of **26** with wild-type SRR. (A) Interaction Diagram. (B) Compound **26** is shown in stick display. (C) Compound **26** is shown in space-filling display.

この相互作用モデルより、左の芳香環のアセトアミド基が Glu283 に水素結合し、リンカー部位の 2 つのカルボニル基がそれぞれ His87, Ser242 と水素結合をしていることがわかる。この結果を踏まえ誘導体合成を行った。

第二節　ヒット化合物の誘導体合成

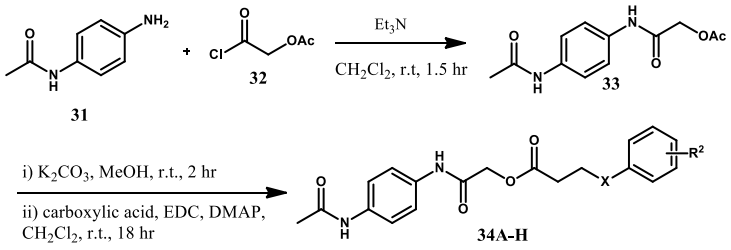
初めに、相互作用モデルで水素結合が見られたアセトアミド基の必要性を確認するため、左の芳香環に対して *o*-ethyl 基（**30A**）、無置換体（**30B**）を持つ化合物を合成した。(Scheme 4)　DL-乳酸を出発物質とし、Ac 保護を行い 2 種類のアニリンと縮合を行いアミド体 **29A, B** とした。その後、アミド体それぞれの Ac 基の脱保護を行い得られたアルコールと文献既知のカルボン酸[46]との縮合を行い誘導体 **30A, B** を得た。



Scheme 4: Synthesis of derivatives **30A, B**

in vitro 評価の結果(Table 3)、**30A, B** はヒット化合物 **26** より wild-type SRR に対する阻害活性をほとんど示さなかった。この結果から、このアセトアミド基は阻害活性発現に必須であることか確認された。相互作用モデルにおいて、リンカー部位の 2 つのカルボニル基も水素結合による相互作用が示唆された。そこで、必須であるアセトアミド基と 2 つのカルボニル基を固定しつつ不斉炭素を回避し、もう一方の芳香環上の置換基の検討を行った。（Scheme 5）

市販のアニリン **31** と酸クロライド **32** を縮合させアミド体 **33** を得た後、上記と同様に脱 Ac 保護を行い、種々のカルボン酸[46-50]と縮合させ誘導体 **34A-H** を得た。



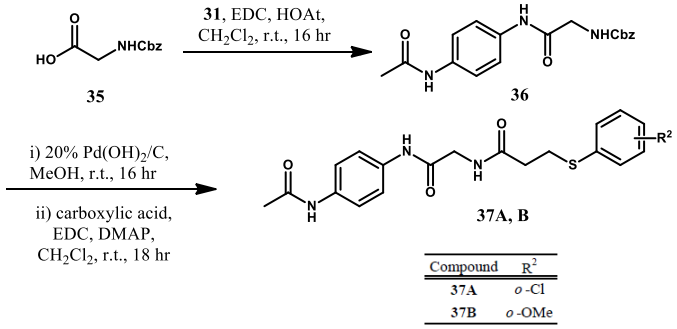
Compound	R^2	X	Compound	R^2	X
34A	*o*-F	S	**34E**	*o*-F	O
34B	*o*-Cl	S	**34F**	*p*-OMe	S
34C	*o*-OMe	S	**34G**	H	S
34D	*m*-Cl	S	**34H**	*m*-OMe	S

Scheme 5: Synthesis of derivatives **34A-H**

in vitro 評価の結果 (Table 3)、**34C** においてヒット化合物 **26** を上回る高い阻害活性が認められた。またチオエーテル結合をエーテル結合に変更した誘導体 **34E** と **34A** の比較からチオエーテル結合の方が優れていることも明らかになった。

この結果を踏まえ、次に、生体内酵素の影響で容易に加水分解されることが予測できるエステル結合をアミド結合に変換した誘導体 **37A** 及び、*in vitro* 評価の高かった **34C** のエステル結合をアミド結合に変換した誘導体 **37B** の合成を行った。(Scheme 6)

市販の *N*-Z-グリシン **35** に対して上記と同様に縮合してアミド体 **36** を得た後、接触還元により Cbz 基の脱保護をしアミンとした後、カルボン酸の縮合を行い誘導体 **37A, B** を得た。



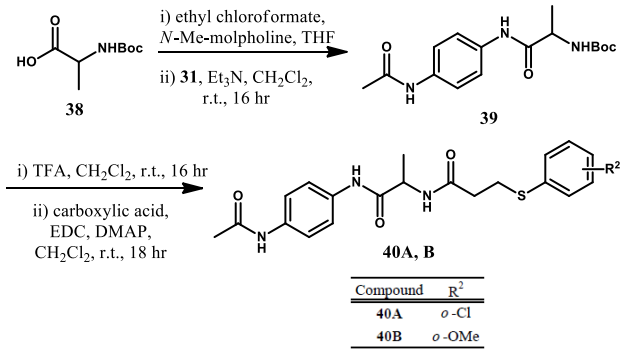
Compound	R^2
37A	*o*-Cl
37B	*o*-OMe

Scheme 6: Synthesis of derivatives **37A, B**

in vitro 評価の結果 (Table 3)、誘導体 **37A, B** ともに全く阻害活性が認められなかった。誘導体 **37A, B** の阻害活性消失は、エステル結合をアミド結合に変換したことによるリンカー部位のコンフォメーションが大きく変化したために起きたことではないかと考え、ヒット化合物 **26** 同様メチル基を付与したアミド誘導体 **40A, B** の合成を検討した。(Scheme 7)

市販の *N*-Boc-アラニン **38** とアニリン **31** とを縮合し、アミド体 **39** を得た。そ

の後、TFA を用いて脱 Boc 保護を行いアミンとし、同様にカルボン酸と縮合を行い誘導体 **40A, B** を得た。



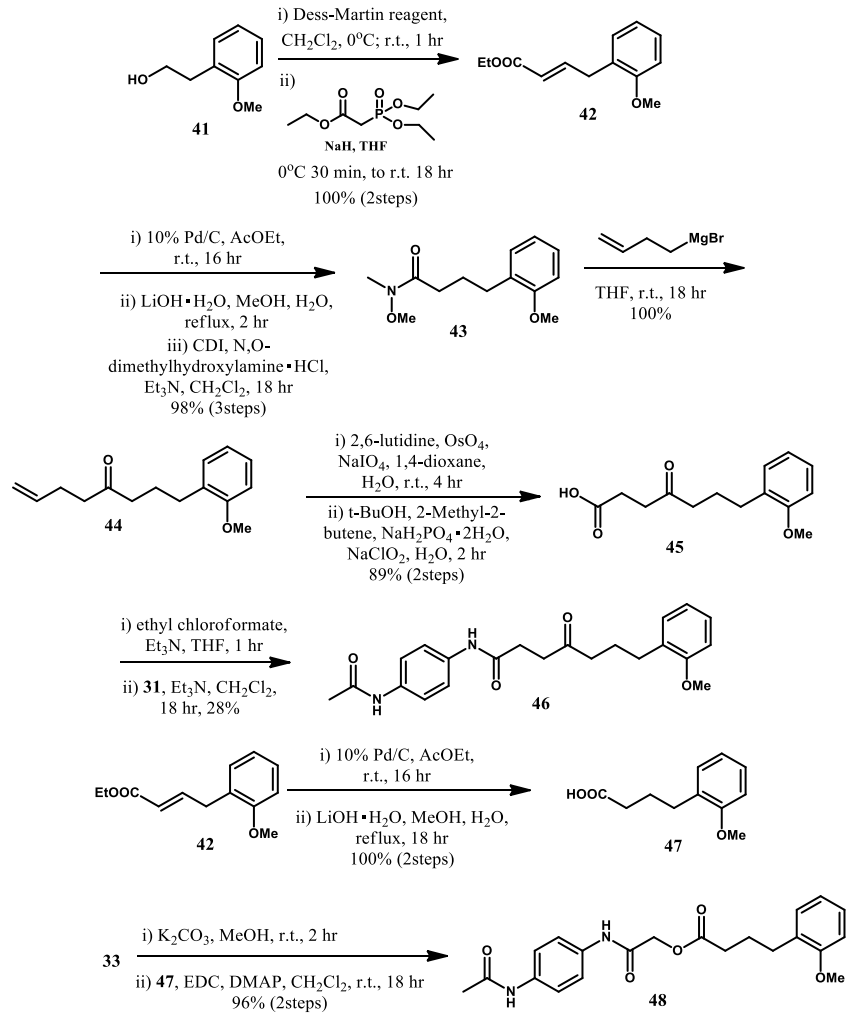
Scheme 7: Synthesis of derivatives **40A, B**

in vitro 評価の結果 (Table 3)、**40A** はヒット化合物 **26** と同等程度の活性が認められたが、**34C** の阻害活性に及ばなかった。

上述のように、エステル結合をアミド結合に変更した化合物は阻害活性の低下を招いたことから、次にエステル結合部をケトンに変更した化合物 **46** の検討を行った。また誘導体 **34A** と **34E** の比較から、**34C** のチオエーテル結合部位の検討余地があると考えられたため、C-誘導体 **48** の合成を行った。(Scheme 8)

市販のアルコール **41** を酸化後、Horner-Wadsworth-Emmons 反応を行い、不飽和エステル **42** を得た。その後 3 工程を経て weinreb's amide **43** とし、Grignard 試薬を用いてブテニルケトン体 **44** を得た。その後、Lemieux-Johnson 酸化、Pinnick 酸化を行い、カルボン酸 **45** としたのち、同様にアニリン **31** と縮合させケトン誘導体 **46** を得た。

また、不飽和エステル **42** に対して接触還元、加水分解と行いカルボン酸 **47** を得た。その後 Ac 体 **33** に対して脱 Ac 保護を行いアルコールとした後、先に合成しておいたカルボン酸 **47** と縮合を行い C-誘導体 **48** を得た。



Scheme 8: Synthesis of derivatives **46** and **48**

しかしながら、ケトン誘導体 **46** は全く阻害活性が認められず、C-誘導体 **48** の阻害活性も誘導体 **34C** に及ばなかった。（Table 3）

ヒット化合物 **26** 及び 15 種類の誘導体について wild-type SRR に対する *in vitro* 阻害活性評価の結果を以下の Table 3 に示した。（評価法は第三節で詳述）溶媒 DMSO のみの場合に産生された D-セリン由来の吸光度を 100 %とし、化合物によって阻害された wild-type SRR により産生される D-セリン由来の吸光度を百分率で表記している。（Table 3）

Compound	Activity [%]	Compound	Activity [%]	Compound	Activity [%]
26	49	**34D**	100	**37B**	100
30A	91	**34E**	88	**40A**	52
30B	91	**34F**	65	**40B**	92
34A	55	**34G**	72	**46**	100
34B	84	**34H**	61	**48**	76
34C	36	**37A**	100		

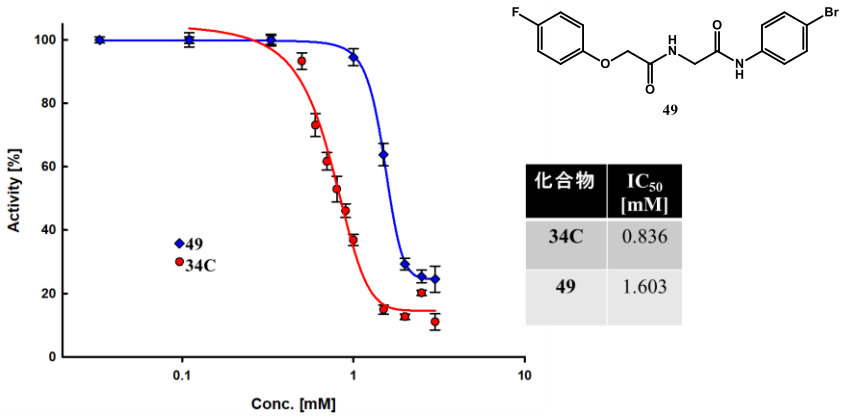
Table 3: Results of *in vitro* assay using wild-type SRR.

第三節　wild-type SRR を用いた *in vitro* 評価

反応の基質として 20 mM L-セリン、また DMSO に溶解した各化合物、及び wild-type SRR を混合し、37 ºC で 30 min 反応させた。その後生成された D-セリンを Dsd1[44] を用いて特異的にピルビン酸へと代謝し、さらに 0.05% DNP/HCl と反応させヒドラゾンを得た。そのヒドラゾンに対し 515 nm の吸光度を測定し、D-セリン生成の阻害活性評価を行った [32]。(Figure 9)

また強い阻害活性を示した化合物 **34C** について、すでに我々のグループが報告している mutant-type SRR の阻害薬 **49**[32] と比較して濃度依存性の評価（IC_{50} value）も行った。(Figure 16)

阻害活性は溶媒の DMSO のみを加え、SRR により産生された D-セリン由来の吸光度を 100%とし、化合物によって阻害された SRR により産生される D-セリン由来の吸光度を示している。



化合物	IC_{50} [mM]
34C	0.836
49	1.603

Figure 16: Dose-response curves of compounds inhibition against the SRR activity. Curves of **49**(◆) and **34C** (●) are indicated. Data are presented as mean ± SD (n = 5-16 in each concentration). The IC_{50} values of **49** and **34C** are 1.603 mM, and 0.836 mM, respectively. The IC_{50} value of **34C** is significantly lower than that of **13** (p =1.80 x 10^{-28}).

