経口投与可能な新規グルコキナーゼ活性化薬 TA-2395 の創製

２０２１年

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第１章　序論

第１節　２型糖尿病と治療薬の現状

糖尿病とは、生体内の血糖値を低下させる唯一のホルモンであるインスリンの作用の不足により生じる、慢性の高血糖を主徴とする代謝症候群である。インスリン作用不足は、膵 β 細胞の機能障害に起因するインスリン分泌の低下、および筋肉、肝臓、脂肪などの血糖を消費する組織におけるインスリン感受性の低下によって引き起こされる[1]。糖尿病は成因により１型糖尿病、２型糖尿病、その他の特定の機序・疾患による糖尿病、妊娠糖尿病の４つに分類される。このうち、主に中高年で発症し、遺伝因子・生活習慣を背景として発症する２型糖尿病が全体の約 95%を占めており、日本国内の成人の約 5 人に 1 人が糖尿病もしくは糖尿病予備軍と推計されている。また、慢性的な糖尿病罹患状態は網膜症、腎症、神経障害など合併症を引き起こし、糖尿病による死亡者は年間約１万 4,000 人に上っている[2]。

２型糖尿病患者の多くは、血糖値をコントールするために生活習慣の改善に加えて血糖降下薬による薬物治療を行っているが、単剤で十分な効果を得ることが難しい場合も多く、複数の薬剤を併用することによる薬物相互作用や、服薬コンプライアンスがよくない場合もあり問題となっている[3]。

以上のことから、2 型糖尿病は医療経済的かつ QOL（ = Quality of Life, 生活の質）向上の観点からも社会的課題の大きい疾患であり、新規作用機序を有する抗糖尿病薬の開発が強く望まれている。

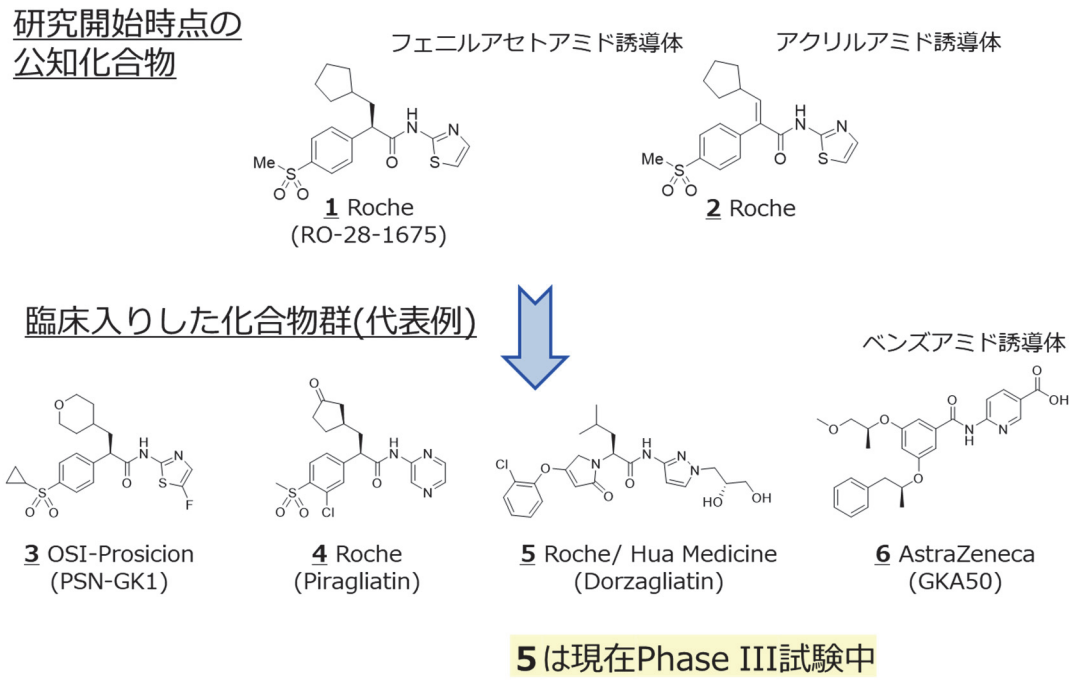
第２節　グルコキナーゼ活性化薬の現状と課題

グルコキナーゼ（GK）は、哺乳動物において見出される４つのヘキソキナーゼの中のひとつであり、グルコース代謝の第一段階であるグルコースをグルコース-6-リン酸へと変換する反応を触媒する。通常の状態では他のヘキソキナーゼに比べてグルコースとの親和性が低いのに対し、グルコース濃度が高い状態で酵素活性を示すことが特徴である。GK タンパクは膵 β 細胞・肝臓・脳など、血糖の調節に関わる組織に多く発現しており、生体内の役割として、血糖値の上昇に伴う肝実質細胞におけるグルコース消費とグリコーゲン産生、および膵 β 細胞でのグルコース応答性インスリン分泌作用が知られている[4,5]。以上の特徴から、肝と膵の両方に作用する GK 活性化薬の創出は、肝糖バランスの異常および膵インスリン分泌不全の両方を改善し、厳格な血糖コントロールを可能にする新しいタイプの血糖降下薬になり得ると考えられる（図表１－１）。

【図表１－１】　2 型糖尿病発症機序と GK 活性化薬の位置づけ

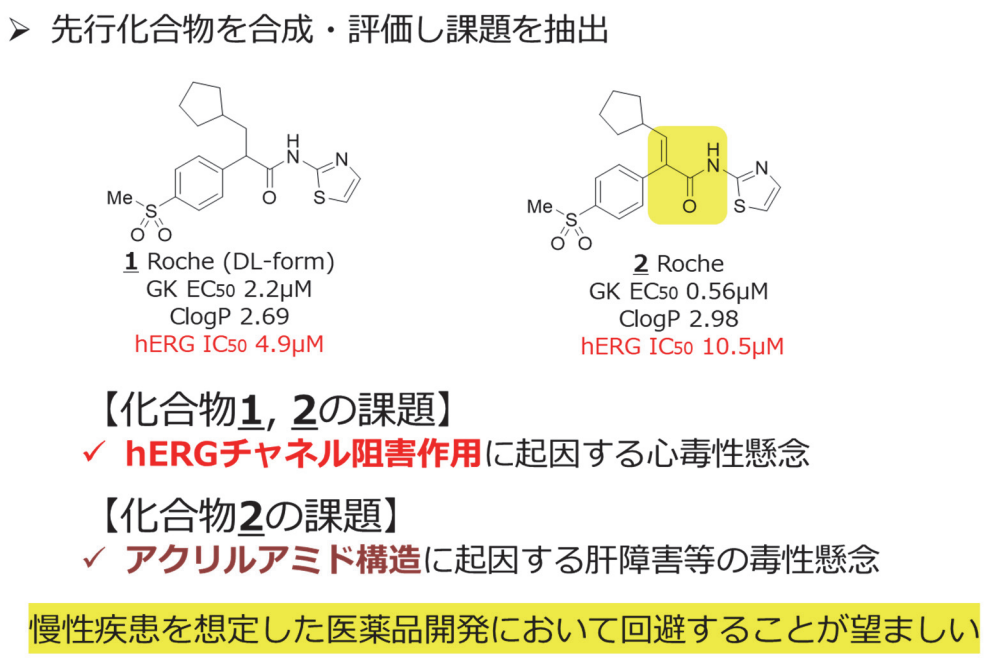
GK 活性化薬開発の現状と代表化合物を図表１－２に示す。著者が研究を開始した 2002 年当初、GK のアロステリック部位に作用する低分子として Roche 社が見いだした、フェニルアセトアミド誘導体 **1**[6]、アクリルアミド誘導体 **2**[7] が公知化合物として知られていた。その後、Roche 社の報告を端緒とした展開から化合物 **3**[8], **4**[9], **5**[10] に加え、AstraZeneca 社が見出したベンズアミド誘導体 **6**[11] などの複数の化合物が臨床段階に進んでいる [12,13]。

【図表１－２】　グルコキナーゼ活性化薬開発の現状と代表化合物



著者はまず先行化合物 **1, 2** を合成し、*in vitro* GK 活性化能を評価した（図表１－３）。その結果、EC_{50} 値で 1 μM 程度の活性を有する事が確認された。しかし、hERG（human Ether-a-go-go Related Gene）K^+チャネルについても同程度の阻害作用を有する事が判明した。hERG K^+チャネルの阻害は致死性の薬物障害性不整脈を誘発するリスクがあることが知られているため、これらの化合物には心毒性の副作用が懸念される [14]。また化合物 **2** はアクリルアミドの部分構造を有しており、生体内のシステインなどの求核性官能基と共有結合を形成することによる肝障害等の毒性が懸念される [15]。

【図表１－３】　先行化合物の課題



構造展開するにあたっての課題設定

著者は化合物 **2** を端緒化合物とし化学構造を展開するに当たり、アクリルアミド構造の回避と hERG K^+チャネル阻害能に影響を及ぼすと考えられる脂溶性の低減を改善課題として設定した。

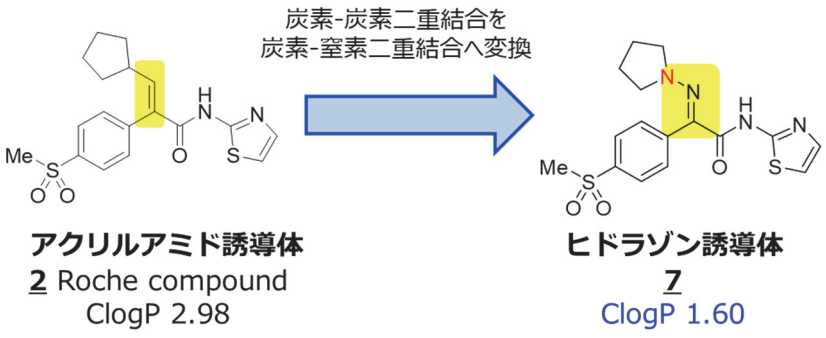
第２章　新規ヒドラゾン誘導体の合成研究

第１節　新規ヒドラゾン化合物のデザイン

第１章 第２節で述べた通り、著者は化合物 **2** を端緒化合物として設定し、アクリルアミド構造の変換と hERG K^+チャネル阻害回避を目指した脂溶性の低減が課題であると考えた。

そこで、これらを満たす変換として、アクリルアミド部位の炭素-炭素二重結合を炭素-窒素二重結合へと置き換えた新規ヒドラゾン誘導体 **7** をデザインした（図表２－１）。

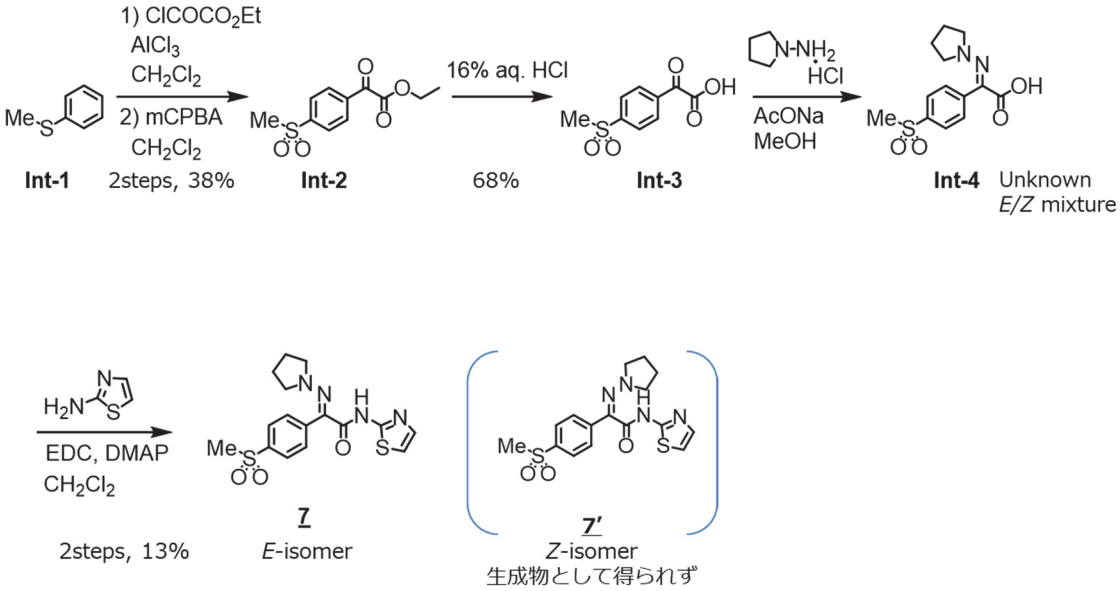
【図表２－１】　新規ヒドラゾン化合物 **7** デザイン



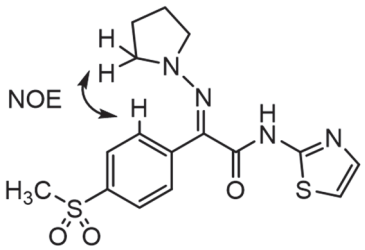
第２節　新規ヒドラゾン化合物の合成研究

まず、新規ヒドラゾン誘導体の端緒となる化合物 **7** の合成を実施した（図表２－２）。チオアニソール **Int-1** を出発原料として、シュウ酸モノエチルクロリドとの Friedel-Crafts 反応、続くスルホンへの酸化によりα-ケトエステル **Int-2** を合成した [16]。続いて、塩酸水による加水分解を経てα-ケト酸 **Int-3** に導いた後、1-アミノピロリジン塩酸塩と酢酸ナトリウムをメタノール中加熱下で作用させることでα-ヒドラゾンカルボン酸 **Int-4** の *E/Z* 混合物を得た。中間体 **Int-4** の *E/Z* 体は結晶化による分離が困難であったために精製は行わずに *E/Z* 混合物のまま 2-アミノチアゾールとの縮合反応を行い、目的の化合物 **7** へと導いた。この時の生成物は単一異性体として得られ、^{1}H NMR の NOESY 測定においてピロリジン 2 位水素原子とベンゼン環水素原子との間に NOE が観測されたことから、化合物 **7** を *E* 体と帰属した（図表２－３）。一方の *Z* 体のアミド化合物 **7'**が得られなかった理由は、ピロリジン残基の立体障害によりカルボン酸の反応性が低下したためと考察している。

【図表２－２】　ヒドラゾン化合物 **7** の合成



【図表２－３】　化合物 **7** の NOESY スペクトル測定結果

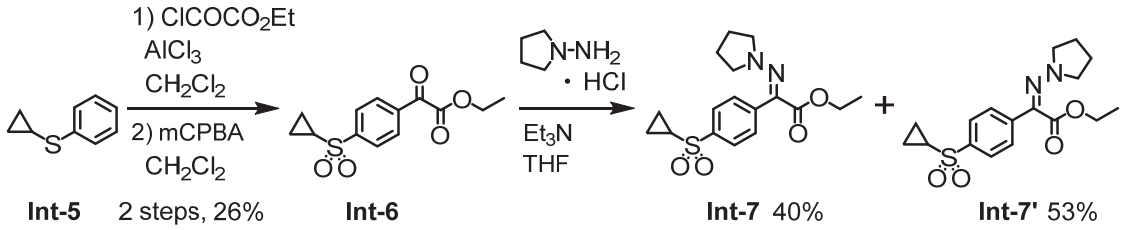


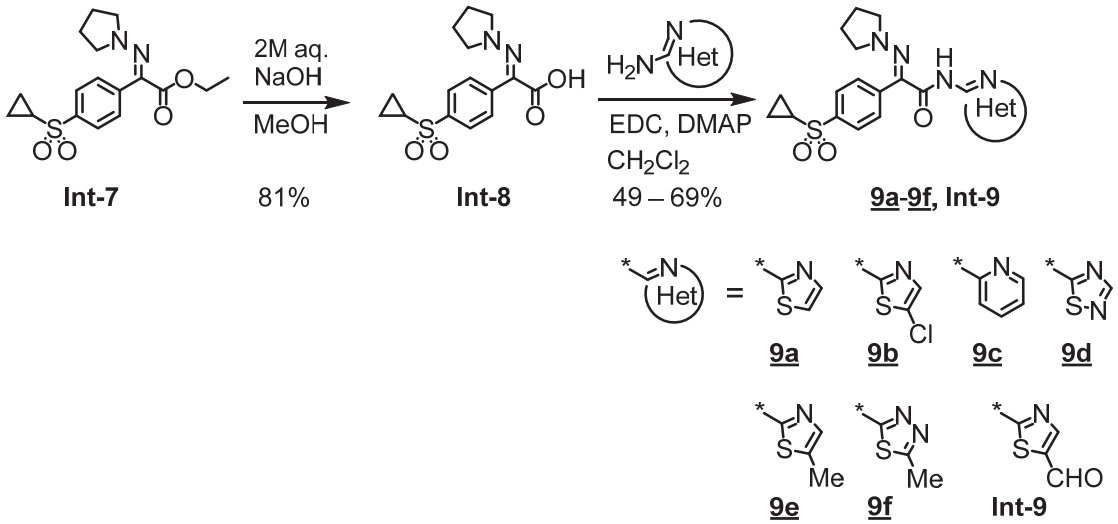
化合物 **7**　（*E*-isomer）

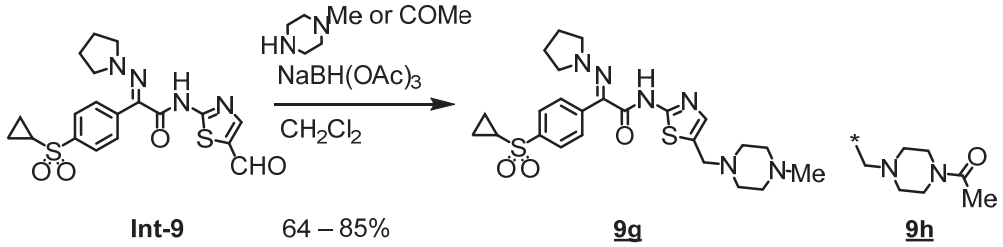
次に、シクロプロピルスルホニル基を有するヒドラゾン化合物 **9a–9h** の合成を実施した（図表２－４）。先の化合物 **7** の合成と同様の手法を用いて、シクロプロピルフェニルスルフィド **Int-5** から２工程でα-ケトエステル **Int-6** へと導いた[17]。ここで図表２－２に示したルートとは異なる手順により、まずα-ケトエステル **Int-6** へのヒドラゾン化を行なった。その結果、α-ヒドラゾンエステル **Int-7** は *E*/*Z* = 1/1 混合物を与えたが、両異性体はカラム精製で分離が可能であった。分離後のエステル中間体 **Int-7** はクロロホルム溶液を室温で撹拌することで *E*/*Z*=1/1 混合物の平衡状態となることから、*E*/*Z* 異性化が進行し易いことを確認した。

得られたα-ヒドラゾンエステル体 **Int-7** に対し塩基による加水分解を行う事で、α-ヒドラゾンカルボン酸 **Int-8** を単一の異性体として得た。この時、^{1}H NMR NOESY スペクトル測定を行い、ピロリジン 2 位水素原子とベンゼン環水素原子との間に NOE が観測されたことから *E* 体と帰属した（図表２－５）。続くアミド化、および還元的アミノ化反応を順次実施し、異性化を伴うことなく目的とする化合物 **9a**－**9h** へと導くことができた。

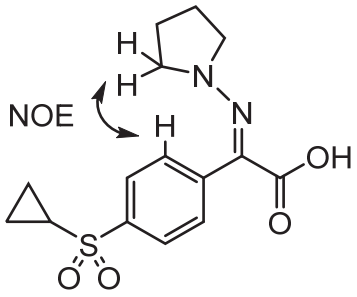
【図表２－４】　ヒドラゾン化合物 **9a**–**9h** の合成







【図表２－５】　中間体 **Int-8** の ^{1}H NMR NOESY スペクトル測定結果



Int-8（*E*-isomer）

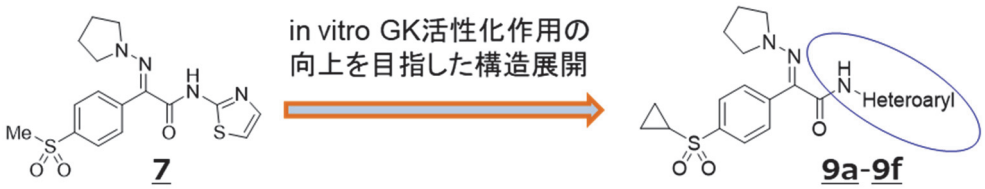
第３節　新規ヒドラゾン化合物のプロファイリング

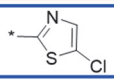
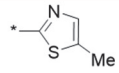
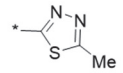
ヒドラゾン化合物の構造活性相関研究を行った結果を図表２－６に示す。

メチルスルホニル基を有する化合物 **7** の活性を評価した結果、EC_{50} = 5.6 μM の *in vitro* GK 活性化能を確認した。しかし、C57BL/6J マウスを用いた 10 mg/kg 経口投与による *in vivo* 薬効試験では血糖降下作用を見出すことが出来なかった。そこで、Bertram らの論文報告 [8a] を参考にメチルスルホニル基をシクロプロピルスルホニル基へと変換したところ、化合物 **9a** は *in vitro* 活性が約 10 倍向上し（EC_{50} = 0.42 μM）、マウス *in vivo* 血糖降下試験においても薬効が確認された。続くチアゾール環 5 位のクロロ基を導入した化合物 **9b** はさらに 10 倍もの GK 活性化能が向上した（EC_{50} = 0.04 μM）。他のヘテロ環への展開や置換基の検討として化合物 **9c**－**9f** を評価した。この中で、チアゾール環 5 位にメチル基を有する化合物 **9e** は *in vitro* GK 活性化能を維持しつつ、hERG 阻害能を回避する事を見出した。一般的に hERG 阻害能の回避は分子全体の脂溶性の低減や極性基の付与により達成するアプローチに加え、脂溶性を維持したまま置換基を導入する事で hERG タンパクへの相互作用を低減させる構造生物学的アプローチが知られている [18]。すなわち、ヒドラゾン化合物 **9e** におけるチアゾール環５位へのメチル基置換効果は、チアゾール５位クロロ置換化合物 **9b** に比べて ClogP 値の

変化が軽微であることから、hERG タンパクへの阻害能の回避を明確に説明することが難しいが、構造生物学的要因による改善ではないかと考えている。

【図表２－６】　ヒドラゾン化合物の構造活性相関研究－１



Compound	Heteroaryl	ClogP	GK activation EC_{50} (μM) [a]		Maximum blood glucose reduction [b]		Solubility in buffer (μg/mL) [d]	hERG inhibition IC_{50} (μM)
7		1.60	5.60		N.A.[c]		5.4	N.D.[e]
9a		2.19	0.42	10倍向上	-36%	in vivo活性を確認	2.4	17.5
9b		2.95	0.04	10倍向上	-37%		0.1	16.7
9c		2.35	2.40		-19%		0.9	N.D.[e]
9d		1.42	0.16		-45%		7.7	5.3
9e		2.69	0.22	活性維持	-32%		1.2	>100 hERG阻害回避
9f		1.60	1.17		-41%		6.5	>100

a) EC_{50} was measured at 5 mM glucose.

b) C57BL/6J fasted mice, 10 mg/kg, p.o. vs vehicle control.

c) N.A. = Not active.

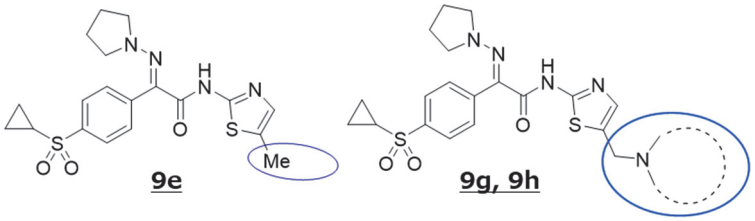
d) Solubility in pH 6.5 phosphate buffer was determined.

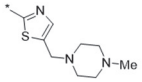
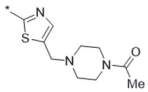
e) N.D. = Not determined.

チアゾール環 5 位へのメチル基導入による hERG 阻害能の回避に着目し、更なる検討を行なった。著者は 5 位メチル基の先に極性基を許容するスペースがあると仮定し、hERG 阻害を回避しつつ脂溶性低減による水溶性の向上を指向し

たピペラジルメチル基の導入を検討した（図表２－７）。その結果、化合物 **9g**, **9h** ともに期待した通り GK 活性化能を維持しつつ、水溶性が大幅に向上することを見出した。特にアセチルピペラジルメチル基を有する化合物 **9h** については、*in vitro* 活性とともにマウス *in vivo* 血糖降下作用を維持しつつ、水溶性向上と hERG 阻害回避を両立することが出来た。

【図表２－７】　ヒドラゾン化合物の構造活性相関研究－２



Compound	Heteroaryl	ClogP	GK activation EC_{50} (μM) [a]	Maximum blood glucose reduction [b]	Solubility in buffer (μg/mL) [d]	hERG inhibition IC_{50} (μM)
9e		2.69	0.22	-32%	1.2	>100
9g		2.50	0.70	-41%	345.0	16.9
9h		1.52	1.92	-42%	78.0	>100

水溶性の大幅向上

in vitro/in vivo活性を維持しつつ水溶性向上とhERG阻害回避を両立した

a) EC_{50} was measured at 5 mM glucose.

b) C57BL/6J fasted mice, 10 mg/kg, p.o. vs vehicle control.

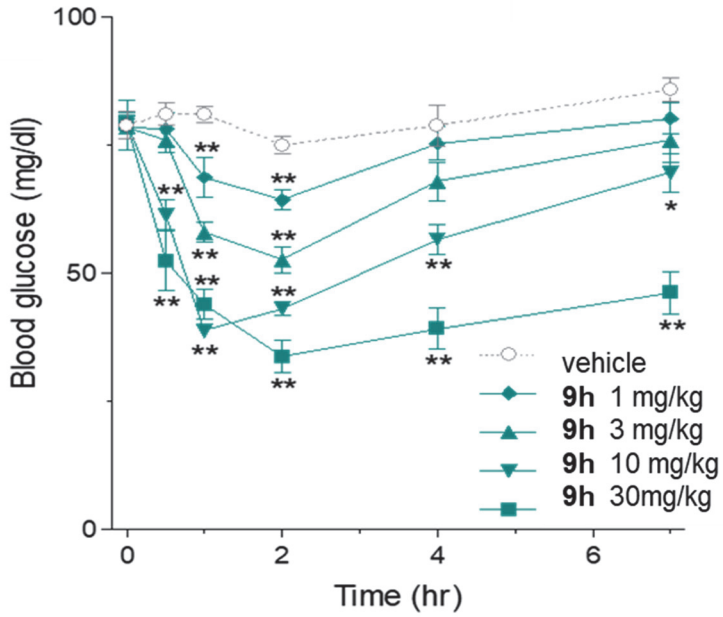
c) N.A. = Not active.

d) Solubility in pH 6.5 phosphate buffer was determined.

e) N.D. = Not determined.