その結果、新たに合成した **34C** は既に報告した SRR 阻害薬 **49** との比較において wild-type SRR に対しては約 2 倍強い阻害活性が認められた。(原著論文 [3])

総括

本研究において我々は、中枢神経系作用薬のより実用的な合成法の確立および、阻害活性を有する新規 SRR 阻害薬を見出した。

文献既知のアミノエステル **1**[18] を出発原料として分子内環化反応を鍵反応とし 10 工程、31%の総収率で目的の Suvorexant の簡便合成法の確立に成功した。

また、*in silico* スクリーニング、化学合成、*in vitro* での生物学的評価を用いて、mutant-type SRR に対しては、既知の SRR 阻害剤であるマロン酸よりも強い阻害活性を有する新規 SRR 阻害薬 **23J** (3-(3,5-Dibromo-phenyl)-*N*-(*N'*-phenylacetylhydrazinocarbothioyl)-acrylamide, IC_{50} = 0.14 mM) を見出した。また *Arc-Luc* Tg HL mice を用いた *in vivo* 評価の結果、**23J** が KA 投与 2 時間後の NMDAR 依存的に発現する Arc タンパク質由来の発光を有意に低下させた。これらの結果から **23J** が脳内で SRR を阻害することで D-セリン濃度を低下させ、NMDAR の機能を抑制し、KA 投与による神経細胞の過剰活性化を抑制した可能性が考えられた。今回の研究により、化合物ライブラリーを用いた *in silico* バーチャルスクリーニング手法が新規 SRR 阻害剤と成りうるヒット化合物の獲得に有用であることが示唆され、化学合成による誘導体合成により、リード化合物と成りうるさらに強力な阻害剤の創製に成功した。また wild-type SRR に対しては既に報告した SRR 阻害薬 **49** より約 2 倍強い阻害活性を有する新規 SRR 阻害薬 **34C** (3-(2-Methoxyphenylsulfanyl)propionic acid (4-acetylaminophenylcarbamoyl)-methyl ester)を見出した。

今回得られた化合物の構造情報や生物学的評価方法を用い、wild-type SRR を標的とした、異なるライブラリーを用いて、さらなる化合物とのドッキングシュミレーション、合成及び評価を行うことで、さらに強力な SRR 阻害剤の創出が期待される。また、SRR 遺伝子の発現量を調節するタンパクをターゲットとした化合物の創製を目的とすることで新しい作用機序の SRR 阻害剤の創出が目指せる。今後違ったアプローチを探索することも大いに可能であり、新たな SRR 阻害剤の創出が期待される。

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実験の部

General: Flash chromatography was performed with Kanto Kagaku silica gel 60N (63-210 mm). NMR spectra were recorded on a JEOL JNM-A 400, JEOL JNM-ECX 500 spectrometer in the solvent indicated. Chemical shifts (δ) are given in ppm downfield from TMS and referenced with $CHCl_3$ (7.26 ppm) or DMSO (2.49 ppm) as an internal standard. Peak multiplicities are designated by the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad and coupling constants are given in (J) Hz. Infrared spectra were obtained with a SHIMADZU FTIR-8400 spectrometer using film KBr pellet technique. High resolution mass spectral data was obtained on a JEOL MStation JMS-700. All commercial reagents were used as received unless otherwise noted. The purities (≥ 95%) of compounds **23A-U**, **24**, and **25** were analyzed by HPLC analysis. Analytical HPLC performed on JASCO PU-2082 plus intelligent HPLC pump with GL Science InertSustain®C18 (5μL, 4.6 x 250 mm) column and 100% MeCN as mobile phase with flow rate of 1-2 mL/min at 30-50 °C.

第一章第二節の実験

(3*R*, α*R*)-3-[Benzyl(1-phenylethyl)amino]-*N*-(2-hydroxyethyl)butyramide (2)

To a stirred solution of **1**[18] (2.13 g, 6.84 mmol) in toluene (15 mL) was added 2-aminoethanol (4.1 mL, 68.4 mmol), and the resulting mixture was heated to reflux. After stirring for 24 hr, 2-aminoethanol (4.1 mL, 68.4 mmol) was added to the reaction mixture, and then the resulting mixture was further refluxed for 24 hr. After cooling, the reaction mixture was diluted with CH_2Cl_2 and sat. $NaHCO_3$ aq., and organic phase was separated, the aqueous mixture was extracted with CH_2Cl_2. The organic phase and extracts were combined, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed on SiO_2 (Hexane–Acetone = 1 : 1) to give **2** (1.90 g, 81%) as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.35-7.16 (10H, m), 4.06 (1H. q, *J* = 6.9 Hz), 3.85 & 3.63 (2H, ABq, *J* =14.0 Hz), 3.50-3.49 (1H, m), 3.42-3.35 (2H, m), 3.10-3.04 (2H, m), 2.97-2.92 (1H, m), 2.46 (1H, dd, *J* = 10.0 Hz, 16.3 Hz), 2.06 (1H, dd, *J* = 4.2 Hz, 16.3 Hz), 1.47 (3H, d, *J* = 7.3 Hz), 1.18 (3H, d, *J* = 6.9 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.15, 143.74, 140.36, 128.57, 128.18, 128.16, 127.59, 126.92, 126.81, 62.09, 57.03, 49.88, 49.24, 42.08, 40.60, 17.26, 17.04; IR (neat): 3305, 2969, 2933, 1643 cm^{-1}; MS (EI) m/z 341 (M+1); HRMS (EI) calcd for $C_{21}H_{28}N_2O_2$: 340.2151 (M+), found: 340.2145; ; $[\alpha]_D^{19}$ -31.5 (*c* 0.9, $CHCl_3$).

(3*R*, α*R*)-{3-[Benzyl(1-phenylethyl)amino]butyl}(2-hydroxyethyl)carbamic acid *tert*-butyl ester (3)

To a stirred solution of **2** (889 mg, 2.61 mmol) in THF (15 mL) was added $LiAlH_4$ (297 mg, 7.83 mmol) at 0 °C, and the resulting suspension was refluxed for 1 hr. After cooling, the reaction mixture was diluted with AcOEt, and then quenched with 10% NaOH aq. at 0 °C. The resulting mixture was filtered off by Celite and the filtrate was dried over Na_2SO_4, and concentrated *in vacuo* to yield corresponding amine. The product was used for the next reaction without further purification. To a stirred solution of the amine obtained above in dioxane (4 mL) and H_2O (2 mL) was added NaOH (355 mg, 8.88 mmol), Boc_2O (1.77 g, 8.09 mmol) at 0 °C, and the resulting mixture was stirred for 2.5 hr at room temperature. The reaction mixture was diluted with AcOEt and organic phase was separated, the aqueous mixture was extracted with AcOEt. The organic phase and extracts were combined, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed on SiO_2 (Hexane–AcOEt = 3 : 1) to give **3** (817 mg, 73% in 2 steps) as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.40-7.19 (10H, m), 3.92 (1H, q, *J* = 6.9 Hz), 3.75 & 3.71 (2H, ABq, *J* = 14.5 Hz), 3.56 (2H, br), 3.08-3.05 (3H, m), 2.79-2.77 (2H, m), 1.68-1.60 (1H, m), 1.34 (3H, d, *J* = 6.9 Hz), 1.31-1.29 (1H, m), 1.10 (3H, d, *J* = 6.9 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 157.43, 144.93, 141.76, 128.37, 128.12, 127.95, 127.66, 126.53, 79.88, 62.50, 56.91, 49.90, 49.69, 46.96, 33.79, 30.84, 28.33, 17.88, 16.85; IR (neat): 2972, 1670 cm^{-1}; MS (EI) m/z 427 (M+1); HRMS (EI) calcd for $C_{26}H_{38}N_2O_3$: 426.2882 (M+), found: 426.2885; $[\alpha]_D^{16}$ +16.8 (*c* 1.0, $CHCl_3$).

(2-Hydroxyethyl)[(*R*)-3-(2-nitrobenzenesulfonylamino)butyl]carbamic acid *tert*-butyl ester (4)

To a stirred solution of **3** (635 mg, 1.49 mmol) in MeOH (10 mL) was added 20% $Pd(OH)_2$/C (8 mg), and the resulting suspension was stirred under a hydrogen atmosphere at 1 atm for 20 hr. The catalyst was removed by filtration and the filtrate was concentrated in vacuo to yield corresponding amine. The product was used for the next reaction without further purification. To a solution of the amine obtained above in THF (5 mL) and sat. $NaHCO_3$ aq. (5 mL) was added 2-nitrobenzenesulfonyl chloride (330 mg, 1.49 mmol) at 0 °C, and the resulting mixture was stirred for 23 hr at same temperature. The reaction mixture was diluted with AcOEt and organic phase was separated, the aqueous mixture was extracted with AcOEt. The organic phase and extracts were combined, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed on SiO_2 (Hexane–AcOEt = 1 : 3) to give **4** (492 mg, 79% in 2 steps) as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.16-8.14 (1H, m), 7.86 (1H, br), 7.75-7.73 (2H, m), 3.73-3.70 (2H, m), 3.53 (1H, br), 3.36-3.23 (4H, m), 1.76-1.72 (2H, m), 1.46 (9H, s), 1.10 (3H, d, *J* = 6.6 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 156.88, 147.74, 134.67, 133.47, 132.82, 130.62, 125.24, 80.23, 62.20, 50.24, 49.13, 45.50, 36.32, 28.32, 21.36; IR (neat): 3441, 1668 cm^{-1}; MS (EI) m/z 417 (M+); HRMS (EI) calcd for $C_{17}H_{27}N_3O_7S$: 417.1570 (M+), found: 417.1575; $[\alpha]_D^{16}$ -94.7 (*c* 1.0, $CHCl_3$).

(*R*)-5-Methyl-4-(2-nitrobenzenesulfonyl)[1,4]diazepane-1-carboxylic acid *tert*-butyl ester (5)

To a stirred solution of **4** (570 mg, 1.37 mmol) in toluene (46 mL) were added PPh_3 (716 mg, 2.73 mmol), bis(2-methoxyethyl)azodicarboxylate (639 mg, 2.73 mmol), and the resulting mixture was stirred for 17 hr at room temperature. The solvent was removed, and the residue was chromatographed on SiO_2 (Hexane–AcOEt = 2:1) to give **5** (524 mg, 96%) as a colorless oil. The ^{1}H NMR and ^{13}C NMR data for this compound

was extremely complicated due to its existence of rotamers.
^{1}H NMR (500 MHz, $CDCl_3$): δ 8.06 (1H, br), 7.71-7.63 (3H, m), 4.20-4.19 (1H, m), 3.98-3.72 (3H, m), 3.30-3.14 (3H, m), 2.19-2.14 (1H, m), 1.66-1.61 (1H, m), 1.45 (9H, s), 1.08-1.03 (3H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 154.82, 154.67, 147.64, 134.02, 133.48, 132.80, 131.98, 131.90, 131.83, 131.72, 130.75, 130.61, 128.44, 128.34, 124.15, 79.84, 79.75, 53.70, 53.38, 51.94, 48.31, 47.58, 45.15, 44.96, 43.67, 42.49, 35.48, 35.13, 29.15, 28.78, 28.28, 23.61, 22.84, 18.55, 17.88; IR (neat): 2976, 2933, 1689 cm^{-1}; MS (EI) m/z 399 (M+); HRMS (EI) calcd for $C_{17}H_{25}N_3O_6S$: 399.1464 (M+), found: 399.1464; $[\alpha]_D^{16}$ -88.3 (*c* 1.0, $CHCl_3$).

(*R*)-5-Methyl[1,4]diazepane-1-carboxylic acid *tert*-butyl ester (6)

To a stirred solution of **5** (880 mg, 2.20 mmol) in CH_3CN (10 mL) were added K_2CO_3 (609 mg, 4.41 mmol), PhSH (0.3 mL, 3.31 mmol), and the resulting mixture was stirred for 17 hr at 60 °C. After cooling, the reaction mixture was diluted with CH_2Cl_2, and filtered off by Celite and the filtrate was concentrated in vacuo. The residue was chromatographed on SiO_2 (CH_2Cl_2–MeOH = 20 : 1) to give **6** (472 mg, 97%) as a yellow oil. The ^{1}H NMR and ^{13}C NMR data for this compound was extremely complicated due to its existence of rotamers.
^{1}H NMR (500 MHz, $CDCl_3$): δ 3.70-3.35 (3H, m), 3.30-3.21 (1H, m), 3.10-3.03 (1H, m), 2.82-2.71 (2H, m), 1.87-1.81 (1H, m), 1.45 (9H, s), 1.44-1.36 (1H, m), 1.13-1.11 (3H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 155.14, 78.96, 78.89, 54.54, 54.37, 49.15, 48.53, 48.06, 47.86, 44.64, 43.66, 37.49, 37.37, 28.20, 22.90; IR (neat): 3422, 2974, 2932, 1669 cm^{-1}; MS (EI) m/z 214 (M+); HRMS (EI) calcd for $C_{11}H_{22}N_2O_2$: 214.1681 (M+), found: 214.1679; $[\alpha]_D^{18}$ +16.4 (*c* 1.3, MeOH).

(*R*)-5-Methyl-4-(5-methyl-2-[1,2,3]triazol-2-ylbenzoyl)[1,4]diazepane-1-carboxylic acid *tert*-butyl ester (7)

To a stirred solution of **6** (184 mg, 0.86 mmol) in DMF (5 mL) were added **8**[17] (174 mg, 0.86 mmol), EDC (198 mg, 1.03 mmol), HOAt (140 mg, 1.03 mmol), and *N*-methylmorpholine (0.5 mL, 4.30 mmol), the resulting mixture was stirred for 16 hr at room temperature. The reaction mixture was diluted with AcOEt, sat. $NaHCO_3$ aq., and organic phase was separated, the aqueous mixture was extracted with AcOEt. The organic phase and extracts were combined, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed on SiO_2 (Hexane–AcOEt = 2 : 1) to give **7** (300 mg, 87%) as a colorless oil. The ^{1}H NMR and ^{13}C NMR data for this compound was extremely complicated due to its existence of rotamers.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.92-7.75 (3H, m), 7.33-7.10 (2H, m), 4.87-2.90 (7H, m), 2.41 (3H, s), 2.19-1.88 (1H, m), 1.71-1.37 (10H, m), 1.31-1.10 (3H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.59, 169.43, 168.98, 168.76, 154.89, 154.69, 154.44, 138.16, 138.11, 137.81, 135.36, 135.24, 133.65, 133.57, 133.30, 130.12, 129.52, 129.44, 129.03, 128.93, 128.27, 128.11, 127.95, 122.22, 122.13, 121.90, 121.83, 79.54, 79.39, 52.02, 51.30, 48.16, 47.53, 46.39, 46.16, 45.49, 45.10, 44.76, 44.41, 43.40, 42.96, 42.43, 42.03, 41.53, 41.16, 39.44, 36.85, 36.40, 35.81, 35.02, 34.76, 34.47, 33.89, 29.40, 28.24, 28.16, 20.73, 20.67, 19.76, 19.5517.96, 17.57, 17.33, 16.67; IR (neat): 1683, 1635 cm^{-1}; MS (EI) m/z 399 (M+); HRMS (EI) calcd for $C_{21}H_{29}N_5O_3$: 399.2270 (M+), found: 399.2273; $[\alpha]_D^{17}$ -34.9 (*c* 0.9, $CHCl_3$).