化合物 **9h** のさらなる薬効プロファイルの取得を目的として、絶食下 Sprague-Dawley（SD）ラットを用いた血糖降下作用について用量依存性試験を実施した（図表２－８）。その結果、1, 3, 10, 30 mg/kg の各投与群において、投与 1-2 時間後に用量依存的かつ有意な血糖降下作用を示した。特に、高用量の 10, 30 mg/kg 投与群においては、投与 7 時間後も持続的な血糖降下作用を示した。以上の結果から、本化合物 **9h** は十分な経口活性を有し、かつ hERG 阻害による心毒性懸念が低いことから、有望な開発品候補化合物であると考えた。

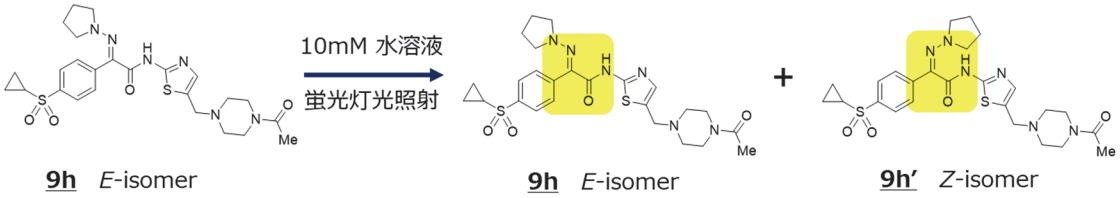
【図表２－８】　正常ラット血糖降下作用試験



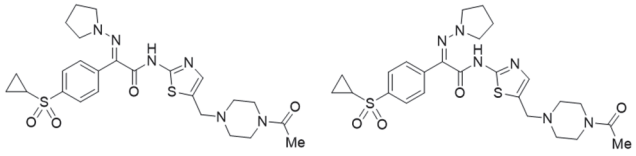
Effect of **9h** on blood glucose level in overnight fasted Sprague-Dawley (SD) rats. 9h or vehicle (10% Gelucire) was orally administered to overnight fasted SD rats at 0 hour. Values are mean ± S.E.M. (n=5). **P<0.01, *P<0.05 vs. control (Dunnett's method)

しかしながら、ヒドラゾン化合物 **9h** の物理化学的性質を検討する中で、光照射下の水溶液中で炭素-窒素二重結合部位の *E*/*Z* 異性化が進行する事が判明した[19]（図表２－９）。この異性化は重アセトニトリル溶液を用いた ^{1}H NMR の経時的変化においても観測され、72 時間後に 8%の *Z* 体への異性化が確認された（図表２－１０）。

【図表２－９】　光照射下の水溶液中における化合物 **9h** の *E*/*Z* 異性化



【図表２－１０】　重アセトニトリル溶液中の化合物 **9h** 光安定性の検証[20]



照射時間 (hr)	累計照射時間 (hr)	積分値		
		A: *E*-isomer CH (δ_H 7.53)	B: *Z*-isomer CH (δ_H 7.65)	A/B [%]
-	-	-	-	-
-	-	2.00	-	-
1	1	2.00	-	-
6	7	2.00	0.05	97.6/ 2.4
17	24	2.00	0.11	94.8/ 5.2
24	48	2.00	0.15	93.0/ 7.0
48	96	2.00	0.18	91.7/ 8.3
72	168	2.00	0.18	91.7/ 8.3

以上の性質は、ヒドラゾン化合物 **9h** を医薬品として開発するにあたり生体内での安定性について不明確な点が多いことが懸念された。また、固体状態の安定性においても不安視された。これらのことを総合的に勘案し、ヒドラゾン化合物の開発に向けたさらなる検討を断念した。

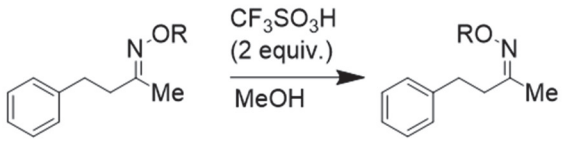
第３章　新規オキシム誘導体の合成研究

第１節　新規オキシム化合物のデザイン

前章で述べたように、ヒドラゾン誘導体では光照射下の溶液状態で顕著な *E*/*Z* 異性化が確認された。そこで炭素-窒素二重結合の *E*/*Z* 異性化が進行しづらいと報告されている *O*-アルキルオキシム誘導体（以下、オキシムと記載）を次の候補として考案した[21,22]（図表３－１）。

【図表３－１】　オキシム *E*/*Z* 異性化に関する文献情報[23]

オキシム**ref-1a**の*anti* / *syn*異性化が速やかに進行する一方、
メチルオキシム**ref-1b**の*anti* / *syn*異性化は観測されなかった

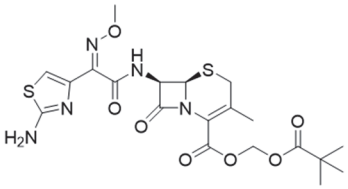


ref-1a/b *anti*　　**ref-2a/b** *syn*

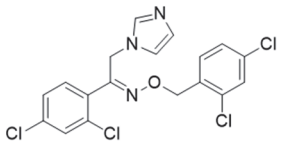
	R	Time	*anti/syn*
ref-1a / **ref-2a**	H	<20 min	2 : 1
ref-1b / **ref-2b**	Me	12 h	100 : 0

また、オキシム骨格を有する低分子化合物はすでに多くの医薬品が製品化されている事から、生体内での安全性が確認されている部分構造であるといえる（図表３－２）。加えて、ヒドラゾン誘導体で得た構造活性相関をオキシム誘導体でも同様に利用できるメリットもあると考え、化合物 **8a** をデザインした（図表３－３）。

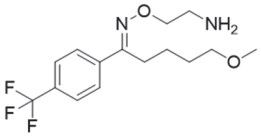
【図表３－２】　オキシム骨格を有する医薬品例 [24-30]



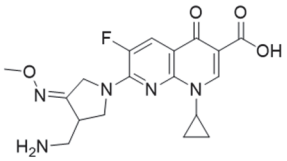
Cefetamet (Roche)
Launched



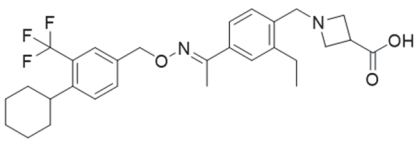
Oxiconazole (Roche)
Launched



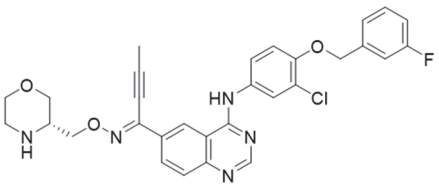
Fluvoxamine (AbbVie)
Launched



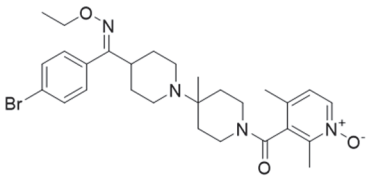
Gemifloxacin (LG Chem)
Launched



Siponimod (Novartis)
Launched

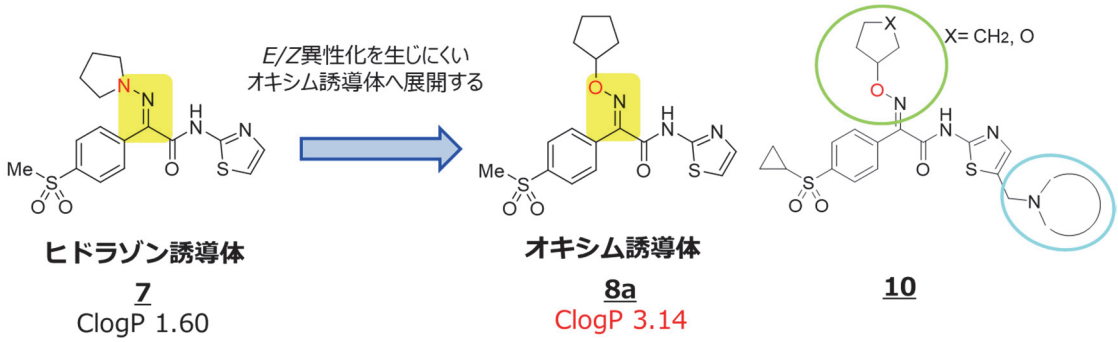


Epertinib (Shionogi)
Phase I/II



Ancriviroc (Merck)
Phase I

【図表３－３】　オキシム化合物のデザイン



【新規オキシム化合物**8a**の課題】

- 分子全体の脂溶性が高いことに起因する毒性懸念

脂溶性低減策としてテトラヒドロフラニル基の導入を検討する(**10**)

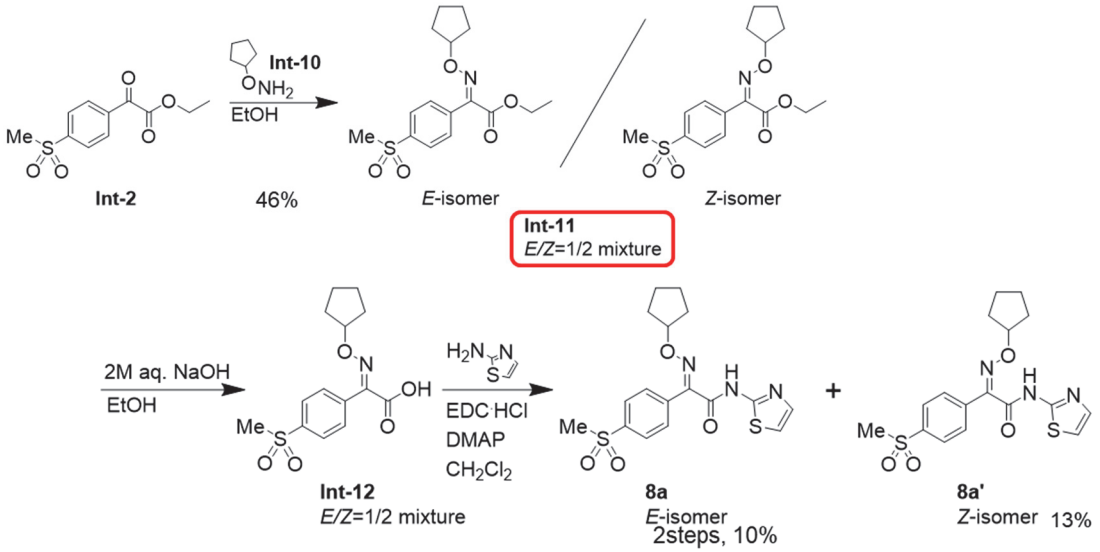
一方、化合物 **8a** のデザイン時点の懸念として、脂溶性が高いことに起因する難水溶性や hERG K^+チャネル阻害が挙げられた。そのため、脂溶性低減策として、シクロペンチル環の炭素原子を酸素原子に置換したテトラヒドロフラニル基を導入することとした（化合物 **10**）。

第２節　新規オキシム化合物の合成研究

まず、オキシム誘導体の端緒となる化合物 **8a** を合成した（図表３－４）。

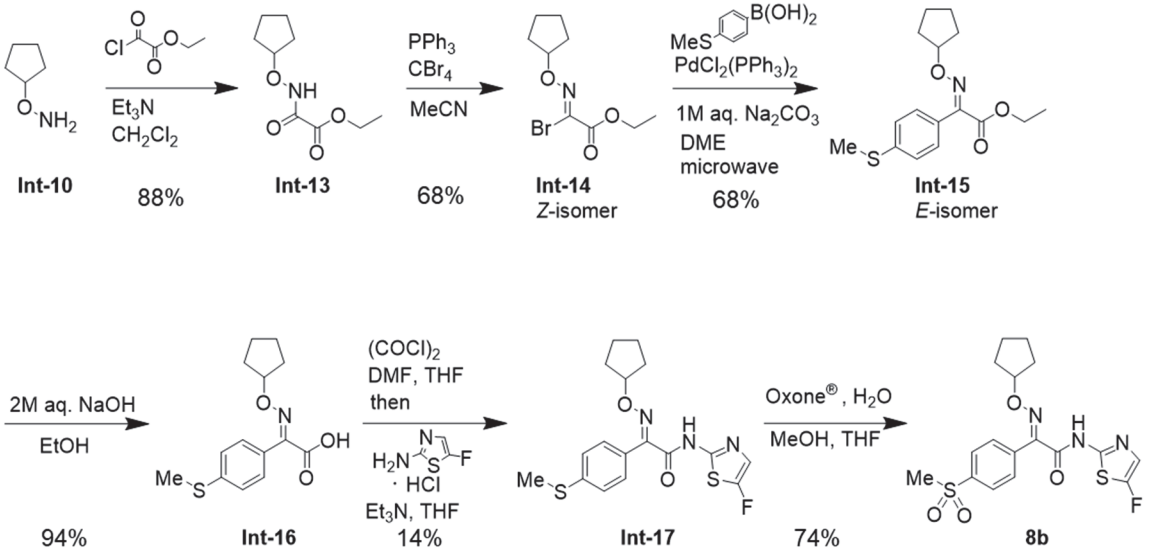
すでに第２章で合成したα-ケトエステル **Int-2** に対してシクロペンチルオキシアミンをエタノール中、室温で作用させることでオキシム化が進行し、幾何異性体の 1/2 混合物を与えた。この時の幾何異性体は *O*-メチルオキシムエステルの合成報告例を参考に *Z* 体が主生成物であると帰属した[21,31]。中間体 **Int-11** は *E*/*Z* 異性体混合物のまま次工程の加水分解、およびアミド化反応を行い目的の *E* 体 **8a** を 2 工程 10%の収率で得ると同時に、*Z* 体 **8a'**についても 13%の収率で得た。このままでは化学効率が低いため、次に *E* 体を選択的に取得する合成方法を検討した。

【図表３－４】　オキシム化合物 **8a** の合成



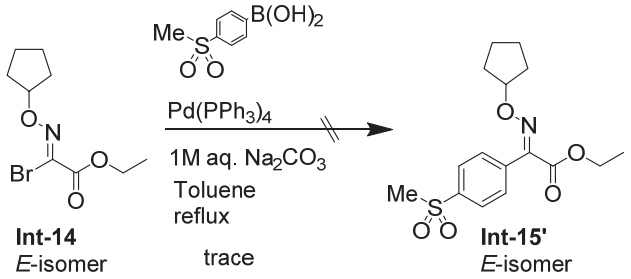
続いて、化合物 **8b** の合成法を図表３－５に示す。シクロペンチルオキシアミン **Int-10** とシュウ酸モノエチルクロリドを反応させて得た中間体 **Int-13** に対して、トリフェニルホスフィン/四臭化炭素をアセトニトリル溶媒中、加熱下で反応させることにより、α位にブロモ基を有するオキシムエステル **Int-14** を単一生成物として得た。この中間体 **Int-14** の幾何異性は、参考文献情報と反応機構から目的の *Z* 体であると帰属した [21,32]。続く 4-メチルチオフェニルボロン酸とパラジウム触媒を用いる鈴木カップリングは立体選択的に反応が進行し、α-オキシムアリール酢酸エステル **Int-15** を得た [33]。次に塩基による加水分解、アミド化を行い、最後にオキソンを用いてスルフィド部分をスルホンへ酸化することにより目的の化合物 **8b** を得た。

【図表３－５】　オキシム化合物 **8b** の合成



ただし、この合成方法は目的の幾何異性である *E* 体を選択的に得るためには有効であったが、用いるボロン酸によっては反応性が著しく低下し、特にスルホニル基などの電子求引性基を有するボロン酸から中間体 **Int-15'**を得ることが出来ないなど、基質一般性に乏しい事が改善課題となった（図表３－６）。

【図表３－６】　電子求引性基を有するボロン酸を用いた **HS-15'**の合成

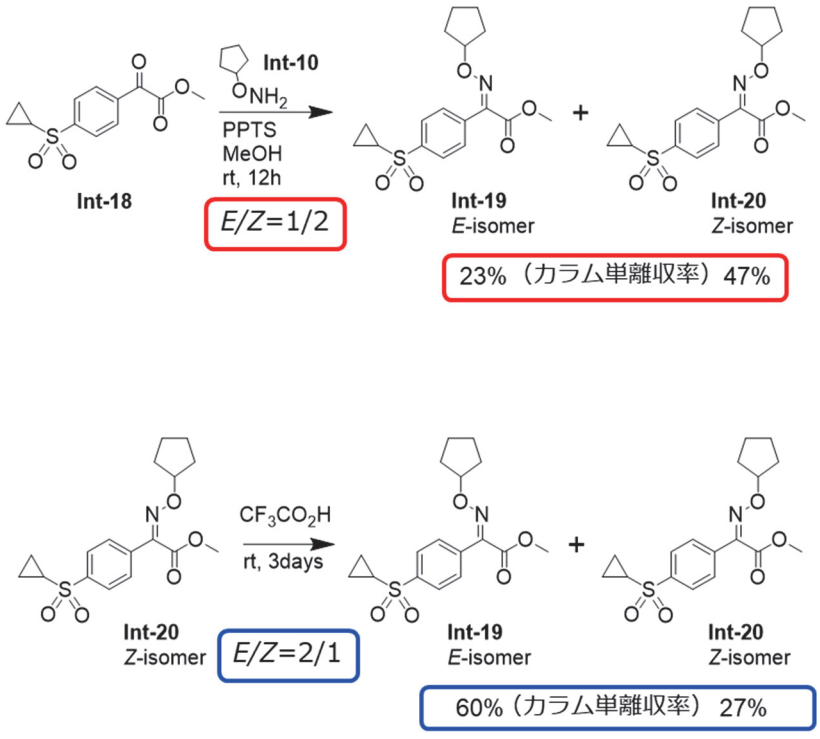


そこで、オキシム体の *E/Z* 混合物を基質とした異性化反応により *E* 体を主として与えるように検討することとした。

まず、エステル中間体 **Int-20** および **Int-26** の *E/Z* 異性化を検討した（図表３－７，８）。α-ケトエステル **Int-18** に対してシクロペンチルオキシアミン **Int-10** を作用させるオキシム化は、約 1/2 の比で *Z* 体を主生成物として与えた。α-オキシムエステル **Int-19** および **Int-20** はヒドラゾン誘導体に比べて非常に安定であ

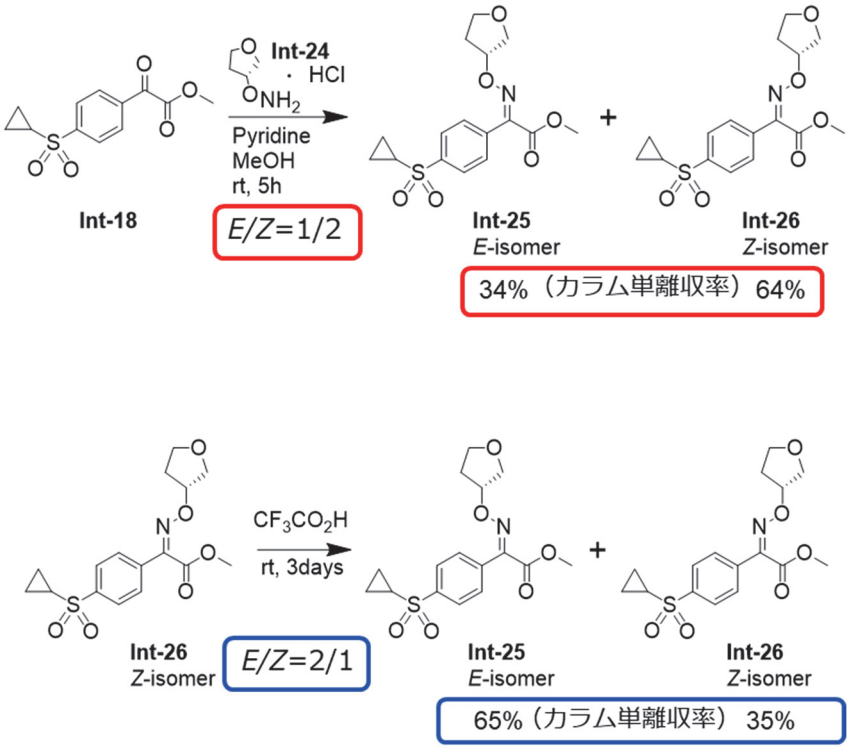
り、中性条件での異性化は進行しなかった。一方、トリフルオロ酢酸を溶媒として用いる条件においては異性化が進行し、室温 3 日間で *E/Z* 比が 2/1 の平衡状態に達することを見出した。これは、最初のオキシム化反応は速度論的支配により *Z* 体を優先的に与え、酸性溶液中の *E/Z* 異性化においては熱力学的に安定な *E* 体へと平衡が移動した結果であると考察している。

【図表３－７】　オキシム *E/Z* 異性化の検討 - 1



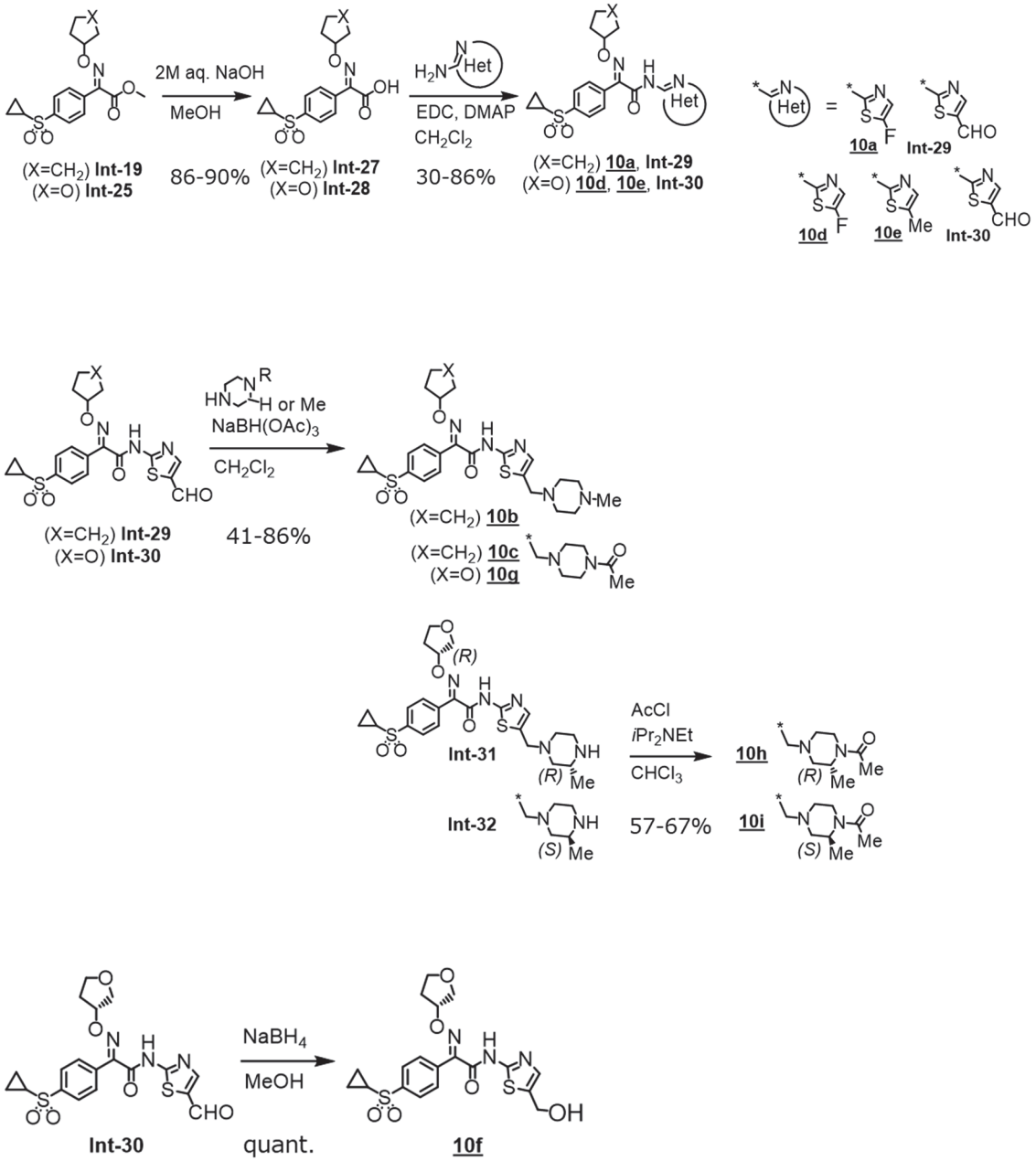
この酸性条件による異性化は、用いるシクロペンチルオキシアミンをテトラヒドロフラニルオキシアミンへと変更しても同様な結果が得られていることから、一般性の高い *E*-オキシム体の合成方法を確立することが出来たと考えている（図表３－８）。

【図表３－８】　オキシム *E/Z* 異性化の検討 - ２



得られた *E*-オキシムエステル体 **Int-19**, **Int-25** に対して、塩基による加水分解、アミド化反応、および還元的アミノ化反応を順次おこない、目的の化合物 **10a-10i** へとそれぞれ導いた（図表３－９）

【図表３－９】　オキシム化合物 **10a–10i** の合成

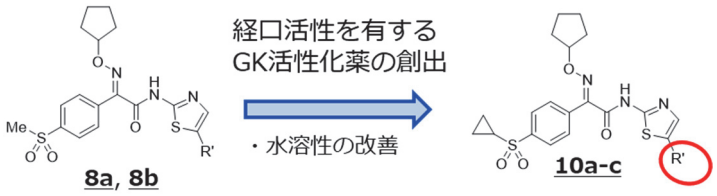


第３節　新規オキシム化合物のプロファイリング

オキシム誘導体の構造活性相関研究を行った（図表３－１０）。

メチルスルホニル基を有する化合物 **8a** の活性を評価した結果、EC_{50}＝0.81 μM の *in vitro* GK 活性化能を確認したが、C57BL/6J マウスへの経口投与による *in vivo* 薬効試験では血糖降下作用を見出せなかった。本オキシム系統においてもヒドラゾン化合物と同様の構造活性相関研究が可能と考え、メチルスルホニル基をシクロプロピルスルホニル基へと変換した化合物 **10a** を評価した結果、EC_{50}＝0.07 μM の非常に強い *in vitro* GK 活性化能を有し、正常マウスへの 10 mg/kg 経口投与において強力な血糖降下作用を示すことを見出した。続くチアゾール環 5 位にアセチルピペラジルメチル基を有する化合物 **10b**, **10c** を評価したところ、水への溶解性は向上した一方、hERG 阻害作用の軽減は見られなかった。前述のように、一般に hERG 阻害作用は分子の脂溶性の影響を受けやすいことが知られており [18]、オキシム誘導体においては分子全体の脂溶性がヒドラゾン誘導体に比べて高い事が原因と考察した。

【図表３－１０】　オキシム化合物の構造活性相関研究－１



Compound	Thiazole-5-substituent	ClogP	GK activation EC_{50} (μM) [a]	Maximum blood glucose reduction [b]	Solubility in buffer (μg/mL) [d]	hERG inhibition IC_{50} (μM)
8a	H	3.14	0.81	N.A.[c]	N.D.[e]	N.D.[e]
8b	F	3.33	0.72	-27%	0.3	6.5
10a	F	3.91	0.07	-39%	0.4	6.5
10b	N-Me	4.03	0.20	-34%	51.0	1.8
10c	N-C(=O)-Me	3.05	0.42	-30%	17.0	3.9

in vitro活性10倍向上

水溶性向上

hERG阻害作用は減弱せず

ヒドラゾン誘導体**9h**に比べて脂溶性が高い事が原因と考察

a) EC_{50} was measured at 5 mM glucose.

b) C57BL/6J fasted mice, 10 mg/kg, p.o. vs vehicle control.