[(*R*)-4-(5-Chlorobenzooxazol-2-yl)-7-methyl[1,4]diazepam-1-yl](5-methyl-2-[1,2,3]-triazol-2-ylphenyl)methanone (Suvorexant)

To a stirred solution of **7** (320 mg, 0.94 mmol) in CH_2Cl_2 (9 mL) was added TFA (9 mL) at room temperature, and the resulting mixture was stirred for 1 hr. The solvent and reagent were removed, and then the product was used for the next reaction without further purification. To a stirred solution of the amine obtained above in CH_3CN (10 mL) was added K_2CO_3 (1.30 g, 9.43 mmol), **9**[17] (263 mg, 1.13 mmol) at room temperature, and the resulting mixture was stirred for 5 hr at 65 °C. The reaction mixture was diluted with CH_2Cl_2 and filtered off by Celite pad, and the filtrate was concentrated in vacuo. The residue was chromatographed on SiO_2 (Hexane– AcOEt = 1 : 1) to give **Suvorexant** (343 mg, 81%) as a white solid. The ^{1}H and ^{13}C NMR data for this compound was extremely complicated due to its existence as rotamers. The ^{1}H and ^{13}C NMR data for the synthesized compound were identical with those of the literature data.[17]

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.95-7.68 (3H, m), 7.35-6.93 (5H, m), 4.56 (1H, d, *J* = 14.3 Hz), 4.24-3.05 (6H, m), 2.43-2.32 (4H, m), 2.14-1.53 (1H, m), 1.30-1.15 (3H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.84, 169.38, 163.02, 162.84, 147.47, 144.73, 144.17, 138.56, 138.16, 135.63, 135.57, 135.53, 134.00, 133.53, 130.57, 129.37, 129.33, 128.89, 128.45, 128.38, 128.07, 122.51, 122.17, 122.07, 120.35, 120.27, 116.31, 116.18, 109.29, 109.18, 109.12, 52.18, 51.47, 48.89, 48.33, 48.06, 47.57, 47.35, 47.02, 45.51, 45.09, 44.87, 44.36, 44.01, 43.80, 41.00 39.86, 36.25, 35.37, 34.21, 33.87, 20.99, 19.88, 17.88, 17.78, 16.67; IR (KBr): 2962, 2925, 1636 cm^{-1}; MS (EI) m/z 450 (M+); HRMS (EI) calcd for $C_{23}H_{23}N_6O_2Cl$: 450.1571 (M+), found: 450.1568; $[\alpha]_D^{16}$ -12.4 (*c* 1.4, MeOH); lit.[8d]: $[\alpha]_D^{25}$ -12.1 (*c* 1.0, MeOH).

第二章第三節の実験

General procedure for the Doebner condensation

To a stirred solution of aldehyde **15a-f** (1.00 mmol) in toluene (5 mL) were added malonic acid (156mg, 1.50 mmol), pyridine (0.12 mL, 1.54 mmol), and aniline (0.01 mL, 0.12 mmol), and the resulting mixture was refluxed for 18 hr. After cooling, the reaction mixture was diluted with AcOEt and 10% HCl aq. and organic phase was separated, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed on SiO_2 (Hexane-Acetone = 100: 1- 40: 1) to give corresponding carboxylic acid **17a-f**.

According to this procedure, the following known carboxylic acids were prepared. 3-(4-Chlorophenyl)acrylic acid (**17a**: 89 %),[51] 3-*p*-Tolylacrylic acid (**17b**: 100 %),[52] 3-(4-Fluoro-phenyl)acrylic acid (**17c**: 86 %),[53] 3-(4-Isopropylphenyl)acrylic acid (**17d**: 90 %),[54] 3-(2,6-Dimethoxyphenyl)acrylic acid (**17e**: 100 %),[55] 3-(4-Trifluoromethylphenyl)acrylic acid (**17f**: 100 %).[56]

General procedure for the Horner-Wadsworth-Emmons reaction

To a stirred suspension of NaH (133 mg, 3.32 mmol) in THF (5 mL) was added triethyl phosphonoacetate (0.66 mL, 3.32 mmol) at 0 °C, and the resulting mixture was stirred at 0 °C. After stirring for 30 min., a solution of aldehyde **15g, h** (2.77 mmol) in THF (5 mL) was added to the reaction mixture, and the resulting mixture was stirred at room temperature for 18 hr. The reaction was quenched by adding H_2O, and the aqeous mixture was extracted with CH_2Cl_2, and the organic phase was separated, the aqueous mixture was extracted with CH_2Cl_2. The organic phase and extracts were combined, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed on SiO_2 (Hexane-Acetone = 90: 1- 80: 1) to give corresponding ethyl ester **16g, h**. To a stirred solution of **16g, h** (2.69 mmol), obtained above, in the mixed solvent (20 mL) with MeOH-H_2O (3: 1) was added $LiOH \cdot H_2O$ (226 mg, 5.38 mmol), and the resulting mixture was refluxed for 2 hr. After cooling, the solvent was removed, and then the residue was acidified with 10% HCl aq. The aqueous mixture was extracted with AcOEt, and the organic extracts were dried over Na_2SO_4, and evaporated to give the carboxylic acid **17g, h**.

According to this procedure, the following known carboxylic acids were prepared. 3-(3,5-Dibromophenyl)acrylic acid (**17g**: 100 %),[57] 3-(2,4,6-Trifluorophenyl)acrylic acid (**17h**: 99 %).[58]

*General procedure for the synthesis of isothiocyanate (**18a-h**, **20c, i**)*

To a stirred solution of carboxylic acid **17a-h** or **19c**, **i** (0.55 mmol) in CH_2Cl_2 for **17a-h** or benzene for **19c**, **i** (3 mL) was added $SOCl_2$ (0.05 mL, 0.66 mmol), and the resulting mixture was stirred at room temperature for 18 hr. The solvent was removed, and then to the residue were added CH_2Cl_2 (3 mL), NH_4SCN (63 mg, 0.82 mmol), and PEG-400 (1 drop), and the resulting mixture was stirred at room temperature for 2 hr. The solvent was removed, and the residue was chromatographed on SiO_2 (Hexane-Acetone = 50: 1- 40: 1) to give corresponding isothiocyanate **18a-h** and **20c**, **i** which were used in the next step immediately.

According to this procedure, the following known isothiocyanates were prepared. 3-(4-Chlorophenyl)-acryloyl isothiocyanate (**18a**: 85%),[59] 3-*p*-Tolylacryloyl isothiocyanate (**18b**: 60%),[60] (4-Fluorophenyl)acetyl isothiocyanate (**20c**: 48%),[61] Phenylacetyl isothiocyanate (**20i**: 75%).[62]

3-(4-Fluorophenyl)acryloyl isothiocyanate (**18c**)

Yield 88%; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 6.43 (1H, d, *J* = 15.8 Hz), 7.13 (2H, t, *J* = 8.6 Hz), 7.57 (2H, dd, *J* = 8.6, 5.3 Hz), 7.73 (1H, d, *J* = 15.8 Hz).

3-(4-Isopropylphenyl)acryloyl isothiocyanate (**18d**)

Yield 79%; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.25 (6H, d, *J* = 7.1 Hz), 2.93 (1H, sept, *J* = 7.1 Hz), 6.45 (1H, d, *J* = 15.7 Hz), 7.27 (2H, d, *J* = 8.2 Hz), 7.48 (2H, d, *J* = 8.2 Hz), 7.73 (1H, d, *J* = 15.7 Hz).

3-(2,6-Dimethoxyphenyl)acryloyl isothiocyanate (**18e**)

Yield 27%; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 3.89 (6H, s), 6.55 (2H, d, *J* = 8.5 Hz), 6.99 (1H, d, *J* = 15.9 Hz), 7.32 (1H, t, *J* = 8.5 Hz), 8.23 (1H, d, *J* = 15.9 Hz).

3-(4-Trifluoromethylphenyl)acryloyl isothiocyanate (**18f**)

Yield 85%; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 6.57 (1H, d, *J* = 15.9 Hz), 7.66-7.71 (4H, m), 7.78 (1H, d, *J* = 15.9 Hz).

3-(3,5-Dibromophenyl)acryloyl isothiocyanate (**18g**)

Yield 63%; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 6.45 (1H, d, *J* = 15.8 Hz), 7.58-7.62 (3H, m), 7.74 (1H, s).

3-(2,4,6-Trifluorophenyl)acryloyl isothiocyanate (**18h**)

Yield 67%; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 6.74-6.79 (3H, m), 7.78 (1H, d, *J* = 16.1 Hz).

*General procedure for the synthesis of acyl hydrazino thiourea (**23A**-**O**)*

To a stirred solution of carboxylic acid **21j-r** (1 mmol) in EtOH (3 mL) was added conc. HCl (1 drop), and the resulting mixture was refluxed for 18 hr. After cooling, the solvent was removed, and then the residue was added sat. $NaHCO_3$ aq., and the aqueous mixture was extracted with CH_2Cl_2. The organic extracts were dried over Na_2SO_4, and the solvent was removed to afford the corresponding ethyl ester, which was used for the next reaction without further purification. To a stirred solution of ethyl ester (1 mmol), obtained above, in EtOH (0.5 mL) was added hydrazine monohydrate (0.05 mL, 1 mmol), and the resulting mixture was refluxed for 18 hr. The solvent was removed to give the corresponding acylhydrazide, which was used for the next reaction without further purification. To a stirred solution of acylhydrazide, obtained above, in CH_2Cl_2 (1 mL) was added a solution of isothiocyanate **18a-h** or **20c, i** (1 mmol) in CH_2Cl_2 (5 mL), and the resulting mixture was stirred at room temperature for 18 hr. The insoluble solid was corrected by filtration, and dried to give acyl hydrazino thiourea **23A-U**.

3-(4-Chlorophenyl)-*N*-[*N'*-(2-thiophen-2-ylacetyl)-hydrazinocarbothioyl]acrylamide **(23A)**

Yield 64%; ^{1}H-NMR (400 MHz, DMSO-d6) δ: 3.85 (2H, s), 6.95-7.01 (3H, m), 7.38 (1H, dd, *J* = 5.0, 1.3 Hz), 7.53 (2H, d, *J* = 8.5 Hz), 7.64 (2H, d, *J* = 8.5 Hz), 7.73 (1H, d, *J* = 15.9 Hz), 11.16 (1H, br), 11.66 (1H, br), 12.57 (1H, br); ^{13}C-NMR (100 MHz, DMSO-d6) δ: 33.90, 120.18, 125.18, 126.65, 126.67, 129.20, 129.90, 132.97, 135.26, 136.19, 143.14, 165.56, 166.26, 176.63; IR (KBr): 1630, 1655, 1684, 3211 cm^{-1}; MS (EI): m/z 379 (M^+); HRMS: calcd for $C_{16}H_{14}{}^{35}ClN_3O_2S_2$ 379.0216, found 379.0193; Mp: 232-233°C (from EtOH). Purity (HPLC) ≥ 98%

N-[*N'*-(2-Thiophen-2-ylacetyl)hydrazinocarbothioyl]-3-p-tolylacrylamide **(23B)**

Yield 77%; ^{1}H-NMR (400 MHz, DMSO-d6) δ: 2.33 (3H, s), 3.85 (2H, s), 6.92-6.98 (3H, m), 7.27 (2H, d, *J* = 7.9 Hz), 7.38 (1H, dd, *J* = 5.1, 1.0 Hz), 7.51 (2H, d, *J* = 7.9 Hz), 7.69 (1H, d, *J* = 15.9 Hz), 11.14 (1H, br), 11.60 (1H, br), 12.59 (1H, br); ^{13}C-NMR (100 MHz, DMSO-d6) δ: 21.09, 33.92, 118.26, 125.20, 126.66, 126.69, 128.30, 129.75, 131.32, 136.22, 140.95, 144.69, 165.96, 166.26, 176.69; IR (KBr): 1630, 1661, 1684, 3227 cm^{-1}; MS (EI): m/z 359 (M^+); HRMS: calcd for $C_{17}H_{17}N_3O_2S_2$ 359.0763, found 359.0766; Mp: 212-213°C (from EtOH). Purity (HPLC) ≥ 98%

3-(4-Fluorophenyl)-*N*-[*N'*-(2-thiophen-2-ylacetyl)-hydrazinocarbothioyl]acrylamide **(23C)**

Yield 89%; ^{1}H-NMR (400 MHz, DMSO-d6) δ: 3.85 (2H, s), 6.93 (1H, d, *J* = 15.9 Hz), 6.96-6.99 (2H, m), 7.31 (2H, t, *J* = 8.8 Hz), 7.38 (1H, dd, *J* = 4.9, 1.5 Hz), 7.69 (2H, dd, *J* = 8.8, 5.6 Hz), 7.74 (1H, d, *J* = 15.9 Hz), 11.15 (1H, br), 11.64 (1H, br), 12.58 (1H, br); ^{13}C-NMR (100 MHz, DMSO-d6) δ: 33.93, 116.25 (d, *J* = 22.3 Hz), 119.25, 125.23, 126.69, 126.72, 130.63 (d, *J* = 8.3 Hz), 130.73 (d, *J* = 3.3 Hz), 136.22, 143.42, 163.46 (d, *J* = 248.9 Hz), 165.73, 166.31, 176.78; IR (KBr): 1628, 1651, 1680, 3186 cm^{-1}; MS (EI): m/z 363 (M^{+}); HRMS: calcd for $C_{16}H_{14}FN_3O_2S_2$ 363.0511, found 363.0513; Mp: 215-216°C (from Acetone). Purity (HPLC) = 96.28%

3-(4-Isopropylphenyl)-*N*-[*N'*-(2-thiophen-2-ylacetyl)-hydrazinocarbothioyl]acrylamide **(23D)**