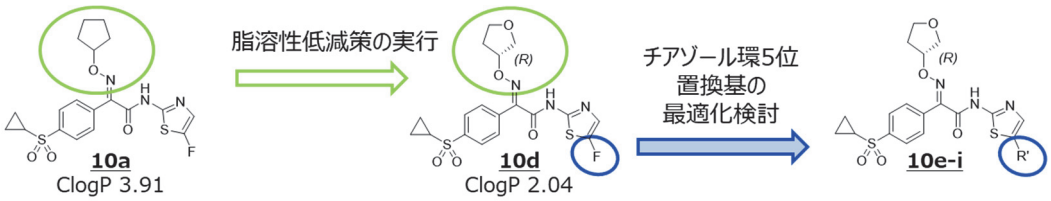
c) N.A. = Not active.

d) Solubility in pH 6.5 phosphate buffer was determined.

e) N.D. = Not determined.

hERG 阻害を目指したさらなる脂溶性低減策として、シクロペンチル基 **10a** をテトラヒドロフラニル基 **10d** へと変更した（図表３－１１）。両エナンチオマーを比較検討した結果、(*R*)体が活性を維持する一方、(*S*)体は活性が約 1/5 程度減弱することが判明した。また、(*R*)体 **10d** は C57BL/6J マウス *in vivo* 薬効においても強力な血糖降下作用を見出し、溶解度についても若干の向上傾向が見られた。引き続き、ヒドラゾン誘導体における構造活性相関の結果（図表２－７）から極性基導入が許容されると考えられるチアゾール環 5 位置換基の最適化検討を、(*R*)-テトラヒドロフラニル基に固定しておこなった。5 位メチル基 **10e** はヒドラゾン誘導体の結果とは異なり hERG 阻害の軽減効果は見出せなかった。5 位ヒドロキシメチル基 **10f** およびアセチルピペラジルメチル基 **10g** は大幅な水溶性の向上と hERG 阻害能の回避を見出したが、マウス *in vivo* 血糖降下作用がやや低下する結果となった。最終的にピペラジン 2 位へメチル基を導入した化合物 **10i** において、良好な *in vitro* / *in vivo* 薬効と hERG 阻害回避の両方を満たす化合物を取得することができた。

【図表３－１１】　オキシム化合物の構造活性相関研究－２



Compound	Thiazole-5-substituent	ClogP	GK activation EC_{50} (μM) [a]	Maximum blood glucose reduction [b]	Solubility in buffer (μg/mL) [d]	hERG inhibition IC_{50} (μM)	
10a		3.91	0.07	-39%	0.4	6.5	
10d	*(R)*-form	2.04	0.25	-44%	5.6	7.4	テトラヒドロフラン環の立体化学とGK活性 ・*(R)*体は活性維持 ・*(S)*体は約5倍減弱
10d'	*(S)*-form	2.04	1.19	-34%	N.D.[e]	4.3	
10e	Me	2.35	0.37	-45%	4.0	3.8	
10f	OH	0.81	0.75	-26%	233.0	29.4	アセチルピペラジルメチル基の導入**10g** ・高い水溶解性 ・hERG阻害作用の減弱
10g		1.18	0.79	-36%	507.0	20.4	
10h		1.70	1.24	-28%	N.D.[e]	N.D.[e]	
10i (TA-2395)		1.70	0.55	-46% in vivo活性向上	112.0	23.5	メチル基の導入**10i** ・in vivo活性が良好

a) EC_{50} was measured at 5 mM glucose.

b) C57BL/6J fasted mice, 10 mg/kg, p.o. vs vehicle control.

c) N.A. = Not active.

d) Solubility in pH 6.5 phosphate buffer was determined.

e) N.D. = Not determined.

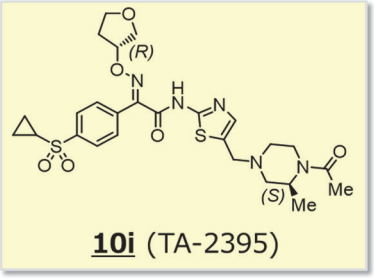
次に化合物 **10i** の高次評価をおこなった（図表３－１２）。

肝細胞および膵 β 細胞を用いた *in vitro* 細胞機能評価試験においては、膵 β-細胞におけるインスリン分泌促進作用（EC_{50} = 0.43 μM）および肝細胞におけるグルコースリン酸化促進作用（EC_{50} = 0.53 μM）をそれぞれ確認することが出来た。これにより化合物 **10i** が肝臓と膵臓の両方に作用しうる GK 活性化薬である性質を有していることを *in vitro* 結果として確認出来た。

薬物動態試験においては、ラットおよびヒト肝ミクロソームにおける代謝安定性が良好であること、タンパク結合率 90%で種差が大きくないことを確認した。さらに、ラット PK 試験の結果、経口剤として十分な分布容積、クリアランス、経口吸収性（バイオアベイラビリティ）43%と良好な値であることを確認した。さらに、化合物 **10i** はげっ歯類およびイヌの毒性試験において薬効量と毒性量に十分な安全域を有することを確認した。

これら非臨床試験成績を総合的に鑑みて、化合物 **10i** は臨床試験候補化合物（開発品番号 TA-2395）として選出された。

【図表３－１２】　オキシム化合物 **10i** プロファイリング



10i (TA-2395)

<in vitro細胞評価試験>

- マウスMIN6細胞 (膵β細胞インスリン分泌促進作用)
  EC_{50} 0.43 μM
- ラット肝細胞 (グルコースリン酸化促進作用)
  EC_{50} 0.53 μM

肝細胞および膵β細胞それぞれ薬効を確認した

<薬物動態試験>

- 肝ミクロソーム代謝安定性試験 (μL/min/mg) ラット: 28, ヒト: 83
- 血漿タンパク結合率 ラット: 93%, ヒト: 89%
- ラットPK試験

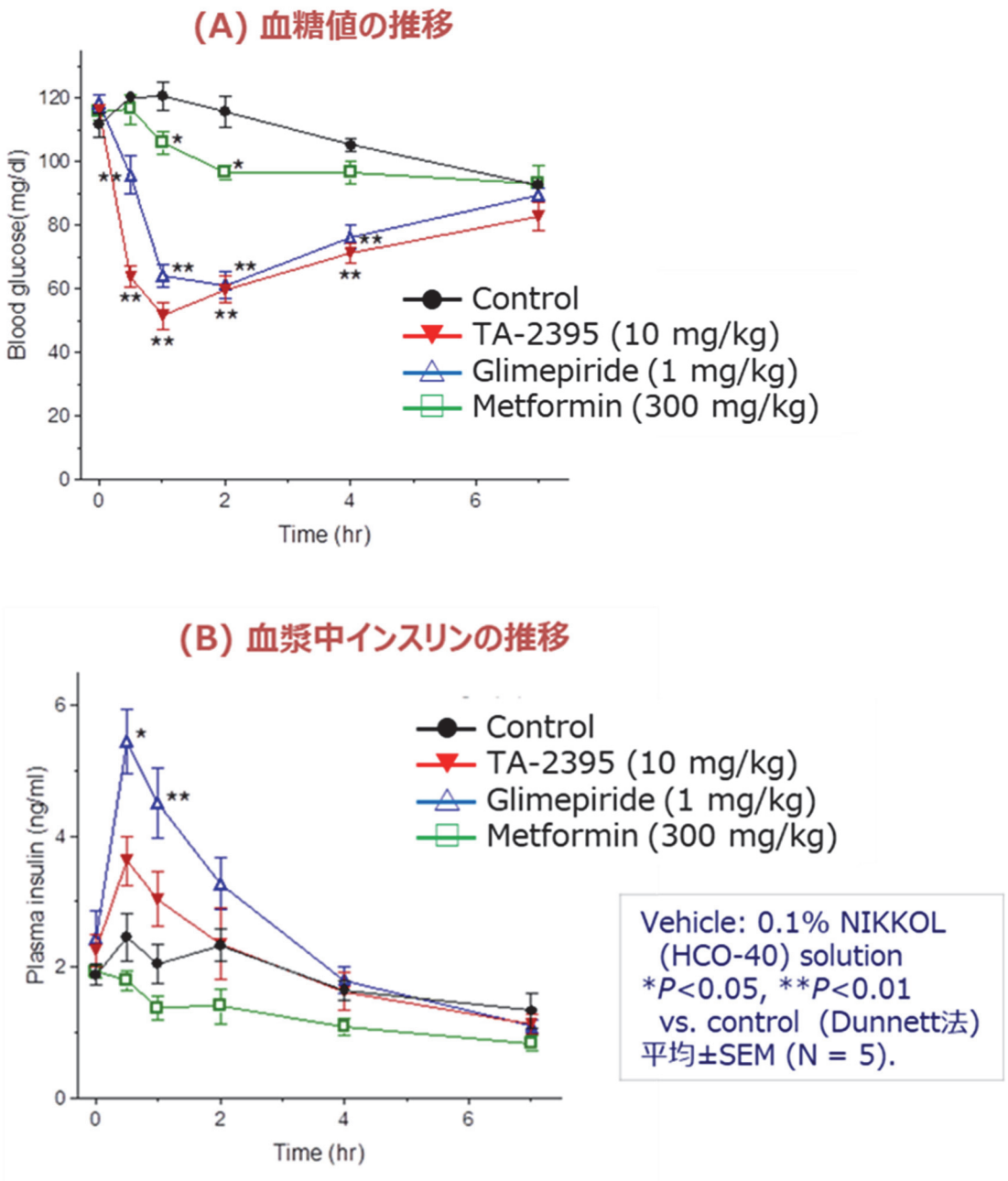
i.v. **3 mg/kg**	CL_{tot}: 1455 mL/hr/kg	Vd_{ss}: 1607 mL/kg	$AUC_{0\text{-}inf}$: 2.08 μg・hr/mL	$t_{1/2}$: 2.0 hr
p.o. **3 mg/kg**	t_{max}: 0.4 hr	C_{max}: 0.74 μmol/L	$AUC_{0\text{-}inf}$: 0.91 μg・hr/mL	BA: 43%

第４節　オキシム化合物 TA-2395(**10i**)のプロファイリング

オキシム化合物 **10i**（TA-2395）の GK 活性化薬としてのコンセプト検証を目的としたラット薬効試験を実施した（図表３－１３）。摂食下 Sprague-Dawley ラットへ TA-2395（10 mg/kg）の経口投与群または control 群と共に、対照薬群としてインスリン分泌促進型の SU 剤であるグリメピリド（1 mg/kg）、およびインスリン非依存型のビグアナイド剤であるメトホルミン（300 mg/kg）の経口投与群を設定し、血糖値の推移と血漿中インスリンの推移をそれぞれ比較した。

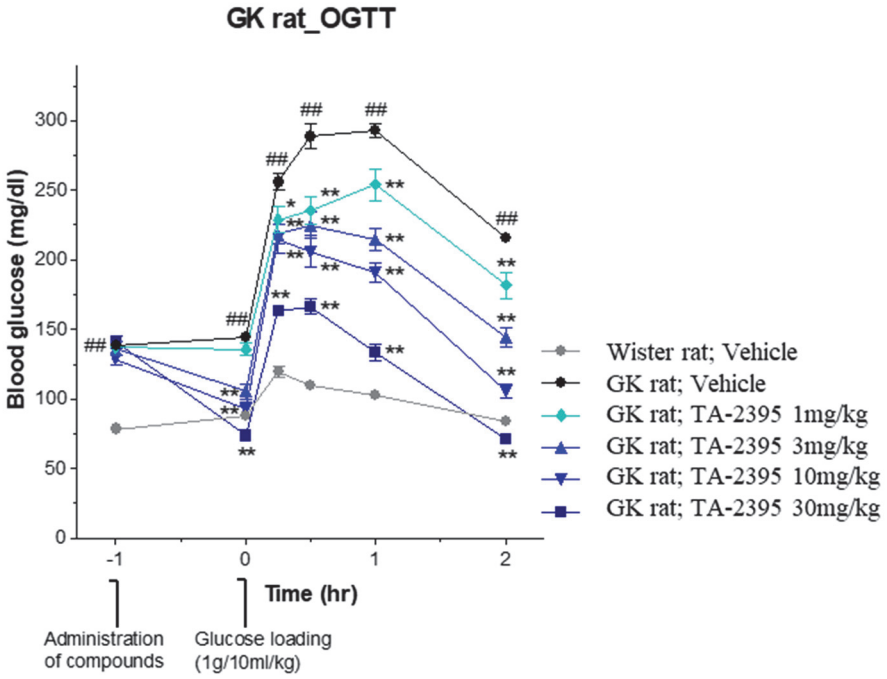
血糖値の推移については、TA-2395 投与群およびグリメピリド投与群それぞれ投与後 1-2 時間後に最大血糖降下作用を示し同等の薬効を見出した。これに対して、メトホルミン投与群の血糖降下作用は軽微な変化に留まった。一方、血漿中インスリンレベルは、グリメピリド投与群でより高い値が認められ、TA-2395 投与群ではインスリンレベルの軽微な上昇傾向が認められた。これは GK 活性化薬 TA-2395 が肝臓における糖取り込み作用と膵臓でのインスリン放出作用の二つの作用機序で血糖値を低下させていることを示唆する結果と考えられる。このことから、当初の想定した GK 活性化薬のコンセプトを *in vivo* 試験においても確認することが出来た。