Yield 50%; ^{1}H-NMR (400 MHz, DMSO-d6) δ: 1.20 (6H, d, *J* = 6.8 Hz), 2.91 (1H, sept, *J* = 6.8 Hz), 3.85 (2H, s), 6.93-6.98 (3H, m), 7.34 (2H, d, *J* = 8.1 Hz), 7.38 (1H, dd, *J* = 5.0, 1.3 Hz), 7.54 (2H, d, *J* = 8.1 Hz), 7.70 (1H, d, *J* = 15.9 Hz), 11.14 (1H, br), 11.62 (1H, br), 12.59 (1H, br); ^{13}C-NMR (100 MHz, DMSO-d6) δ: 23.56, 33.40, 33.89, 118.37, 125.18, 126.64, 126.67, 127.12, 128.40, 131.73, 136.20, 144.63, 151.64, 165.92, 166.24, 176.78; IR (KBr): 1605, 1626, 1682, 3210 cm^{-1}; MS (EI): m/z 387 (M^{+}); HRMS: calcd for $C_{19}H_{21}N_3O_2S_2$ 387.1075, found 387.1078; Mp: 201-202°C (from AcOEt). Purity (HPLC) ≥ 98%

3-(2,6-Dimethoxyphenyl)-*N*-[*N'*-(2-thiophen-2-ylacetyl)hydrazinocarbothioyl]-acrylamide **(23E)**

Yield 59%; ^{1}H-NMR (400 MHz, DMSO-d6) δ: 3.87 (8H, s), 6.71 (2H, d, *J* = 8.3 Hz), 6.96-6.98 (2H, m), 7.34-7.38 (3H, m), 8.06 (1H, d, *J* = 15.9 Hz), 11.12 (1H, br), 11.67 (1H, br), 12.73 (1H, br); ^{13}C-NMR (100 MHz, DMSO-d6) δ: 33.90, 55.92, 104.07, 110.99, 120.95, 125.18, 126.62, 126.67, 132.55, 135.32, 136.23, 136.86, 159.82, 166.17, 167.53, 176.86; IR (KBr): 1611, 1653, 1670, 3219 cm^{-1}; MS (EI): m/z 405 (M^{+}); HRMS: calcd for $C_{18}H_{19}N_3O_4S_2$ 405.0804, found 405.0826; Mp: 220-221°C (from Hexane and Acetone). Purity (HPLC) ≥ 98%

N-[*N'*-(2-Thiophen-2-ylacetyl)hydrazinocarbothioyl]-3-(2,4,6-trifluorophenyl)-acrylamide **(23F)**

Yield 69%; ^{1}H-NMR (400 MHz, DMSO-d6) δ: 3.85 (2H, s), 6.96-6.98 (2H, m), 7.20

(1H, d, *J* = 16.0 Hz), 7.36-7.40 (3H, m), 7.60 (1H, d, *J* = 16.0 Hz), 11.17 (1H, br), 11.87 (1H, br), 12.57 (1H, br); ^{13}C-NMR (100 MHz, DMSO-d6) δ: 33.90, 101.73 (td, *J* = 26.9, 2.5 Hz), 108.66 (td, *J* = 14.9, 5.0 Hz), 124.65, 125.19, 126.65, 126.68, 129.11, 136.19, 161.39 (ddd, *J* = 253.9, 15.7, 9.1 Hz), 163.01 (dt, *J* = 251.4, 16.5 Hz), 165.39, 166.27, 176.44; IR (KBr): 1624, 1653, 1684, 3190 cm^{-1}; MS (EI): m/z 399 (M^+); HRMS: calcd for $C_{16}H_{12}F_3N_3O_2S_2$ 399.0323, found 399.0315; Mp: 207-208°C. Purity (HPLC) ≥ 98%

3-(4-Fluorophenyl)-*N*-(*N'*-phenylacetylhydrazino-carbothioyl)acrylamide **(23G)**
Yield 89%; ^{1}H-NMR (400 MHz, DMSO-d6) δ: 3.62 (2H, s), 6.93 (1H, d, *J* = 15.7 Hz), 7.25-7.32 (7H, m), 7.69 (2H, dd, *J* = 8.8, 5.6 Hz), 7.73 (1H, d, *J* = 15.7 Hz), 11.10 (1H, s), 11.62 (1H, s), 12.56 (1H, s); ^{13}C-NMR (100 MHz, DMSO-d6) δ: 39.57, 116.20 (d, *J* = 21.5 Hz), 119.25, 126.61, 128.25, 129.20, 130.57 (d, *J* = 9.1 Hz), 130.71 (d, *J* = 3.3 Hz), 135.26, 143.33, 163.41 (d, *J* = 248.9 Hz), 165.66, 167.29, 176.77; IR (KBr): 1629, 1651, 1681, 3191 cm^{-1}; MS(EI): m/z 357 (M+); HRMS: calcd for $C_{18}H_{16}FN_3O_2S$ 357.0947 , found: 357.0957; Mp: 212-213°C. Purity (HPLC) ≥ 98%

N-[*N'*-(2-Thiophen-2-ylacetyl)hydrazinocarbothioyl]-3-(4-trifluoromethylphenyl)-acrylamide **(23H)**
Yield 83%; ^{1}H-NMR (400 MHz, DMSO-d6) δ: 3.86 (2H, s), 6.95-6.99 (2H, m), 7.11 (1H, d, *J* = 15.9 Hz), 7.38 (1H, dd, *J* = 4.9, 1.5 Hz), 7.76-7.83 (5H, m), 11.17 (1H, br), 11.72 (1H, br), 12.53 (1H, br); ^{13}C-NMR (100 MHz, DMSO-d6) δ: 33.93, 122.34, 124.00 (q, *J* = 272.4 Hz), 125.22, 126.02 (q, *J* = 4.1 Hz), 126.70, 127.83, 128.86, 130.24 (q, *J* = 32.0 Hz), 136.19, 138.03 (q, *J* = 1.7 Hz), 142.66, 165.28, 166.34, 176.62; IR (KBr): 1636, 1661, 1684, 3196 cm^{-1}; MS (EI): m/z 413 (M^+); HRMS: calcd for $C_{17}H_{14}F_3N_3O_2S_2$ 413.0479, found 413.0487; Mp: 224-225°C. Purity (HPLC) ≥ 98%

N-(*N'*-Phenylacetylhydrazinocarbothioyl)-3-(2,4,6-trifluorophenyl)acrylamide **(23I)**
Yield 75%; ^{1}H-NMR (400 MHz, DMSO-d6) δ: 3.62 (2H, s), 7.19 (1H, d, *J* = 16.1 Hz), 7.23-7.40 (7H, m), 7.59 (1H, d, *J* = 16.1 Hz), 11.13 (1H, br), 11.86 (1H, br), 12.53 (1H, br) ; ^{13}C-NMR (100 MHz, DMSO-d6) δ: 39.54, 101.71 (td, *J* = 27.3, 3.3 Hz), 108.64 (td, *J* = 16.5, 5.0 Hz), 124.65, 126.60, 128.25, 129.07, 129.18, 135.25, 161.36 (ddd, *J* = 254.7, 15.7, 9.9 Hz), 162.99 (dt, *J* = 252.2, 17.2 Hz), 165.35, 167.28, 176.47; IR (KBr): 1628, 1651, 1687, 3215 cm^{-1}; MS (EI): m/z 393 (M^+); HRMS: calcd for $C_{18}H_{14}F_3N_3O_2S$ 393.0759, found 393.0741; Mp: 210-211°C. Purity (HPLC) ≥ 98%

3-(3,5-Dibromophenyl)-*N*-(*N'*-phenylacetylhydrazino-carbothioyl)acrylamide **(23J)**

Yield 70%; ^{1}H-NMR (400 MHz, DMSO-d6) δ: 3.62 (2H, s), 7.06 (1H, d, *J* = 15.9 Hz), 7.23-7.32 (5H, m), 7.66 (1H, d, *J* = 15.9 Hz), 7.84 (2H, s), 7.92 (1H, s), 11.13 (1H, br), 11.57 (1H, br), 12.47 (1H, br) ; ^{13}C-NMR (100 MHz, DMSO-d6) δ: 39.59, 122.79, 123.12, 126.66, 128.30, 129.24, 129.80, 134.88, 135.26, 138.32, 141.06, 164.99, 167.42, 176.69; IR (KBr): 1632, 1655, 1684, 3180 cm^{-1}; MS (EI): m/z 495 (M^{+}); HRMS: calcd for $C_{18}H_{15}Br_2N_3O_2S$ 494.9252, found 494.9232; Mp: 192-193°C. Purity (HPLC) ≥ 98%

3-(4-Fluorophenyl)-*N*-[*N'*-(thiophene-2-carbonyl)-hydrazinocarbothioyl]acrylamide **(23K)**

Yield 85%; ^{1}H-NMR (400 MHz, DMSO-d6) δ: 6.98 (1H, d, *J* = 15.7 Hz), 7.21 (1H, t, *J* = 4.5 Hz), 7.32 (2H, t, *J* = 8.7 Hz) , 7.69-7.72 (2H, m), 7.77 (1H, d, *J* = 15.7 Hz), 7.87 (1H, d, *J* = 4.5 Hz), 7.90 (1H, d, *J* = 4.5 Hz), 11.10 (1H, br), 11.67 (1H, br), 12.14 (1H, br); ^{13}C-NMR (100 MHz, DMSO-d6) δ: 116.24 (d, *J* = 22.3 Hz), 119.41, 128.21, 129.60, 130.63 (d, *J* = 9.1 Hz), 130.76 (d, *J* = 3.3 Hz), 132.00, 136.63, 143.47, 159.48, 163.45 (d, *J* = 248.9 Hz), 165.44, 177.93; IR (KBr): 1598, 1632, 1684, 3172 cm^{-1}; MS (EI): m/z 349 (M^{+}); HRMS: calcd for $C_{15}H_{12}FN_3O_2S_2$ 349.0355, found 349.0347; Mp: 217-218°C. Purity (HPLC) ≥ 98%

3-(2,6-Dimethoxyphenyl)-*N*-(*N'*-phenylacetylhydrazino-carbothioyl)acrylamide **(23L)**

Yield 73%; ^{1}H-NMR (400 MHz, DMSO-d6) δ: 3.62 (2H, s), 3.89 (6H, s), 6.71 (2H, d, *J* = 8.3 Hz), 7.25-7.39 (7H, m), 8.05 (1H, d, *J* = 15.9 Hz), 11.07 (1H, s), 11.65 (1H, s), 12.70 (1H, br); ^{13}C-NMR (100 MHz, DMSO-d6) δ: 39.59, 55.94, 104.09, 111.02, 121.00, 126.63, 128.29, 129.21, 132.57, 135.19, 135.31, 159.84, 167.26, 167.53, 177.00; IR (KBr): 1609, 1660, 1668, 3227cm^{-1}; MS (EI): m/z 399 (M^{+}); HRMS: calcd for $C_{20}H_{21}N_3O_4S$ 399.1253, found 399.1246; Mp: 211-212°C. Purity (HPLC) ≥ 98%

3-(2,6-Dimethoxyphenyl)-*N*-[*N'*-(thiophene-2-carbonyl)-hydrazinocarbothioyl]-acrylamide **(23M)**

Yield 26%; ^{1}H-NMR (400 MHz, DMSO-d6) δ: 3.88 (6H, s), 6.72 (2H, d, *J* = 8.5 Hz), 7.21 (1H, t, *J* = 5.1 Hz), 7.37-7.42 (2H, m), 7.84-7.90 (2H, m), 8.09 (1H, d, *J* = 15.9 Hz), 11.07 (1H, br), 11.68 (1H, br), 12.14 (1H, br); ^{13}C-NMR (125 MHz, DMSO-d6) δ: 56.43, 104.57, 109.48, 111.51, 121.58, 128.69, 130.02, 132.47, 133.07, 135.78, 160.34, 167.77, 170.80, 171.20; IR (KBr): 1595, 1609, 1674, 3223 cm^{-1}; MS (EI): m/z 391 (M^{+}); HRMS: calcd for $C_{17}H_{17}N_3O_4S_2$ 391.0660, found 391.0657; Mp: 209-210°C. Purity (HPLC) ≥ 98%

3-(3,5-Dibromophenyl)-*N*-{*N'*-[2-(4-fluorophenyl)acetyl]hydrazinocarbothioyl}-acrylamide **(23N)**

Yield 28%; ^{1}H-NMR (400 MHz, DMSO-d6) δ: 3.61 (2H, s), 7.06 (1H, d, *J* = 16.0 Hz), 7.11-7.35 (4H, m), 7.66 (1H, d, *J* = 16.0 Hz), 7.84 (2H, s), 7.92 (1H, s), 11.11 (1H, br), 11.56 (1H, br), 12.44 (1H, br); ^{13}C-NMR (100 MHz, DMSO-d6) δ: 38.72, 115.06 (d, *J* = 21.5 Hz), 122.79, 123.17, 129.86, 130.95 (d, *J* = 8.3 Hz), 131.45 (d, *J* = 2.5 Hz), 134.94, 138.34, 141.15, 165.63, 166.23 (d, *J* = 241.5 Hz), 167.50, 176.07; IR (KBr): 1630, 1650, 1682, 3196 cm^{-1}; MS (EI): m/z 515 (M^+); HRMS: calcd for $C_{18}H_{14}{}^{79}Br_2FN_3O_2S$ 512.9153, found 512.9162; Mp: 195-196°C. Purity (HPLC) ≥ 98%

3-(3,5-Dibromophenyl)-*N*-[*N'*-(3-phenylpropionyl)hydrazinocarbothioyl]acrylamide **(23O)**

Yield 23%; ^{1}H-NMR (400 MHz, DMSO-d6) δ: 2.57 (2H, t, *J* = 7.8 Hz), 2.87 (2H, t, *J* = 7.8 Hz), 7.06 (1H, d, *J* = 16.0 Hz), 7.18-7.28 (5H, m), 7.67 (1H, d, *J* = 16.0 Hz), 7.84 (2H, d, *J* = 1.5 Hz), 7.92 (1H, t, *J* = 1.5 Hz), 10.98 (1H, br), 11.54 (1H, br), 12.47 (1H, br); ^{13}C-NMR (100 MHz; DMSO-d6) δ: 30.57, 34.46, 122.80, 123.10, 125.99, 128.22, 128.30, 129.76, 129.51, 132.25, 138.30, 140.80, 168.68, 176.29, 180.24; IR (KBr): 1636, 1650, 1685, 3233 cm^{-1}; MS (EI): m/z 511 (M^+); HRMS: calcd for $C_{19}H_{17}{}^{79}Br_2N_3O_2S$ 508.9409, found 508.9407; Mp: 213-214°C. Purity (HPLC) ≥ 98%

2-Phenyl-*N*-[*N'*-(2-thiophen-2-ylacetyl)hydrazinocarbothioyl]acetamide **(23P)**

Yield 63%; ^{1}H-NMR (400 MHz, DMSO-d6) δ: 3.74 (2H, s), 3.81 (2H, s), 6.93-6.95 (2H, m), 7.24-7.37 (6H, m), 11.06 (1H, br), 11.73 (1H, br), 12.30 (1H, br); ^{13}C-NMR (100 MHz, DMSO-d6) δ: 33.94, 42.11, 125.24, 126.70, 126.74, 127.08, 128.32, 128.51, 129.41, 134.30, 166.39, 167.43, 176.90; IR (KBr): 1537, 1676, 1697, 3179cm^{-1}; MS (EI): m/z 333 (M^+); HRMS: calcd for $C_{15}H_{15}N_3O_2S_2$ 333.0606, found 333.0607; Mp: 161-162°C. Purity (HPLC) ≥ 98%

N-{*N'*-[2-(4-Fluorophenyl)acetyl]hydrazinocarbothioyl}-2-phenylacetamide **(23Q)**

Yield 55%; ^{1}H-NMR (400 MHz, DMSO-d6) δ: 3.57 (2H, s), 3.74 (2H, s), 7.11 (2H, t, *J* = 8.7 H), 7.20-7.40 (7H, m), 10.10 (1H, br), 11.02 (1H, br), 11.73 (1H, br); ^{13}C-NMR (100 MHz, DMSO-d6) δ: 38.64, 42.07, 114.98 (d, *J* = 21.5 Hz), 127.01, 128.45, 129.37, 131.06 (d, *J* = 8.3 Hz), 131.43 (d, *J* = 2.5 Hz), 134.30, 161.16 (d, *J* = 242.3 Hz), 167.33, 172.57, 177.11; IR (KBr): 1589, 1674, 1699, 3194 cm^{-1}; MS (EI): m/z 345 (M^+); HRMS: calcd for $C_{17}H_{16}FN_3O_2S$ 345.0947, found 345.0951; Mp: 182-183°C. Purity (HPLC) ≥ 98%

2-Phenyl-*N*-[*N'*-(2-m-tolylacetyl)hydrazinocarbothioyl]acetamide **(23R)**
Yield 24%; ^{1}H-NMR (400 MHz, DMSO-d6) δ: 2.26 (3H, s), 3.53 (2H, s), 3.74 (2H, s), 7.09-7.29 (9H, m), 11.01 (1H, br), 11.72 (1H, br), 12.28 (1H, br); ^{13}C-NMR (100 MHz, DMSO-d6) δ: 20.96, 40.08, 42.04, 126.24, 127.00, 127.22, 128.15, 128.44, 129.36, 129.78, 134.29, 135.13, 137.27, 167.33, 172.55, 176.75; IR (KBr): 1590, 1653, 1691, 3191 cm^{-1}; MS (EI): m/z 341 (M^+); HRMS: calcd for $C_{18}H_{19}N_3O_2S$ 341.1198, found 341.1198; Mp: 149-150°C. Purity (HPLC) ≥ 98%

N-{*N'*-[2-(2,6-Difluorophenyl)acetyl]hydrazinocarbothioyl}-2-phenylacetamide **(23S)**
Yield 88%; ^{1}H-NMR (400 MHz, DMSO-d6) δ: 3.67 (2H, s), 3.74 (2H, s), 7.07 (2H, t, *J* = 7.4 Hz), 7.22-7.40 (6H, m), 11.07 (1H, br), 11.73 (1H, br), 12.23 (1H, br); ^{13}C-NMR (100 MHz, DMSO-d6) δ: 26.73, 42.06, 110.87 (t, *J* = 20.7 Hz), 111.26 (dd, *J* = 19.0, 6.6 Hz), 127.00, 128.44, 129.35, 129.40 (t, *J* = 10.3 Hz), 134.29, 161.08 (dd, *J* = 246.5, 8.3 Hz), 165.60, 172.53, 177.32; IR (KBr): 1628, 1663, 1700, 3180 cm^{-1}; MS (EI): m/z 363 (M^+); HRMS: calcd for $C_{17}H_{15}F_2N_3O_2S$ 363.0857, found 363.0851; Mp: 174-175°C. Purity (HPLC) ≥ 98%

N-{*N'*-[2-(3,5-Difluorophenyl)acetyl]hydrazinocarbothioyl}-2-phenylacetamide **(23T)**
Yield 4%; ^{1}H-NMR (400 MHz, DMSO-d6) δ: 3.74 (2H, s), 4.03 (2H, s), 7.03-7.12 (3H, m), 7.23-7.34 (5H, m), 11.02 (1H, br), 11.74 (1H, br), 12.18 (1H, br); ^{13}C-NMR (100 MHz, DMSO-d6) δ: 40.79, 42.04, 102.17 (t, *J* = 25.6 Hz), 112.43 (dd, *J* = 18.6, 7.0 Hz), 126.99, 128.43, 129.35, 134.28, 139.58 (t, *J* = 10.3 Hz), 162.12 (dd, *J* = 245.2, 13.6 Hz), 166.42, 172.43, 177.72; IR (KBr): 1627, 1653, 1684, 3187 cm^{-1}; MS (EI): m/z 363 (M^+); HRMS: calcd for $C_{17}H_{15}F_2N_3O_2S$ 363.0838, found 363.0849; Mp: 178-179°C. Purity (HPLC) ≥ 98%

2-(4-Fluorophenyl)-*N*-{*N'*-[2-(4-fluorophenyl)acetyl]hydrazinocarbothioyl}acetamide **(23U)**
Yield 35%; ^{1}H-NMR (400 MHz, DMSO-d6) δ: 3.57 (2H, s), 3.74 (2H, s), 7.12-7.32 (8H, m), 11.03 (1H, br), 11.72 (1H, br), 12.28 (1H, br); ^{13}C-NMR (100 MHz, DMSO-d6) δ: 38.60, 41.08, 114.93 (d, *J* = 19.8 Hz), 115.15 (d, *J* = 19.8 Hz), 130.38 (d, *J* = 2.5 Hz), 130.01 (d, *J* = 8.3 Hz), 131.32 (d, *J* = 7.4 Hz), 131.40 (d, *J* = 3.3 Hz), 161.13 (d, *J* = 242.3 Hz), 161.32 (d, *J* = 242.3 Hz), 167.24, 172.38, 176.98; IR (KBr): 1610, 1669, 1700, 3194 cm^{-1}; MS (EI): m/z 363 (M^+); HRMS: calcd for $C_{17}H_{15}F_2N_3O_2S$ 363.0853, found 363.0857; Mp: 178-179°C. Purity (HPLC) ≥ 98%

*Synthesis of N-(4-benzyloxyphenyl)-2-(2-p-tolylethene-sulfonylamino)acetamide **(24)***

To a stirred solution of (*p*-Benzyloxyphenylcarbamoyl)- methylcarbamic acid *tert*-butyl ester[63] (140 mg, 0.39 mmol) in CH_2Cl_2 (3mL) was added TFA (0.30 mL, 3.95 mmol), and the resulting mixture was stirred at room temperature for 18 hr. The reaction was quenched with sat. $NaHCO_3$ aq, and the organic phase was separated. The aqueous layer was extracted with CH_2Cl_2. The organic phase and extracts were combined, dried over K_2CO_3, and concentrated in vacuo to yeild corresponding amine (80 mg, 84 %), which was used for the next reaction without further purification. To a solution of the amine, obtained above, in CH_2Cl_2 (5 mL) were added Et_3N (0.17 mL, 1.22 mmol), DMAP (41 mg, 0.33 mmol) at room temperature, and the resulting mixture was added 2-*p*-tolylethene-sulfonyl chloride[64] at 0 °C, and then the resulting mixture was stirred at room temperature for 18 hr. The solvent was removed, and the residue was chromatographed on SiO_2 (Hexane-Acetone = 6 : 1) to give sulfoneamide **24** (94 mg, 66 %).

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.36 (3H, s), 3.80 (2H, d, *J* = 6.1 Hz), 5.00 (2H, s), 5.29 (1H, br), 6.68 (1H, d, *J* = 15.4 Hz), 6.88 (2H, d, *J* = 9.0 Hz), 7.17 (2H, d, *J* = 7.8 Hz), 7.25-7.45 (9H, m), 7.49 (1H, d, *J* = 15.4 Hz), 8.02 (1H, br); ^{13}C-NMR (125 MHz, DMSO-d6) δ: 20.99, 45.59, 69.33, 114.79, 120.80, 125.89, 127.64, 127.77, 128.31, 128.39, 129.51, 130.07, 131.90, 137.13, 139.05, 140.35, 154.38, 166.45; IR (KBr): 1142, 1316, 1511, 1662, 3287 cm^{-1}; MS (EI): m/z 436 (M^+); HRMS: calcd for $C_{24}H_{24}N_2O_4S$ 436.1457, found 436.1457; Mp: 178-179°C. Purity (HPLC) ≥ 98%

*Synthesis of N-{2-[3-(3,4-dichlorophenyl)ureido]phenyl}-4-methylbenzenesulfonamide **(25)***

To a stirred solution of *N*-(2-aminophenyl)-4-methylbenzene-sulfonamide[65] (100 mg, 0.38 mmol) in CH_2Cl_2 (5 mL) was added 3,4-dichlorophenylisocyanate (72 mg, 0.38 mmol), and the reaction mixture was stirred at room temperature for 18 hr. The insoluble solid was corrected by filtration, and dried to provide sulfonamide **25** (129 mg, 75 %).

^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.40 (3H, s), 6.68 (1H, dd, *J* = 7.8, 1.2 Hz), 6.92 (1H, td, *J* = 7.8, 1.2 Hz), 7.06 (1H, br), 7.10 (1H, br), 7.18 (1H, dd, *J* = 9.0, 2.5 Hz), 7.20-7.27 (3H, m), 7.30 (1H, d, *J* = 9.0 Hz), 7.57 (1H, br), 7.58 (1H, d, *J* = 2.5 Hz), 7.64 (2H, d, *J* = 8.6 Hz), 7.79 (1H, dd, *J* = 7.8, 1.2 Hz); ^{13}C-NMR (100 MHz, DMSO-d6) δ: 21.00, 118.14, 119.16, 121.27, 122.41, 123.10, 125.35, 127.20, 127.32, 127.61, 129.52, 130.63, 131.11, 136.26, 136.46, 140.08, 143.30, 152.18; IR (KBr): 1154, 1324, 1545, 1658,

3327 cm^{-1}; MS (EI): m/z 449 (M^+); HRMS: calcd for $C_{20}H_{17}{}^{35}Cl_2N_3O_3S$ 449.0372, found 449.0364; Mp: 204-205°C. Purity (HPLC) = 96.51%

第二章第四節の実験

For the assay of compounds **23A**–**U**, **24**, **25** recombinant human SRR (8 μg) with C2D2 mutation[36] in a final volume of 125 μL of 100 mM HEPES (pH 8.0), 10 μM PLP, 1 mM $MgCl_2$, 5 mM DTT, 1 mM ATP, 20 mM L-Ser, and 1 mM inhibitors. Reactions were proceeded more than 8 hr at 37 °C. The reaction mixture (25 μL) was further incubated in 100 mM HEPES (pH 8.0), 10 μM PLP, and recombinant Dsd1 in a final volume of 50 μL for 30 min at 30 °C. The reaction mixture was mixed with 50 μL of 0.05% DNP in 2 M HCl aq. After the incubation for 5 min at 30 °C, 100 μL of EtOH and 125 μL of 10 M NaOH aq. were sequentially added. Absorbance at 515 nm of the resultant hydrazine was measured after 10 min incubation at room temperature. The activity of the inhibitors was evaluated with the percentage of the D-serine production compared with that without inhibitors.

第二章第五節の実験

The Arc-Luc HL Tg mice[45] were anesthetized by inhalation of 1.5% isoflurane before and during imaging. After the i.p. injection of luciferin EF (Promega) dissolved in PBS (pH 7.4) at 150 mg/kg BW, the bioluminescence signal intensity in Arc-Luc HL Tg mice was measured using an *in vivo* imaging system (Clairvivo OPT; Shimadzu Co., Kyoto, Japan). Bioluminescence images were taken for 60 s with 4 x 4 binning without using an optical filter. The bioluminescence intensity at each time point was adopted the strongest intensity in measurement result about 5 times. Pseudocolored luminescent images representing the spatial distribution of emitted photons were overlaid on photographs of mice taken in the chamber under a dim light. The ROIs, including the lower end of the ear to corner of the eye were selected. Data were expressed as the mean number of photons in the ROI. Bioluminescence signal intensity was calculated from bioluminescence images by ROI analysis of NIH ImageJ. After the first imaging, we administered vehicle (PEG-400) or **23J** dissolved in PEG-400 at 15 mg/kg BW p.o. under anesthesia. 3 hours after the administration of **23J** or vehicle, we measured bioluminescence signals as a basal level and i.p. injected KA (Tocris, Bristol, UK) dissolved in saline at 20 mg/kg BW under anesthesia. The fold changes of the bioluminescence signals intensity are calculated from the measured photon intensity of ROIs at each time point.

第三章第二節の実験

To a stirred solution of DL-lactic acid **28** (100 mg, 1.11 mmol) in pyridine (5 mL) was added acetic anhydride (136 mg, 1.33 mmol), and the resulting mixture was stirred at room temperature for 18 hr. The reaction mixture was diluted with AcOEt and 10 % HCl aq., and organic phase was separated, the aqueous mixture was extracted with AcOEt. The organic phase and extracts were combined, dried over Na_2SO_4, and concentrated in vacuo to yield acetate, which was used for the next reaction without further purification. To a stirred solution of acetate in CH_2Cl_2 (5 mL) was added CDI (198 mg, 1.22 mmol), and the resulting mixture was stirred at room temperature for 1 hr. After then, to the resulting mixture was added aniline (1.11 mmol), and the reaction mixture was stirred at room temperature for 16 hr. The reaction mixture was diluted with AcOEt and10 % HCl aq. and organic phase was separated, the aqueous mixture was extracted with AcOEt. The organic phase and extracts were combined, dried over Na_2SO_4, and concentrated in vacuo.The residue was chromatographed on SiO_2 (Hexane-Acetone = 15: 1-10: 1) to give corresponding amide **29A**, **B**.