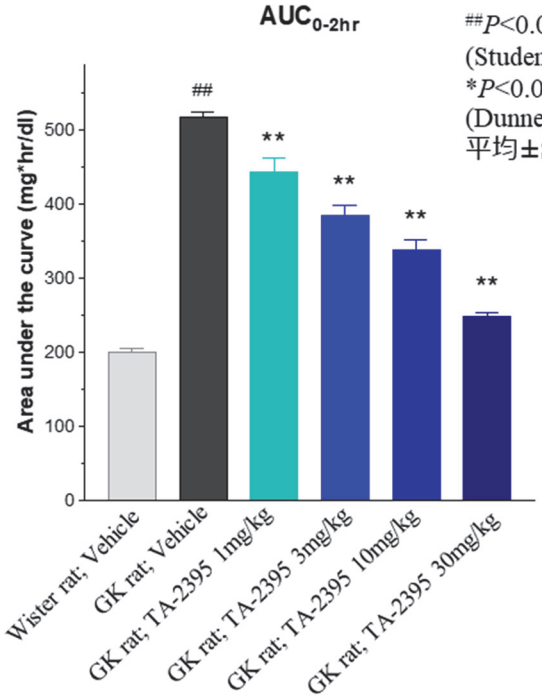
【図表３－１３】　正常ラットにおける血糖値および血漿中インスリンレベルの作用



続いて糖尿病モデルラット糖負荷試験（OGTT）をおこなった（図表３－１４）。非肥満型で幼齢時から高血糖を示す Goto-Kakizaki ラット（GK ラット）[34]に対して、TA-2395 あるいは vehicle の経口投与を行い、1 時間後に経口ブドウ糖負荷をおこなった。また正常動物群として Wister ラットへ vehicle の投与を行った。その結果、TA-2395 は用量依存的かつ 1 mg/kg の低用量群から血中グルコース濃度上昇を有意に抑制する事が明らかとなり、糖尿病治療薬候補としての強力なポテンシャルを示すことが出来た。

【図表３－１４】　糖尿病モデルラットへの糖負荷作用と血糖降下作用



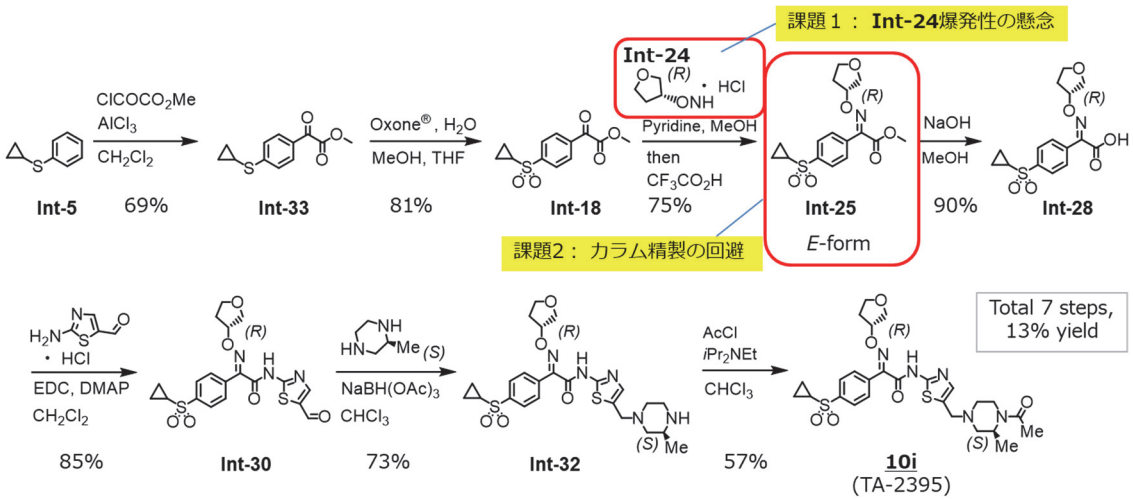


##P<0.01 vs. 'Wistar rat; Vehicle' (Student's t-test),
*P<0.05, **P<0.01 vs. 'GK rat; Vehicle' (Dunnett's method)
平均±SEM (N = 8).

第４章　TA-2395 (10i)のスケールアップ合成研究

臨床試験に用いる製造レベルでの合成を視野に入れつつ、安全性試験などの前臨床試験で必要な kg レベルでの化合物 **10i** の大量合成法を検討した。探索段階時の小スケール合成ルートではアルコキシアミン **Int-24** を使用していたが、本化合物には爆発性の懸念があるため大量合成には使用できないと判断した（図表４－１）。また、*E/Z* 異性体を分離するための中間体 **Int-25** のカラム精製は、溶媒を大量に使用することによるコストや環境負荷が大きい方法であり、これを回避することが望ましいと考えた。そこで大量合成に先立ち、ケトエステル中間体 **Int-18** からオキシムエステル中間体 **Int-25** を得る合成法の改良が必要となった。

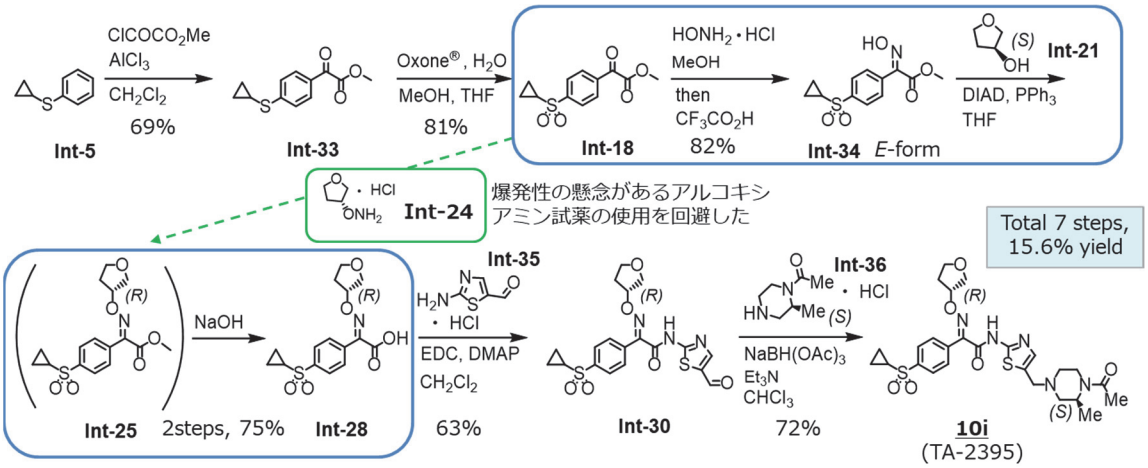
【図表４－１】　オキシム化合物 **10i** 探索段階ルート



スケールアップ研究結果後の改良した合成スキームを図表４－２に示す。

出発原料 **Int-5** より導いたα-ケトエステル **Int-18** に対して、ヒドロキシルアミン塩酸塩をメタノール加熱条件下作用させることによりオキシム化を進行させ、反応液を濃縮した。得られた *Z* 体過剰の粗生成物をトリフルオロ酢酸に作用させることでオキシムの *E/Z* 異性化を促進させ、溶媒濃縮後の再結晶により望む *E* 体のα-ヒドロキシイミノエステル **Int-34** を得た。続いて(*S*)-3-テトラヒドロフラノール **Int-21** を DIAD, PPh_3 を用いる光延反応条件で作用させることにより、目的の(*R*)-テトラヒドロフラニル基を有するα-アルコキシイミノエステル体 **Int-25** へと導くことに成功した[35]。また、エステル体 **Int-25** の単離精製を行うことなく、反応液中に過剰量の水酸化ナトリウム水溶液を添加することによりカルボン酸 **Int-28** へとワンポット反応で導いた。塩基性の反応溶液を有機溶媒で洗浄後、塩酸水で酸性とした後に有機相へ抽出する操作をおこなうことによりカラム精製を用いることなくカルボン酸 **Int-28** を結晶化のみで精製できる方法を見出した。その結果、原料から目的物まで全７工程、総収率 15.6%の安全性と操作性に優れた大量合成ルートを確立できた。

【図表４－２】　オキシム化合物 **10i** スケールアップ合成ルート



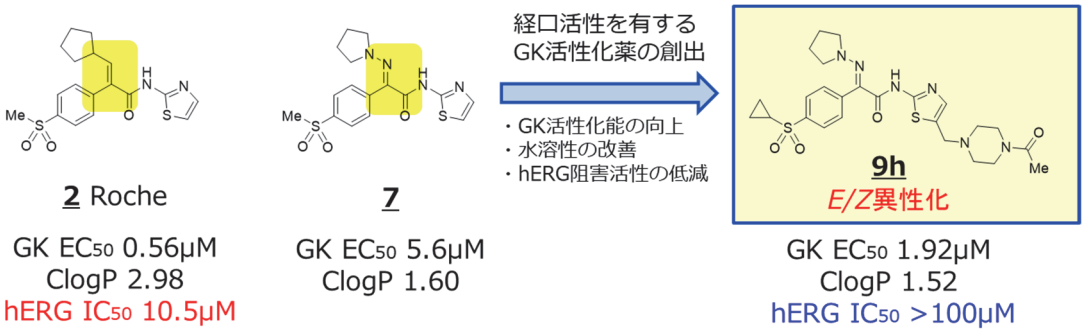
第５章　総括

2 型糖尿病患者の治療目標は、血糖値を正常レベルに厳格に管理することにあるが、現在でも多くの患者で血糖管理目標を達成するには至っていない。そのため、既存の血糖降下薬とは異なる新規作用機序を有する治療薬の開発が望まれていた。このような背景の中、肝と膵の両方に作用するグルコキナーゼ活性化薬の創出は、肝糖バランスの異常および膵インスリン分泌不全の両方を改善し、厳格な血糖コントロールを可能にする新しいタイプの血糖降下薬として注目を集め、精力的な研究開発が進められていた。

筆者は先行するアクリルアミド誘導体 **2** を端緒化合物と設定し、hERG 阻害作用とアクリルアミド構造の回避を解決すべき課題と考え、アクリルアミド部分の炭素-炭素二重結合を炭素-窒素二重結合へ変換した新規ヒドラゾン誘導体および新規オキシム誘導体に着目した。経口投与で強力な薬効を示すとともに安全性にも優れた化合物の探索研究を行い、臨床試験化合物 **10i** (TA-2395)を創出する事に成功した。本研究の概要を以下の様に纏めた。

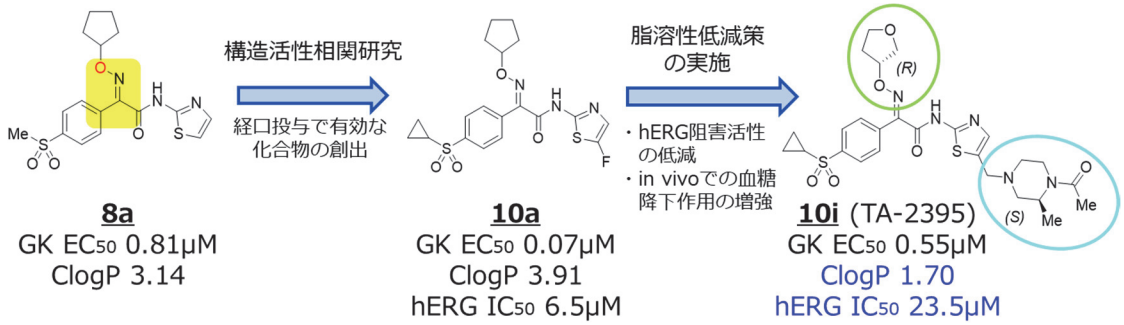
第２章においては、新規ヒドラゾン誘導体に関する研究結果を論述した。すなわち、GK 活性と溶解度の向上を目的とした構造活性相関研究を行い、チアゾール環 5 位側鎖にアセチルピペラジルメチル基を有するヒドラゾン化合物 **9h** を取

得した。化合物 **9h** は hERG 阻害作用を回避するとともに、ラット *in vivo* 試験において低用量の 1mg/kg から有意な血糖降下作用を示した。しかし、**9h** の光照射下の溶液安定性試験で *E* 体から *Z* 体への異性化が観測されたことから臨床開発に向けた更なる検討を断念した。