2-(acetyloxy)-*N*-(2-ethylphenyl)propanamide (**29A**)
Yield 69 %; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.25 (3H, t, *J* = 7.4 Hz), 1.59 (3H, d, *J* = 6.9 Hz), 2.21 (3H, s), 2.61 (2H, q, *J* = 7.4 Hz), 5.39 (1H, q, *J* = 6.9 Hz), 7.14 (1H, d, *J* = 7.0 Hz), 7.21-7.23 (2H, m), 7.86 (1H, br), 7.91 (1H, d, *J* = 7.0 Hz)

2-(acetyloxy)-*N*-phenylpropanamide (**29B**: 71 %) [66]

To a stirred solution of amide **29A**, **B** (0.84 mmol) in MeOH (5 mL) was added K_2CO_3 (175 mg, 1.67 mmol), and the resulting mixture was stirred at room temperature for 2 hr. The reaction was quenched with 10 % HCl aq. and the aqueous mixture was extracted with AcOEt. The organic extracts were combined, dried over Na_2SO_4, and concentrated in vacuo to afford the alcohol, which was used for the next reaction without further purification. To a stirred solution of the alcohol, obtained above, in CH_2Cl_2 (5 mL) were added EDC (323 mg, 1.69 mmol), DMAP (10 mg, 0.08 mmol) and 3-[(2-chlorophenyl)thio]propanoic acid[46] (183 mg, 0.84 mmol), and the resulting mixture was stirred at room temperature for 18 hr. The resulting mixture was diluted with CH_2Cl_2 and sat. $NaHCO_3$ aq., and organic phase was separated, the aqueous mixture was extracted with CH_2Cl_2. The organic phase and extracts were combined, dried over Na_2SO_4, and concentrated in vacuo.The residue was chromatographed on

SiO_2 (Hexane-Acetone = 15: 1-10: 1) to give corresponding amide **30A**, **B**.

3-(2-Chlorophenylsulfanyl)propionic acid 1-(2-ethylphenylcarbamoyl)ethyl ester (**30A**)
Yield 69 %; mp: 118-120 °C; IR (KBr): 1179, 1246, 1370, 1456, 1539, 1665, 1733, 3251 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.20 (3H, t, *J* = 7.5 Hz), 1.60 (3H, d, *J* = 6.7 Hz), 2.57 (2H, q, *J* = 7.5 Hz), 2.80 (2H, t, *J* = 7.0 Hz), 3.27 (2H, t, *J* = 7.0 Hz), 5.42 (1H, q, *J* = 6.7 Hz), 7.13-7.23 (5H, m), 7.35 (1H, d, *J* = 8.0 Hz), 7.38 (1H, d, *J* = 8.0 Hz), 7.81 (1H, d, *J* = 7.9 Hz), 7.86 (1H, br); ^{13}C-NMR (100 MHz, DMSO-d6): δ 14.25, 17.58, 23.80, 26.21, 33.01, 70.35, 125.99, 126.27, 126.62, 126.76, 127.67, 127.90, 128.60, 129.59, 131.51, 134.78, 135.00, 138.86, 169.10, 170.61; MS (EI): m/z 391 (M^+); HRMS: calcd for $C_{20}H_{22}{}^{35}ClNO_3S$ 391.9116, found 391.1018.

3-(2-Chlorophenylsulfanyl)propionic acid 1-phenylcarbamoylethyl ester (**30B**)
Yield 40 %; mp: 122-124 °C; IR (KBr): 1178, 1371, 1445, 1539, 1668, 1732, 3261 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.59 (3H, d, *J* = 7.0 Hz), 2.79-2.84 (2H, m), 3.27-3.31 (2H, m), 5.40 (1H, q, *J* = 7.0 Hz), 7.13 (1H, t, *J* = 7.6 Hz), 7.16-7.25 (2H, m), 7.32 (2H, t, *J* = 7.6 Hz), 7.36-7.42 (2H, m), 7.55 (2H, d, *J* = 7.6 Hz), 8.04 (1H, br); ^{13}C-NMR (100 MHz, DMSO-d6): δ 17.41, 26.23, 32.95, 70.41, 119.51, 123.62, 126.78, 127.72, 127.90, 128.73, 129.59, 131.54, 134.94, 138.48, 168.57, 170.63; MS (EI): m/z 363 (M^+); HRMS: calcd for $C_{18}H_{18}{}^{35}ClNO_3S$ 363.8584, found 363.0694.

To a stirred solution of 4'-aminoacetanilide **31** (100 mg, 0.67 mmol) in CH_2Cl_2 (5 mL) were added Et_3N (0.19 mL, 1.33 mmol) and acetoxyacetyl chloride **32** (0.08 mL, 0.73 mmol) at 0 °C, and the resulting mixture was stirred at room temperature for 1.5 hr. The reaction was quenched with satd. $NaHCO_3$ aq., and the organic phase was separated, the aqueous mixture was extracted with CH_2Cl_2. The organic phase and extracts were combined, dried over Na_2SO_4, and concentrated in vacuo.The residue was chromatographed on SiO_2 (CH_2Cl_2-MeOH = 40: 1) to give amide **33**.

N-[4-(acetylamino)phenyl]-2-(acetyloxy)acetamide (**33**)
Yield 80 %; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.18 (3H, s), 2.24 (3H, s), 4.69 (2H, s), 7.20 (1H, br), 7.45 (4H, s), 7.77 (1H, br)

To a stirred solution of amide **33** (0.42 mmol) in MeOH (4 mL) was added K_2CO_3 (88 mg, 0.64 mmol), and the resulting mixture was stirred at room temperature for 2 hr. The resulting mixture was quenched with H_2O and the aqueous mixture was extracted with

AcOEt. The organic extracts were combined, dried over Na_2SO_4, and concentrated in vacuo to afford the alcohol, which was used for the next reaction without further purification. To a stirred solution alcohol, obtained above, in CH_2Cl_2 (5 mL) were added EDC (163 mg, 0.85 mmol), DMAP (5 mg, 0.04 mmol) and various propanoic acid (0.42 mmol), and the resulting mixture was stirred at room temperature for 18 hr. The resulting mixture was diluted with CH_2Cl_2 and quenched 10 % HCl aq., and organic phase was separated, the aqueous mixture was extracted with CH_2Cl_2. The organic phase and extracts were combined, dried over Na_2SO_4, and concentrated in vacuo.The residue was chromatographed on SiO_2 (CH_2Cl_2-MeOH = 70: 1) to give corresponding amide **34A-H**.

3-(2-Fluorophenylsulfanyl)propionic acid (4-acetylaminophenylcarbamoyl)methyl ester (**34A**)

Yield: 53 %; mp: 158-159 °C; IR (KBr): 1186, 1247, 1310, 1408, 1474, 1517, 1559, 1565, 1660, 1734, 1740, 3297, 3304 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d6): δ 2.00 (3H, s), 2.73 (2H, t, *J* = 7.2 Hz), 3.19 (2H, t, *J* = 7.2 Hz), 4.62 (2H, s), 7.19-7.34 (3H, m), 7.44-7.50 (5H, m), 9.87 (1H, s), 9.96 (1H, s); ^{13}C-NMR (100 MHz, DMSO-d6): δ 23.90, 27.02, 33.48, 62.73, 115.68(d, *J* = 22.0 Hz), 119.35, 119.98, 122.07(d, *J* = 17.3 Hz), 125.21(d, *J* = 2.9 Hz), 128.57(d, *J* = 7.7 Hz), 131.03, 133.50, 135.19, 160.22(d, *J* = 242.5 Hz), 164.91, 168.02, 170.75; MS (EI): m/z 390 (M^+); HRMS: calcd for $C_{19}H_{19}FN_2O_4S$ 390.4286, found 390.1044.

3-(2-Chlorophenylsulfanyl)propionic acid (4-acetylaminophenylcarbamoyl)methyl ester (**34B**)

Yield 44 %; mp: 160-161 °C; IR (KBr): 1169, 1308, 1407, 1517, 1550, 1663, 1735, 3295 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d6): δ 2.00 (3H, s), 2.80 (2H, t, *J* = 7.2 Hz), 3.25 (2H, t, *J* = 7.2 Hz), 4.65 (2H, s), 7.21(1H, td, *J* = 7.6, 1.3 Hz), 7.35 (1H, td, *J* = 7.6, 1.3 Hz), 7.42-7.51 (6H, m), 9.87 (1H, s), 9.97 (1H, s); ^{13}C-NMR (100 MHz, DMSO-d6): δ 23.91, 26.18, 32.87, 62.79, 119.35, 119.87, 126.76, 127.62, 127.93, 129.61, 131.47, 133.50, 134.97, 135.20, 164.93, 168.02, 170.80; MS (EI): m/z 406 (M^+); HRMS: calcd for $C_{19}H_{19}{}^{35}ClN_2O_4S$ 406.8832, found 406.0746.

3-(2-Methoxyphenylsulfanyl)propionic acid (4-acetylaminophenylcarbamoyl)methyl ester (**34C**)

Yield 75 %; mp: 143-145 °C; IR (KBr): 1242, 1311, 1407, 1521, 1558, 1564, 1661, 1680, 1740, 3301, 3305cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d6): δ 2.00 (3H, s), 2.71 (2H,

t, *J* = 7.1 Hz), 3.11 (2H, t, *J* = 7.1 Hz), 3.80 (3H, s), 4.63 (2H, s), 6.94 (1H, td, *J* = 7.7, 1.2 Hz), 6.99 (1H, d, *J* = 7.7 Hz), 7.21 (1H, td, *J* = 7.7, 1.4 Hz), 7.27 (1H, dd, *J* = 7.7, 1.4 Hz), 7.45 (2H, d, *J* = 7.8), 7.50 (2H, d, *J* = 7.8), 9.87 (1H, s), 9.96 (1H, s); ^{13}C-NMR (100 MHz, DMSO-d6): δ 23.90, 25.79, 33.32, 55.67, 62.71, 111.02, 119.35, 119.87, 121.08, 123.31, 127.15, 128.31, 133.51, 135.19, 156.67, 164.98, 168.03, 170.96; MS (EI): m/z 402 (M^+); HRMS: calcd for $C_{20}H_{22}N_2O_5S$ 402.4641, found 402.1249.

3-(3-Chlorophenylsulfanyl)propionic acid (4-acetylaminophenylcarbamoyl)methyl ester (**34D**)

Yield 47 %; mp: 161-162 °C; IR (KBr): 1186, 1250, 1309, 1408, 1521, 1558, 1660, 1739, 3308, 3316cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d6): δ 2.00 (3H, s), 2.75 (2H, t, *J* = 7.1 Hz), 3.25 (2H, t, *J* = 7.1 Hz), 4.64 (2H, s), 7.26 (1H, dt, *J* = 7.4, 1.7 Hz), 7.30 (1H, dt, *J* = 7.4, 1.7 Hz), 7.33 (1H, t, *J* = 7.4 Hz), 7.41 (1H, t, *J* = 1.7 Hz), 7.45 (2H, d, *J* = 8.8 Hz), 7.49 (2H, d, *J* = 8.8 Hz), 9.87 (1H, s), 9.97 (1H, s); ^{13}C-NMR (100 MHz, DMSO-d6): δ 23.89, 27.10, 33.27, 62.74, 119.33, 119.86, 125.84, 126.66, 127.24, 130.73, 133.50, 133.78, 135.19, 138.17, 164.91, 168.00, 170.80; MS (EI): m/z 406 (M^+); HRMS: calcd for $C_{19}H_{19}{}^{35}ClN_2O_4S$ 406.8832, found 406.0746.

3-(2-Fluorophenoxy)propionic acid (4-acetylaminophenylcarbamoyl)methyl ester (**34E**)

Yield 67 %; mp: 172-174 °C; IR (KBr): 1199, 1308, 1410, 1517, 1561, 1684, 1724, 3301 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d6): δ 2.00 (3H, s), 2.94 (2H, t, *J* = 5.8 Hz), 4.29 (2H, t, *J* = 5.8 Hz), 4.68 (2H, s), 6.92-6.97 (1H, m), 7.12 (1H, t, *J* = 8.1 Hz), 7.18 (1H, dd, *J* = 8.1, 1.3 Hz), 7.20 (1H, td, *J* = 8.1, 1.3 Hz), 7.45 (2H, d, *J* = 8.8 Hz), 7.49 (2H, d, *J* = 8.8 Hz), 9.87 (1H, s), 9.99 (1H, s); ^{13}C-NMR (100 MHz, DMSO-d6): δ 23.91, 33.69, 62.75, 64.39, 115.25, 116.05(d, *J* = 18.2 Hz), 119.37, 119.85, 121.38(d, *J* = 7.7 Hz), 124.88(d, *J* = 2.9 Hz), 133.55, 135.20, 146.14(d, *J* = 10.5 Hz), 151.72(d, *J* = 243.4 Hz), 164.95, 168.04, 170.34; MS (EI): m/z 374 (M^+); HRMS: calcd for $C_{19}H_{19}FN_2O_5$ 374.3630, found 374.1274.

3-(4-Methoxyphenoxy)propionic acid (4-acetylaminophenylcarbamoyl)methyl ester (**34F**)

Yield 85 %; mp: 160-161 °C; IR (KBr): 1241, 1406, 1518, 1513, 1583, 1672, 1662, 1734, 3300 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d6): δ 2.00 (3H, s), 2.64 (2H, t, *J* = 7.0 Hz), 3.05 (2H, t, *J* = 7.0 Hz), 3.74 (3H, s), 4.62 (2H, s), 6.92 (2H, dd, *J* = 11.6, 3.0 Hz), 7.37 (2H, dd, *J* = 11.6, 3.0 Hz), 7.45 (2H, d, *J* = 9.2 Hz), 7.49 (2H, d, *J* = 9.2 Hz), 9.87 (1H, s), 9.96 (1H, s); ^{13}C-NMR (100 MHz, DMSO-d6): δ 23.90, 29.85, 33.68, 55.20,

62.65, 114.86, 119.35, 119.84, 124.80, 133.13, 133.53, 135.18, 158.77, 164.96, 168.02, 170.92; MS (EI): m/z 402 (M^+); HRMS: calcd for $C_{20}H_{22}N_2O_5S$ 402.4641, found 402.1255.

3-Phenylsulfanylpropionic acid (4-acetylaminophenylcarbamoyl)methyl ester (**34G**)
Yield 64 %; mp: 166-167 °C; IR (KBr): 1409, 1520, 1558, 1660, 1679, 1733, 1737, 3283cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d6): δ 2.00 (3H, s), 2.73 (2H, t, *J* = 7.0 Hz), 3.19 (2H, t, *J* = 7.0 Hz), 4.63 (2H, s), 7.22 (1H, tt, *J* = 6.7, 1.8 Hz), 7.31-7.37 (4H, m), 7.45 (2H, d, *J* = 9.2 Hz), 7.49 (2H, d, *J* = 9.2 Hz), 9.87 (1H, s), 9.97 (1H, s); ^{13}C-NMR (100 MHz, DMSO-d6): δ 23.91, 27.53, 33.48, 62.71, 119.35, 119.87, 126.13, 128.68, 129.19, 133.52, 135.19, 135.28, 164.95, 168.02, 170.89; MS (EI): m/z 372 (M^+); HRMS: calcd for $C_{19}H_{20}N_2O_4S$ 372.4381, found 372.1148.

3-(3-Methoxyphenylsulfanyl)propionic acid (4-acetylaminophenylcarbamoyl)methyl ester (**34H**)
Yield 78 %; mp: 116-118 °C; IR (KBr): 1246, 1408, 1520, 1566, 1589, 1661, 1740, 3308cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d6): δ 2.00 (3H, s), 2.73 (2H, t, *J* = 7.2 Hz), 3.20 (2H, t, *J* = 7.2 Hz), 3.74 (3H, s), 4.64 (2H, s), 6.78 (1H, dd, *J* = 8.0, 2.4 Hz), 6.89 (1H, t, *J* = 2.4 Hz), 6.91 (1H, d, *J* = 8.0 Hz), 7.24 (1H, t, *J* = 8.0 Hz), 7.45 (2H, d, *J* = 9.2 Hz), 7.50 (2H, d, *J* = 9.2 Hz), 9.87 (1H, s), 9.97 (1H, s); ^{13}C-NMR (100 MHz, DMSO-d6): δ 23.91, 27.31, 33.46, 55.16, 62.73, 111.87, 113.70, 119.36, 119.87, 120.50, 130.10, 133.52, 135.20, 136.65, 159.69, 164.96, 168.04, 170.91; MS (EI): m/z 402 (M^+); HRMS: calcd for $C_{20}H_{22}N_2O_5S$ 402.4641, found 402.1243.

To a stirred solution of *N*-(tert-butoxycarbonyl)-DL-alanine **35** (100 mg, 0.53 mmol) in THF (5 mL) were added ethyl chloroformate (0.06 mL, 0.64 mmol) and *N*-methylmorpholine (0.07 mL, 0.64 mmol), and the resulting mixture was stirred at room temperature for 1 hr. The reaction mixture was filtered off by Celite and the filtrate was dried over Na_2SO_4, and concentrated in vacuo to afford the mixed acid anhydrate, which was used for the next reaction without further purification. To a stirred solution of anhydrate, obtained above, in CH_2Cl_2 (6 mL) were added 4'-aminoacetanilide **31** (79 mg, 0.53 mmol) and Et_3N (0.15 mL, 1.06 mmol), and the resulting mixture was stirred at room temperature for 16 hr. The reaction was quenched with 10 % HCl aq., and organic phase was separated, the aqueous mixture was extracted with CH_2Cl_2. The organic phase and extracts were combined, dried over Na_2SO_4, and concentrated in vacuo.The residue was chromatographed on SiO_2 (CH_2Cl_2-MeOH = 40:

1) to give amide **36**.

[1-(4-Acetylaminophenylcarbamoyl)ethyl]carbamic acid *tert*-butyl ester (**36**)
Yield 91 %; ^{1}H-NMR (400 MHz, DMSO-d6): δ 1.23 (3H, d, *J* = 7.1 Hz), 1.37 (9H, s), 2.00 (3H, s), 4.07 (1H, quin, *J* = 7.1 Hz), 7.02 (1H, d, *J* = 7.1 Hz), 7.48 (4H, s), 9.82 (1H, s), 9.85 (1H, s)

To a stirred solution of amide **36** (120 mg, 0.37 mmol) in CH_2Cl_2 (4 mL) was added TFA (4 mL), and resulting mixture was stirred at room temperature for 18 hr. The solvent and reagent were removed, and then the residue were added 10 % NaOH aq. and CH_2Cl_2, and organic phase was separated, the aqueous mixture was extracted with CH_2Cl_2. The organic phase and extracts were combined, dried over Na_2SO_4, and concentrated in vacuo to afford the amine, which was used for the next reaction without further purification. To a stirred solution of amine, obtained above, in CH_2Cl_2 (4 mL) were added various carboxylic acid (0.37 mmol), EDC (143 mg, 0.75 mmol) and DMAP (5 mg, 0.04 mmol), and the resulting mixture was stirred at room temperature for 18 hr. The reaction mixture was diluted with 10 % HCl aq. and CH_2Cl_2, and organic phase was separated, the aqueous mixture was extracted with CH_2Cl_2. The organic phase and extracts were combined, dried over Na_2SO_4, and concentrated in vacuo.The residue was chromatographed on SiO_2 (CH_2Cl_2-Acetone-MeOH = 50: 1: 1) to give amide **37A**, **B**.

N-[1-(4-Acetylaminophenylcarbamoyl)ethyl]-3-(2-chlorophenylsulfanyl)propionamide (**37A**)
Yield 45 %; mp: 214-215 °C; IR (KBr): 1516, 1555, 1639, 1661, 3285 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d6): δ 1.26 (3H, d, *J* = 7.3 Hz), 2.00 (3H, s), 2.53 (2H, t, *J* = 7.2 Hz), 3.18 (2H, t, *J* = 7.2Hz), 4.41 (1H, quin, J = 7.3 Hz), 7.18 (1H, td, J = 7.9, 1.7Hz), 7.34 (1H, td, J = 7.9, 1.4 Hz), 7.39 (1H, dd, J = 7.9, 1.7 Hz), 7.44 (1H, dd, J = 7.9, 1.4 Hz), 7.48 (4H, s), 8.28 (1H, d, J = 7.3 Hz), 9.85 (1H, s), 9.89 (1H, s); ^{13}C-NMR (100 MHz, DMSO-d6): δ 18.77, 24.43, 27.34, 34.56, 49.53, 119.79, 120.16, 126.87, 127.66, 128.37, 130.01, 131.61, 134.68, 135.44, 136.18, 168.48, 170.34, 171.39; MS (EI): m/z 419 (M^+); HRMS: calcd for $C_{20}H_{22}{}^{35}ClN_3O_3S$ 419.1070, found 419.1077.

N-[1-(4-Acetylaminophenylcarbamoyl)ethyl]-3-(2-methoxyphenylsulfanyl)-propionamide (**37B**)
Yeild 31 %; mp: 205-207 °C; IR (KBr):1242, 1406, 1477, 1514, 1556, 1660, 3290 cm^{-1};

^{1}H-NMR (400 MHz, DMSO-d6): δ 1.25 (3H, d, *J* = 7.1 Hz), 2.00 (3H, s), 2.44-2.49 (2H, m), 3.04 (2H, t, *J* = 7.3 Hz), 3.78 (3H, s), 4.38 (1H, quin, *J* = 7.1 Hz), 6.94 (1H, t, *J* = 7.8 Hz), 6.95 (1H, d, *J* = 7.8 Hz), 7.17 (1H, td, *J* = 7.8, 1.6 Hz), 7.23 (1H, dd, *J* = 7.8, 1.6 Hz), 7.48 (4H, s), 8.24 (1H, d, *J* = 7.1 Hz); ^{13}C-NMR (100 MHz, DMSO-d6): δ 18.21, 23.92, 26.34, 34.56, 49.07, 55.67, 110.88, 119.31, 119.67, 121.12, 124.28, 126.58, 127.43, 134.21, 134.94, 156.32, 168.04,170.17, 170.97; MS (EI): m/z 415 (M^{+}); HRMS: calcd for $C_{21}H_{25}N_3O_4S$ 415.1566, found 415.1577

To a stirred solution of 4'-aminoacetanilide **31** (105 mg, 0.67 mmol) in CH_2Cl_2 were added *N*-Carbobenzoxyglycine **38** (138 mg, 0.67 mmol), EDC (255 mg, 1.33 mmol) and HOAt (181 mg, 1.33 mmol), and the resulting mixture was stirred at room temperature for 16 hr. The reaction mixture was diluted with 10 % HCl aq. and CH_2Cl_2, and organic phase was separated, the aqueous mixture was extracted with CH_2Cl_2. The organic phase and extracts were combined, dried over Na_2SO_4, and concentrated in vacuo.The residue was chromatographed on SiO_2 (CH_2Cl_2-MeOH = 100: 1) to give amide **39**.

[(4-Acetylaminophenylcarbamoyl)methyl]carbamic acid benzyl ester (**39**)
Yield 100 %; IR (KBr):1250, 1298, 1410, 1520, 1539, 1570, 1668, 1686, 1697, 3265, 3350 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d6): δ 2.00 (3H, s), 3.77 (2H, d, *J* = 6.3 Hz), 5.04 (2H, s), 7.24-7.37 (5H, m), 7.48 (4H, s), 7.52 (1H, t, *J* = 6.3. Hz), 9.85 (1H, s), 9.88 (1H, s); ^{13}C-NMR (400 MHz, DMSO-d6): δ 23.89, 44.01, 65.50, 119.39, 119.49, 127.74, 127.81, 128.37, 134.15, 134.85, 137.06, 156.60, 167.63, 167.99

To a stirred solution of amide **39** (100 mg, 0.293 mmol) in MeOH (10 mL) was added 20 % $Pd(OH)_2$/C, and the resulting suspension was stirred under a hydrogen atmosphere at 1 atm for 16 hr. The catalyst was removed by filtration and the filtrate was concentrated in vacuo to afford the amine, which was used for the next reaction without further purification. To a stirred solution of amine, obtained above, in CH_2Cl_2 (5 mL) were added EDC (112 mg, 0.586 mmol), DMAP (7 mg, 0.06 mmol) and carboxylic acid (64 mg, 293 mmol), and the resulting solution was stirred at room temperature for 18 hr. The reaction mixture was diluted with 10 % HCl aq. and CH_2Cl_2, and organic phase was separated, the aqueous mixture was extracted with CH_2Cl_2. The organic phase and extracts were combined, dried over Na_2SO_4, and concentrated in vacuo.The residue was chromatographed on SiO_2 (CH_2Cl_2-MeOH = 120: 1-110:1) to give amide **40A**, **B**.

N-[(4-Acetylaminophenylcarbamoyl)methyl]-3-(2-chlorophenylsulfanyl)propionamide

(**40A**)

Yield 41 %; mp: 212-213 °C; IR (KBr): 1254, 1406, 1516, 1574, 1651, 1680, 3306 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d6): δ 2.00 (3H, s), 2.56 (2H, t, *J* = 7.4 Hz), 3.20 (2H, t, *J* = 7.4 Hz), 3.87 (2H, d, *J* = 5.6 Hz), 7.19 (1H, td, *J* = 7.6, 1.5 Hz), 7.35 (1H, td, *J* = 7.6, 1.2 Hz), 7.40 (1H, dd, *J* = 7.6, 1.2 Hz), 7.44-7.47 (5H, m), 8.31 (1H, t, *J* = 5.8 Hz), 9.85 (2H, s); ^{13}C-NMR (100 MHz, DMSO-d6): δ 23.89, 26.78, 34.12, 42.69, 119.36, 119.59, 126.36, 127.09, 127.87, 129.51, 131.06, 134.04, 134.90, 135.63, 167.31, 167.97, 170.47; MS (EI): m/z 405 (M^{+}); HRMS: calcd for $C_{19}H_{20}{}^{35}ClN_3O_3S$ 405.0914, found 405.0909.

N-[(4-Acetylaminophenylcarbamoyl)methyl]-3-(2-methoxyphenylsulfanyl)-propionamide (**40B**)

Yield 29 %; mp: 199-201 °C; IR (KBr): 1242, 1518, 1541, 1576, 3277cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d6): δ 1.99 (3H, s), 2.48 (2H, t, *J* = 7.2 Hz), 3.06 (2H, t, *J* = 7.2 Hz), 3.80 (3H, s), 3.85 (2H, d, *J* = 5.9 Hz), 6.94 (1H, td, *J* = 7.7, 1.2 Hz), 6.97 (1H, d, *J* = 7.7 Hz), 7.17 (1H, td, *J* = 7.7, 1.6 Hz), 7.24 (1H, dd, *J* = 7.7, 1.2 Hz), 7.47 (4H, s), 9.83 (1H, s), 9.85 (1H, s); ^{13}C-NMR (100 MHz, DMSO-d6): δ23.89, 26.30, 34.62, 42.68, 55.64, 110.85, 119.35, 119.57, 121.09, 124.21, 126.55, 127.37, 134.04, 134.89, 156.28, 167.36, 167.97, 170.73; MS (EI): m/z 401 (M^{+}); HRMS: calcd for $C_{20}H_{23}N_3O_4S$ 401.1409, found 401.1407.

To a stirred solution of 2-methoxtphenethyl alcohol **16** (100 mg, 0.66 mmol) in CH_2Cl_2 (5 mL) was added Dess-Martin reagent (293 mg, 0.69 mmol) at 0 °C, and the resulting mixture was stirred at room temperature for 1 hr. The reaction mixture was washed with sat. $Na_2S_2O_3$ aq. and 10 % NaOH aq. and the organic phase was separated, dried over Na_2SO_4, and concentrated in vacuo to afford the aldehyde, which was used for the next reaction without further purification. To a stirred suspension of 60 % NaH (32 mg, 0.79 mmol) in THF (3 mL) was added triethyl phosphonoacetate (177 mg, 0.79 mmol) at 0 °C, and the resulting mixture was stirred at 0 °C for 30 min. The reaction mixture was added aldehyde, obtained above, in THF (3 mL) by cannula at 0 °C, and the resulting mixture was stirred at room temperature for 18 hr. The reaction mixture was diluted with AcOEt and H_2O, and organic phase was separated, the aqueous mixture was extracted with AcOEt. The organic phase and extracts were combined, dried over Na_2SO_4, and concentrated in vacuo.The residue was chromatographed on SiO_2 (Hexane-Acetone = 20: 1) to give ester **42**.