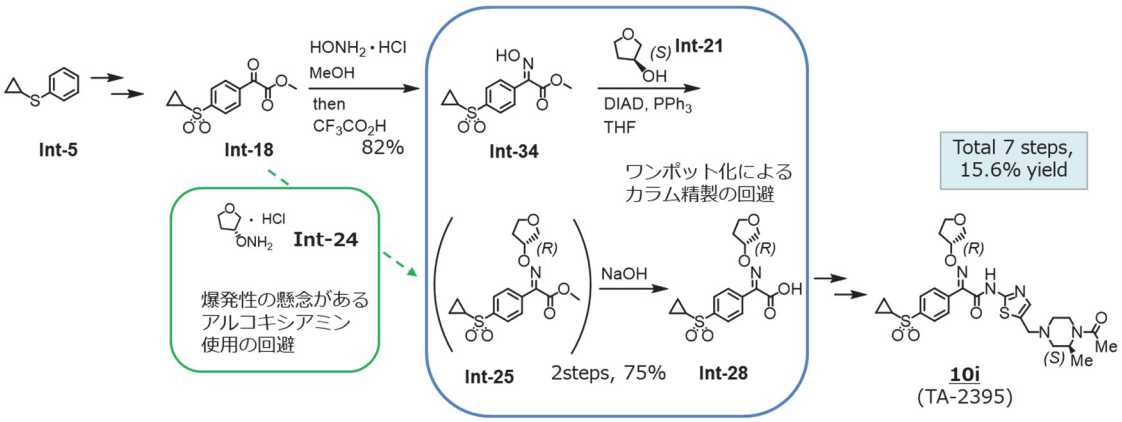


第３章においては、主に新規オキシム誘導体に着目した探索研究結果について論述した。すなわち、ヒドラゾン誘導体の構造活性相関をもとにオキシム誘導体の初期展開を行い、*in vitro* で高活性な化合物 **10a** を見出した。水溶解性の向上と hERG 阻害の回避に向けた脂溶性低減による最適化検討の結果、マウスおよびラット *in vivo* 試験で十分な血糖降下作用を有し、hERG 阻害作用を低減した化合物 **10i** を取得した。さらに、化合物 **10i** は肝糖バランスの異常および膵インスリン分泌不全の両方を改善する新しいタイプの血糖降下薬となる可能性を *in vitro* および *in vivo* の双方において確認することが出来た。化合物 **10i** はげっ歯類およびイヌの動物試験において薬効量と毒性量に十分な安全域を有する事

も確認され、これら非臨床試験成績を総合的に鑑みて、臨床試験候補化合物（開発品番号 TA-2395）として選出した。



第４章においては、臨床試験候補化合物として選出された **10i** の大量合成法の開発研究について論述した。すなわち、安全性試験などの前臨床試験で用いる化合物 **10i** の kg レベルでのプロセス合成ルートへの改良研究を行い、原料から目的物まで全７工程、中間体のカラム精製を不要とする総収率 15.6%の安全性と操作性に優れた大量合成ルートを確立した。



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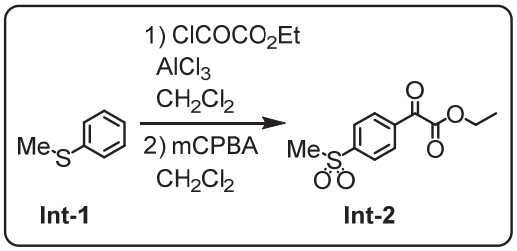
最後に教育の機会を与えてくださった両親、姉、そして研究生活を激励し、常に支えとなってくれた妻　奈津佳、そして三人の娘たちに深く感謝致します。

実験の部

All reactions were carried out under inert gas or with $CaCl_2$ tube and reaction mixtures were stirred magnetically. All reagents and solvents were purchased from commercial suppliers and used without further purification unless otherwise noted. Reaction products were monitored by TLC using 0.25 mm E. Merck silica gel plates (60 F254) and were visualized using UV light or 5% phosphomolybdic acid in 95% EtOH. NMR spectra were collected on JEOL JNM-ECX400P and Varian UNITY INOVA500 spectrometers. Chemical shifts were given in parts per million (ppm) downfield from internal reference tetramethylsilane standard; coupling constants (*J* value) were given in hertz (Hz). Infrared spectra were measured on Perkin-Elmer PARAGON1000. APCI- and ESI-MS spectra were obtained on Finnigan MAT SSQ7000C or ThermoQuest LCQ Advantage eluting with 10 mM $AcONH_4$ / MeOH. Analytical HPLC spectra were reported using Agilent 1100 with a UV detector measuring absorbance at 210 nm or 220 nm. All compounds were found to be >95% pure by HPLC analysis unless otherwise noted.

第２章第２節に関する実験の部

(4-methylsulfonylphenyl)-2-oxo-acetic acid ethyl ester (Int-2)[16]

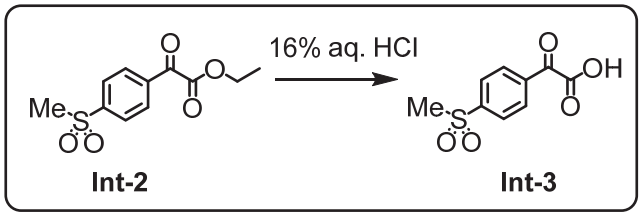


To a solution of aluminum chloride (96.0 g, 720 mmol) in CH_2Cl_2 (280 mL) was added chloroglyoxylic acid ethyl ester (86.0 g, 630 mmol) dropwise over 1 hour under ice-cooling bath temperature, and the mixture was stirred for 15 minutes at the same temperature. Then, a solution of thioanisole (**Int-1**: 74.5 g, 600 mmol) in CH_2Cl_2 (70 mL) was added dropwise to the above mixture over 1 hour. After the ice-cooling bath was removed, the reaction mixture was stirred at room temperature for 2 hours. Water was carefully added to the reaction mixture under ice-cooling, then the whole was extracted with CH_2Cl_2 three times, and the combined organic layers were dried over anhydrous $MgSO_4$, and concentrated in vacuo to give ethyl 2-(4-methylsulfanylphenyl)-2-oxo-acetate as a yellow oil (128.4 g, 95% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.42 (t, J = 7.1 Hz, 3H), 2.54 (s, 3H), 4.44 (q, J = 7.1 Hz, 2H), 7.29 (d, J = 8.8 Hz, 2H), 7.92 (d, J = 8.8 Hz, 2H). MS (APCI, *m/z*) 225 $[M+H]^+$.

To a solution of ethyl 2-(4-methylsulfanylphenyl)-2-oxo-acetate (127.0 g, 566 mmol) in CH_2Cl_2 (2.8 L) was added 70% *m*-chloroperoxybenzoic acid (mCPBA, 277.0g, 1.13 mol) portionwise over 20 minutes under ice-cooling bath temperature. The reaction mixture was stirred for 1 hour at the same temperature, and then stirred additional 3 hour at room temperature. Insoluble materials were removed by filtration and the filtrate was extracted with CH_2Cl_2. The organic layer was washed with 10% aq Na_2SO_3, 10% aq Na_2CO_3 and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residual solid was triturated in Et_2O and filtered to give **Int-2** (36.7 g, 25% yield) as a colorless powder. The filtrate was concentrated in vacuo and purified by column chromatography on silica gel (hexane/EtOAc = 3/2) to give additional **Int-2** as a colorless powder (21.3 g, 15% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44 (t, J = 7.1 Hz, 3H), 3.10 (s, 3H), 4.48 (q, J = 7.1 Hz, 2H), 8.09 (d, J = 8.8 Hz, 2H), 8.24 (d, J = 8.8 Hz, 2H). MS (APCI, *m/z*)

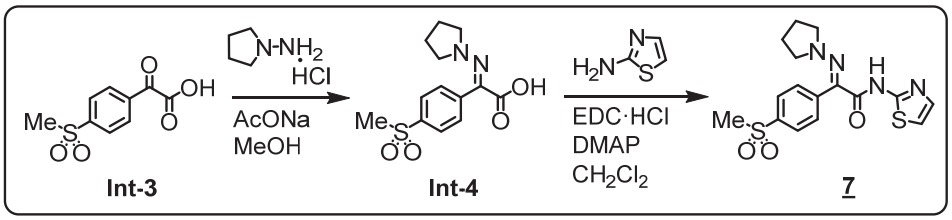
274 $[M+NH_4]^+$. IR (nujol, cm^{-1}) 1689, 1729.

(4-methylsulfonylphenyl)-oxo-acetic acid (**Int-3**)



A mixture of (4-methylsulfonylphenyl)-2-oxo-acetic acid ethyl ester (**Int-2**: 12.56 g, 49.01 mmol) and 16% aq HCl (125 mL) was refluxed for 24 h. After cooling to room temperature, the resulting suspension was concentrated in vacuo. The residue solid was recrystallized from THF – toluene to give **Int-3** (9.86 g, 88% yield) as a colorless powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.11 (s, 3H), 8.08 (d, *J* = 8.6 Hz, 2H), 8.28 (d, *J* = 8.8 Hz, 2H).

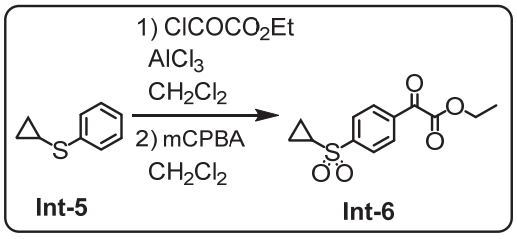
(2*E*)-2-(4-methylsulfonylphenyl)-2-pyrrolidin-1-ylimino-*N*-thiazol-2-yl-acetamide (**7**)



To a solution of **Int-3** (228 mg, 1.0 mmol) in MeOH (5 mL) were added 1-aminopyrrolidine hydrochloride (345 mg, 4.0 mmol) and sodium acetate (656 mg, 8.0 mmol), and the mixture was stirred at room temperature for 5 hours. 1 M aqueous HCl was added to the reaction mixture and extracted with CH_2Cl_2 three times. The combined organic layers were washed with water and brine, and then dried over sodium sulfate and concentrated in vacuo to give (2*E*)-2-(4-methylsulfonylphenyl)-2-pyrrolidin-1-ylimino-acetic acid **Int-4** as a crude material. To a solution of **Int-4** and 2-aminothiazole (400mg, 4.0 mmol) in CH_2Cl_2 (5 mL) was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl, 395 mg, 2 mmol) and catalytic amount of 4-dimethylaminopyridine (DMAP) at 0°C. The mixture was stirred for 30 min at 0°C, and for 5 hours at ambient temperature. After the reaction mixture was concentrated in vacuo, the resulting residue was diluted with EtOAc, and the organic layer was washed with 10% aq citric acid, water and brine, dried over anhydrous $MgSO_4$ and concentrated in vacuo. The resulting residue was purified by column chromatography on NH-silica gel

(Hexane/AcOEt = 60/40 – 20/80) to give **7** (42 mg, 13% yield) as a colorless powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.80 – 1.90 (m, 4H), 3.08 (s, 3H), 3.15 – 3.25 (m, 4H), 6.92 (d, *J* = 3.6 Hz, 1H), 7.45 (d, *J* = 3.6 Hz, 1H), 7.56 (d, *J* = 8.5 Hz, 2H), 7.95 (d, *J* = 8.5 Hz, 2H), 10.20 (s, 1H). MS (ESI, *m/z*) 379 $[M+H]^+$.

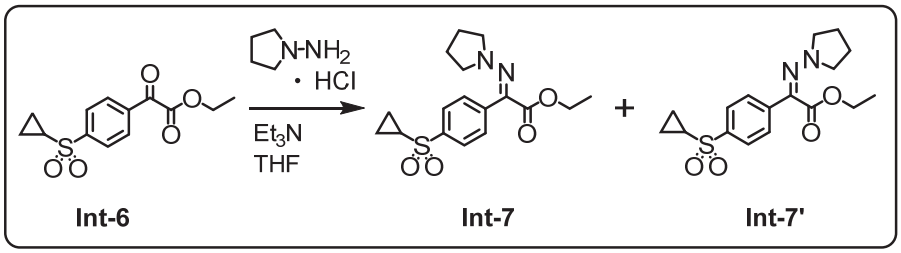
(4-cyclopropylsulfonylphenyl)-2-oxo-acetic acid ethyl ester (**Int-6**)



To a solution of aluminum chloride (106.47 g, 798.7 mmol) in CH_2Cl_2 (560 mL) was added chloroglyoxylic acid ethyl ester (13.98 g, 102.4 mmol) dropwise over 15 minutes under ice-cooling bath temperature, and the mixture was stirred for 15 minutes at the same temperature. Then, a solution of cyclopropyl phenyl sulfide (**Int-5**: 100.0 g, 665.6 mmol) in CH_2Cl_2 (140 mL) was added dropwise to the above mixture over 90 minutes. After the ice-cooling bath was removed, the reaction mixture was stirred at room temperature for 2 hours. Water was carefully added to the reaction mixture under ice-cooling, then the whole was extracted with $CHCl_3$ three times, and the combined organic layers were dried over anhydrous $MgSO_4$, and concentrated in vacuo to give ethyl 2-(4-cyclopropylsulfanylphenyl)-2-oxo-acetate as a yellow oil (17.81 g, quantitative yield). MS (APCI, *m/z*) 251 $[M+H]^+$.