4-(2-Methoxy-phenyl)but-2-enoic acid ethyl ester (**42**)
Yield 100 %; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.26 (3H, t, *J* = 7.2 Hz), 3.51 (2H, dd, *J* = 7.2, 1.8 Hz), 3.82 (3H, s), 4.61 (2H, q, *J* = 7.2 Hz), 5.77 (1H, dt, *J* = 14.8, 1.8 Hz), 6.87 (1H, d, *J* = 7.8 Hz), 6.90 (1H, td, *J* = 7.8, 1.2 Hz), 7.10 (1H, d, *J* = 7.8 Hz), 7.11 (1H, dt, *J* = 14.8, 7.2 Hz), 7. 23 (1H, td, *J* = 7.8, 1.6 Hz); ^{13}C-NMR (400 MHz, $CDCl_3$): δ 14.03, 32.72, 55.02, 59.89, 110.18, 120.38, 121.57, 125.98, 127.84, 129.92, 147.11,157.04, 166.44

To a stirred solution of unsaturated ester **42** (199 mg, 0.90 mmol) in AcOEt (6 mL) was added 10 % Pd/C, and the resulting suspension was stirred under a hydrogen atmosphere at 1 atm for 16 hr. The catalyst was removed by filtration and the filtrate was concentrated in vacuo to afford ester, which was used for the next reaction without further purification. To a stirred solution of ester in MeOH (6 mL) and H_2O (2 mL) was added $LiOH \cdot H_2O$ (76 mg, 1.81 mmol), and the resulting mixture was reflused for 2 hr. The solvent was removed and the residue was added 10 % HCl aq. and AcOEt, and the organic phase was separated, and the aquoeus mixture was extracted with AcOEt. The organic phase and extracts were combined, dried over Na_2SO_4, and concentrated in vacuo to afford the carboxylic acid, which was used for the next reaction without further purification. To a stirred solution of carboxylic acid, obtained above, in CH_2Cl_2 (5 mL) was added CDI (220 mg, 1.36 mmol), and the resulting mixture was stirred at room temperature for 1 hr. The reaction mixture were added *N, O*-dimethylhydroxylammine (132 mg, 1.36 mmmol) and Et_3N (0.19 mL, 1.36 mmol),and the resulting mixture was stirred at room temperature for 18 hr.The reaction mixture was diluted with 10 % HCl aq. and CH_2Cl_2, and the organic phase was separated, and the aquoeus mixture was extracted with CH_2Cl_2. The organic phase and extracts were combined, dried over Na_2SO_4, and concentrated in vacuo.The residue was chromatographed on SiO_2 (Hexane-Acetone = 10: 1) to give amide **43**.

N-Methoxy-4-(2-methoxyphenyl)-*N*-methylbutyramide (**43**)
Yield 98 %; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.93 (2H, quint, *J* = 7.5 Hz), 2.45 (2H, t, *J* = 7.5 Hz), 2.67 (2H, t, *J* = 7.5 Hz), 3.17 (3H, s), 3.64 (3H, s), 3.81 (3H, s), 6.84 (1H, d, *J* = 7.4 Hz), 7.14 (1H, d, *J* = 7.4 Hz), 7.17 (1H, t, *J* = 7.4 Hz); ^{13}C-NMR (400 MHz, $CDCl_3$): δ 24.64, 29.80, 31.59, 32.27, 55.26, 61.19, 110.25, 120.39, 127.18, 130.02, 130.21, 157.57, 174.81

To a stirred solution of amide **43** (351 mg,1.48 mmol) in THF (6 mL) were added

butenyl magnesium bromide (3.698 mmol) in THF (6 mL) by cannula, and the resulting mixture was stirred at room temperature for 18 hr. The reactionwas quenched with sat. NH_4Cl aq. and diluted with AcOEt, and the organic phase was seperated, and the aquoeus mixture was extracted with AcOEt. The organic phase and extracts were combined, dried over Na_2SO_4, and concentrated in vacuo.The residue was chromatographed on SiO_2 (Hexane-Acetone = 30: 1) to give ketone **44**.

1-(2-Methoxyphenyl)oct-7-en-4-one (**44**)
Yield 100 %; ^{1}H-NMR (400 MHz, $CDCl_3$): 1.88 (2H, quint, *J* = 7.5 Hz), 2.31 (2H, q, *J* = 6.8 Hz), 2.42 (2H, t, *J* = 7.5 Hz), 2.49 (2H, t, *J* = 6.8 Hz), 2.62 (2H, t, *J* = 7.5 Hz), 3.81 (3H, s), 4.97 (1H, d, *J* = 10.4 Hz), 5.02 (1H, d, *J* = 17.0 Hz), 5.80 (1H, ddt, *J* = 17.0, 10.4, 6.8 Hz), 6.84 (1H, d, *J* = 7.7 Hz), 6.88 (1H, t, *J* = 7.7 Hz), 7.11 (1H, d, *J* = 7.7 Hz), 7.18 (1H, t, *J* = 7.7 Hz); ^{13}C-NMR (400 MHz, $CDCl_3$): δ 23.78, 27.70, 29.44, 41.63, 42.31, 55.14, 110.17, 115.05, 120.31, 127.14, 129.91, 137.21, 157.42, 210.27

To a stirred solution of ketone **44** (114 mg, 0.49 mmol) in 1, 4-dioxane (6 mL) and H_2O (2 mL) was added 2, 6-lutidine (0.11mL, 0.98 mmol) at room temperature, and the resulting mixture were added 2 % OsO_4 in H_2O (0.25 mL, 0.10 mmol) and $NaIO_4$ (420 mg, 1.96 mmol) at 0 °C, and the resulting mixture was stirred at room temperature for 4 hr. The reaction was quenched with hypo $NaHCO_3$ aq., and diluted with CH_2Cl_2, and organic phase was separated. The organic mixture was added 10 % HCl aq., and organic phase was separated, and the aquoeus mixture was extracted with CH_2Cl_2. The organic phase and extracts were combined, dried over Na_2SO_4, and concentrated in vacuo.The residue was chromatographed on SiO_2 (Hexane-Acetone = 20: 1) to give aldehyde. To a stirred solution of aldehyde in *tert*-BuOH (6 mL) were added $NaH_2PO_4 \cdot 2H_2O$ (766 mg, 4.91 mmol), 2-methyl-2-butene (1 mL, 9.81 mmol) and H_2O (2 mL) at room temperature, and the resulting mixture was added 70 % $NaClO_2$ (381 mg, 2.95 mmol) at 0 °C, and the resulting mixture was stirred at room temperature for 2 hr. The reaction was quenched with sat. $NaHSO_3$ aq. and 10 % HCl aq., and diluted with AcOEt, and organic phase was separated, and the aquoeus mixture was extracted with AcOEt. The organic phase and extracts were combined, dried over Na_2SO_4, and concentrated in vacuo.The residue was chromatographed on SiO_2 (Hexane-Acetone = 4: 1) to give carboxylic acid **45**.

7-(2-Methoxy-phenyl)-4-oxoheptanoic acid (**45**)
Yield 89 %; ^{1}H-NMR (400 MHz, $CDCl_3$): 1.89 (2H, quint, *J* = 7.3 Hz), 2.45 (2H, t, *J* =

7.3 Hz), 2.61 (2H, t, *J* = 6.3 Hz), 2.62 (2H, t, *J* = 7.3 Hz), 2.70 (2H, t, *J* = 6.3 Hz), 3.81 (3H, s), 6.84 (1H, d, *J* = 7.7 Hz), 6.87 (1H, t, *J* = 7.7 Hz), 7.10 (1H, dd, *J* = 7.7, 1.8 Hz), 7.18 (1H, td, *J* = 7.7, 1.8 Hz); ^{13}C-NMR (400 MHz, $CDCl_3$): δ 23.74, 27.76, 29.38, 36.75, 42.08, 55.16, 110.20, 120.33, 127.17, 129.79, 129.95, 157.40, 177.96, 209.06

To a stirred solution of carboxylic acid **45** (110 mg, 0.44 mmol) in THF (5 mL) were added ethyl chloroformate (0.06 mL, 0.66 mmol) and Et_3N (0.09 mL, 0.66 mmol), and the resulting mixture was stirred at room temperature for 1 hr. The reaction mixture was filtered off by Celite and the filtrate was dried over Na_2SO_4, and concentrated in vacuo to affoed the mixed acid anhydrate, which was used for the next reaction without further purification. To a stirred solution of anhydrate, obtained above, in CH_2Cl_2 (5 mL) were added 4'-aminoacetanilide **31** (66 mg, 0.44 mmol) and Et_3N (0.12 mL, 0.88 mmol), and the resulting mixture was stirred at room temperature for 18 hr. The reaction was quenched with 10 % HCl aq., and organic phase was separated, the aqueous mixture was extracted with CH_2Cl_2. The organic phase and extracts were combined, dried over Na_2SO_4, and concentrated in vacuo.The residue was chromatographed on SiO_2 (CH_2Cl_2-AcOEt-MeOH = 100: 50: 1) to give amide **46**.

7-(2-Methoxyphenyl)-4-oxoheptanoic acid (4-acetylaminophenyl)amide (**46**)
Yield 28 %; mp: 182-184 °C;IR (KBr):1244, 1404, 1516, 1558, 1656, 1701, 3302cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d6): δ 1.71 (2H, quint, *J* = 7.4 Hz), 1.99 (3H, s), 2.44 (2H, t, *J* = 7.4 Hz), 2.48-2.52 (4H, m), 2.68 (2H, t, *J* = 6.7 Hz), 3.75 (3H, s), 6.85 (1H, td, *J* = 7.7, 1.2 Hz), 6.92 (1H, d, *J* = 7.7 Hz), 7.10 (1H, dd, *J* = 7.7, 1.6 Hz), 7.16 (1H, td, *J* = 7.7, 1.6 Hz), 7.45 (4H, s), 9.85 (1H, s), 9.88 (1H, s); ^{13}C-NMR (400 MHz, DMSO-d6): δ 23.57, 23.87, 28.90, 29.94, 36.72, 41.45, 55.16, 110.56, 119.25, 119.33, 120.22, 127.22, 129.48, 129.61, 134.55, 134.60, 157.05, 167.90, 169.96, 209.33; MS (EI): m/z 382 (M^+); HRMS: calcd for $C_{22}H_{26}N_2O_4$ 382.4528, found 382.1894.

To a stirred solution of unsaturated ester **42** (169 mg, 0.77 mmol) in AcOEt (5 mL) was added 10 % Pd/C, and the resulting suspension was stirred under a hydrogen atmosphere at 1 atm for 16 hr. The catalyst was removed by filtration and the filtrate was concentrated in vacuo to afford the ester, which was used for the next reaction without further purification. To a stirred solution of ester in MeOH (6 mL) and H_2O (2 mL) was added $LiOH \cdot H_2O$ (64 mg, 1.54 mmol), and the resulting mixture was reflused for 18 hr. The solvent was removed and the residue was added 10 % HCl aq. and AcOEt, and the organic phase was separated, and the aquoeus mixture was extracted with

AcOEt. The organic phase and extracts were combined, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed on SiO_2 (Hexane-Acetone = 3: 1) to give carboxylic acid **47**.[67]

To a stirred solution of amide **33** (90 mg, 0.36 mmol) in MeOH (5 mL) was added K_2CO_3 (75 mg, 0.54 mmol), and the resulting mixture was stirred at room temperature for 2 hr. The reaction was quenched with H_2O and extracted with AcOEt, dried over Na_2SO_4, and concentrated in vacuo to afford the alcohol, which was used for the next reaction without further purification. To stirred solution alcohol, obtained above, in CH_2Cl_2 (4 mL) were added EDC (138 mg, 0.72 mmol), DMAP (4 mg, 0.04mmol) and carboxylic acid **22** (70 mg, 0.36 mmol), and the resulting mixture was stirred at room temperature for 18 hr. The reaction mixture was diluted with CH_2Cl_2 and quenched 10% HCl aq., and organic phase was separated, the aqueous mixture was extracted with CH_2Cl_2. The organic phase and extracts were combined, dried over Na_2SO_4, and concentrated in vacuo.The residue was chromatographed on SiO_2 (CH_2Cl_2-MeOH = 70: 1) to give amide **48**.

4-(2-Methoxyphenyl)butyric acid (4-acetylaminophenylcarbamoyl)methyl ester (**48**)
Yield 96 %; mp: 212-213 °C; IR (KBr):1242, 1408, 1510, 1585, 1665, 1744, 3069, 3244cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d6): δ 1.80 (2H, quint, *J* = 7.5 Hz), 2.38 (2H, t, *J* = 7.5 Hz), 2.59 (2H, t, *J* = 7.5 Hz), 3.76 (3H, s), 4.61 (2H, s), 6.86 (1H, t, *J* = 7.5 Hz), 6.93 (1H, d, *J* = 7.5 Hz), 7.13 (1H, dd, *J* = 7.5, 1.5 Hz), 7.17 (1H, td, *J* = 7.5, 1.5 Hz), 7.45 (2H, d, *J* = 8.7 Hz), 7.49 (2H, d, *J* = 8.7 Hz), 9.87 (1H, s), 9.99 (1H, s); ^{13}C-NMR (400 MHz, DMSO-d6): δ 23.90, 24.65, 28.79, 32.79, 55.18, 62.39, 110.60, 119.36, 119.73, 120.23, 127.35, 129.10, 129.75, 133.63, 135.11, 157.07, 165.18, 168.00, 172.48; MS (EI): m/z 384 (M^+); HRMS: calcd for $C_{21}H_{24}N_2O_5$ 384.4257, found 384.1693.

第三章第三節の実験

For the assay of compounds **26**, **27**, **30A**, **B**, **34A-H**, **37A**, **B**, **40A**, **B**, **46** and **48**, enzyme activities using 1.25 μg wild-type SRR in a final volume of 125 μL of 100 mM HEPES (pH 8.0), 10 μM PLP, 1 mM $MgCl_2$, 5 mM DTT, 1 mM ATP, 20 mM L-Ser, and 1 mM inhibitors. The reaction mixtures were incubated for 30 min at 37 °C. The reaction mixtures (25 μL) were further incubated in 100 mM HEPES (pH 8.0), 10 μM PLP, and recombinant Dsd1 in a final volume of 50 μL for 30 min at 30 °C. The reaction mixtures were mixed with 50 μL of 0.05 % DNP in 2 M HCl aq. and incubated for 5 min at 30 °C. To the reaction mixtures, 100 μL of EtOH and 125 μL of 10 M NaOH aq. were sequentially added and then the mixtures were incubated for 10 min at room temperature. Absorbance at 515 nm of the resultant hydrazine was measured. The activity of the inhibitors was evaluated with the percentage of the D-serine production compared with that without inhibitors.

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