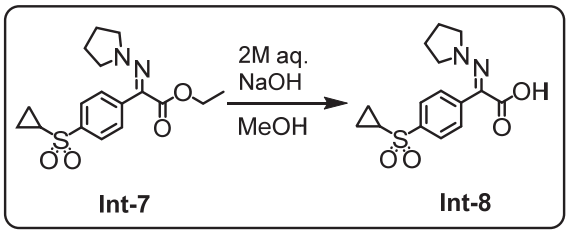
To a solution of ethyl 2-(4-cyclopropylsulfanylphenyl)-2-oxo-acetate (25.0 g, 100.0 mmol) in CH_2Cl_2 (500 mL) was added 70% mCPBA (59.0g, 240.0 mmol) portionwise over 15 minutes under ice-cooling bath temperature. The reaction mixture was stirred for 30 minutes at the same temperature, and then stirred additional 12 hours at room temperature. Insoluble materials were removed by filtration and the filtrate was extracted with CH_2Cl_2. The extract was washed with 10% aq Na_2SO_3, 10% aq Na_2CO_3 and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (hexane/EtOAc = 2/1) to give **Int-6** as a colorless powder (7.44 g, 26% yield). MS (APCI, *m/z*) 283 $[M+H]^+$.

(2*E*)-(4-cyclopropylsulfonylphenyl)-2-pyrrolidin-1-ylimino-acetic acid ethyl ester (**Int-7**) and **(2*Z*)-(4-cyclopropylsulfonylphenyl)-2-pyrrolidin-1-ylimino-acetic acid ethyl ester** (**Int-7'**)



A mixture of (4-cyclopropylsulfonylphenyl)-2-oxo-acetic acid ethyl ester (**Int-6**: 25 g, 88.6 mmol), 1-aminopyrrolidine hydrochloride (25 g, 204 mmol), and triethylamine (28.4 mL, 204 mmol) in THF (350 mL) was stirred at reflux temperature for 3 days. The reaction was cooled to room temperature, diluted with water, and the whole was extracted with EtOAc. The organic layer was washed with water, brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure, and purified by silica gel column chromatography (Hexane/EtOAc = 1/1) to give less polar isomer **Int-7'** (16.56 g, 53% yield) and more polar isomer **Int-7** (12.43 g, 40% yield). (**Int-7**) ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98 – 1.12 (m, 2H), 1.32 (t, *J* = 7.1 Hz, 3H), 1.34 – 1.42 (m, 2H), 1.74 – 1.83 (m, 4H), 2.48 (tt, J = 8.0 Hz, 4.8 Hz, 1H), 3.12 – 3.20 (m, 4H), 4.28 (q, *J* = 7.1 Hz, 2H), 7.47 (d, *J* = 8.4 Hz, 2H), 7.84 (d, *J* = 8.6 Hz, 2H). MS (APCI, *m/z*) 351 $[M+H]^+$. (**Int-7'**) ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95 – 1.05 (m, 2H), 1.28 – 1.40 (m, 2H), 1.38 (t, *J* = 7.1 Hz, 3H), 1.90 – 2.00 (m, 4H), 2.44 (tt, *J* = 8.0 Hz, 4.8 Hz, 1H), 3.44 – 3.53 (m, 4H), 4.37 (q, *J* = 7.1 Hz, 2H), 7.64 (d, *J* = 8.8 Hz, 2H), 7.79 (d, *J* = 9.0 Hz, 2H). MS (APCI, *m/z*) 351 $[M+H]^+$.

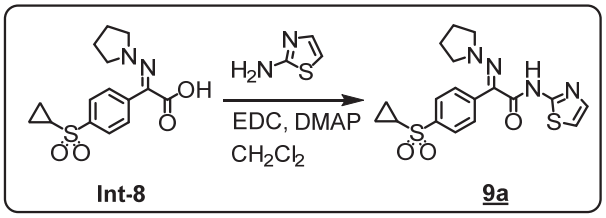
(2*E*)-2-(4-cyclopropylsulfonylphenyl)-2-pyrrolidin-1-ylimino-acetic acid (Int-8)



To an MeOH (200 mL) solution of **Int-7** (12.37 g, 35.3 mmol) was added with 2 M aqueous NaOH (150 mL, 300 mmol). The mixture was stirred at room temperature for 4 hours and then concentrated in vacuo. The residue was diluted with water and washed with Et_2O. The aqueous layer was diluted with $CHCl_3$, ice-cooled, and neutralized with 2 M aq HCl (70 mL), then extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue solid was triturated in THF – Et_2O to give **Int-8** (9.18 g, 81% yield) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.97 – 1.15 (m, 2H), 1.30 – 1.44 (m, 2H), 1.80 – 1.91 (m, 4H), 2.45-2.51 (m, 1H), 3.14 – 3.25 (m,

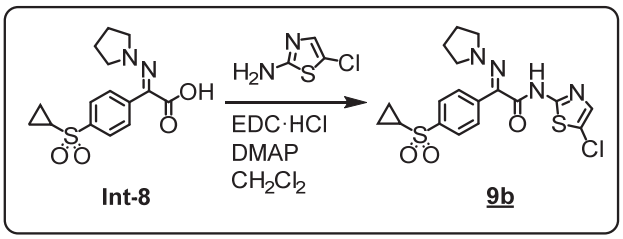
4H), 7.50 (d, *J* = 8.5 Hz, 2H), 7.88 (d, *J* = 8.5 Hz, 2H), 8.5 - 10.5 (br s, 1H). MS (ESI, *m/z*) 321 $[M-H]^-$.

(2*E*)-2-(4-Cyclopropylsulfonylphenyl)-2-pyrrolidin-1-ylimino-*N*-thiazol-2-yl-acetamide (9a)



To an ice-cooling solution of **Int-8** (130 mg, 0.40 mmol), 2-aminothiazole (121 mg, 1.2 mmol) and DMAP (73 mg, 0.60 mmol) in CH_2Cl_2 (5 mL) was added EDC (105 μL, 0.60 mmol) in one portion and the mixture was stirred for 30 min. After being warmed to room temperature and stirred for 24 hours, the reaction mixture was quenched with additional water and the whole was extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4, treated with activated charcoal, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 100/0 – 95/5) to give **9a** (113 mg, 69% yield) as a yellowish powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.99 – 1.13 (m, 2H), 1.31 – 1.44 (m, 2H), 1.79 – 1.90 (m, 4H), 2.44 – 2.51 (m, 1H), 3.14 – 3.25 (m, 4H), 6.92 (d, *J* = 3.6 Hz, 1H), 7.45 (d, *J* = 3.6 Hz, 1H), 7.53 (d, *J* = 8.5 Hz, 2H), 7.90 (d, *J* = 8.5 Hz, 2H), 10.20 (s, 1H). MS (ESI, *m/z*) 405 $[M+H]^+$.

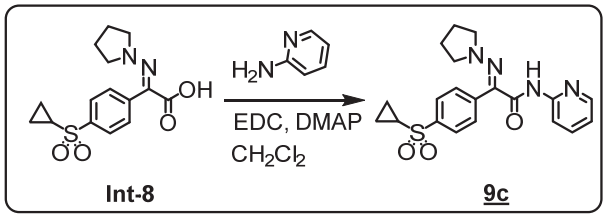
(2*E*)-*N*-(5-Chlorothiazol-2-yl)-2-(4-cyclopropylsulfonylphenyl)-2-pyrrolidin-1-ylimino-acetamide (9b)



To an ice-cooling solution of **Int-8** (100 mg, 0.31 mmol), 2-amino-5-chlorothiazole (125 mg, 0.93 mmol) and DMAP (57 mg, 0.46 mmol) in CH_2Cl_2 (3 mL) was added EDC·HCl (71 mg, 0.46 mmol) in one portion and the mixture was stirred for 30 min. After being warmed to room temperature and stirred for 24 hours, the reaction mixture was quenched with additional water and the whole was extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4, treated with activated charcoal, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography

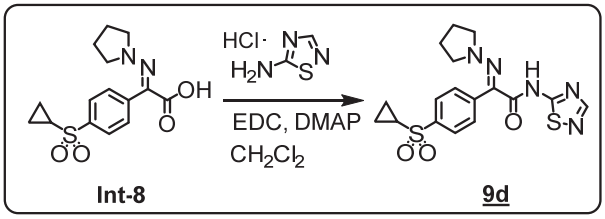
($CHCl_3$/MeOH = 100/0 – 90/10) to give **9b** (113 mg, 69% yield) as a yellowish powder. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.00 – 1.14 (m, 2H), 1.32 – 1.44 (m, 2H), 1.80 – 1.90 (m, 4H), 2.45 – 2.51 (m, 1H), 3.14 – 3.24 (m, 4H), 7.26 (s, 1H), 7.51 (d, *J* = 8.5 Hz, 2H), 7.90 (d, *J* = 8.5 Hz, 2H), 10.06 (s, 1H). MS (ESI, *m/z*) 439/441 $[M+H]^+$.

(2*E*)-2-(4-Cyclopropylsulfonylphenyl)-*N*-(2-pyridyl)-2-pyrrolidin-1-ylimino-acetamide (**9c**)



To an ice-cooling solution of **Int-8** (130 mg, 0.40 mmol), 2-aminopyridine (113 mg, 1.2 mmol) and DMAP (73 mg, 0.60 mmol) in CH_2Cl_2 (5 mL) was added EDC (105 μL, 0.60 mmol) in one portion and the mixture was stirred for 30 min. After being warmed to room temperature and stirred for 24 hours, the reaction mixture was quenched with additional water and the whole was extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4, treated with activated charcoal, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 100/0 – 95/5) to give **9c** (95 mg, 59% yield) as a yellowish powder. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.99 – 1.13 (m, 2H), 1.31 – 1.45 (m, 2H), 1.76 – 1.86 (m, 4H), 2.45 – 2.52 (m, 1H), 3.12 – 3.23 (m, 4H), 7.00 (ddd, *J* = 5.0, 2.5, 1.1 Hz, 1H), 7.54 (d, *J* = 8.5 Hz, 2H), 7.66 (td, *J* = 5.2, 2.0 Hz, 1H), 7.89 (d, *J* = 8.5 Hz, 2H), 8.25 (dt, *J* = 8.7, 1.0 Hz, 1H), 8.31 (ddd, *J* = 5.0, 2.0, 0.8 Hz, 1H), 9.53 (s, 1H). MS (ESI, *m/z*) 399 $[M+H]^+$.

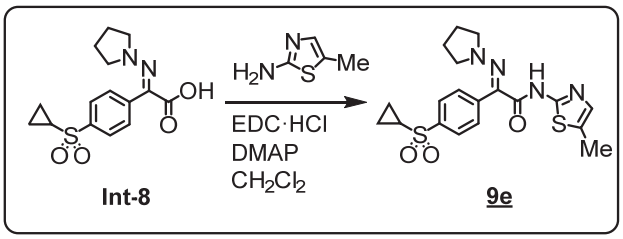
(2*E*)-2-(4-Cyclopropylsulfonylphenyl)-2-pyrrolidin-1-ylimino-*N*-(1,2,4-thiadiazol-5-yl)acetamide (**9d**)



To an ice-cooling solution of **Int-8** (100 mg, 0.31 mmol), 1,2,4-thiadiazol-5-ylamine hydrochloride (130 mg, 0.93 mmol) and DMAP (57 mg, 0.46 mmol) in CH_2Cl_2 (3 mL) was added EDC (80 μL, 0.46 mmol) in one portion and the mixture was stirred for 30 min. After being warmed to room temperature and stirred for 24 hours, the reaction

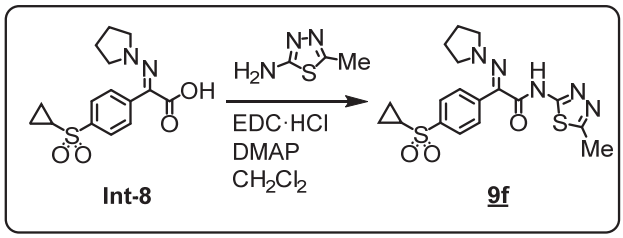
mixture was quenched with additional water and the whole was extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4, treated with activated charcoal, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 100/0 – 90/10) to give **9d** (61 mg, 49% yield) as a yellowish powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.00 – 1.14 (m, 2H), 1.31 – 1.44 (m, 2H), 1.82 – 1.93 (m, 4H), 2.47 – 2.52 (m, 1H), 3.19 – 3.29 (m, 4H), 7.52 (d, *J* = 8.5 Hz, 2H), 7.92 (d, *J* = 8.5 Hz, 2H), 8.30 (s, 1H), 10.45 (s, 1H). MS (APCI, *m/z*) 406 $[M+H]^+$.

(2*E*)-2-(4-Cyclopropylsulfonylphenyl)-*N*-(5-methylthiazol-2-yl)-2-pyrrolidin-1-ylimino-acetamide (9e)



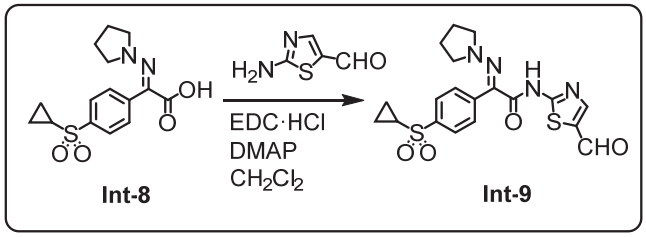
To an ice-cooling solution of **Int-8** (100 mg, 0.31 mmol), 2-amino-5-methylthiazole (106 mg, 0.93 mmol) and DMAP (57 mg, 0.46 mmol) in CH_2Cl_2 (3 mL) was added EDC·HCl (71 mg, 0.46 mmol) in one portion and the mixture was stirred for 30 min. After being warmed to room temperature and stirred for 24 hours, the reaction mixture was quenched with additional water and the whole was extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4, treated with activated charcoal, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 100/0 – 90/10) to give **9e** (86 mg, 67% yield) as a yellow powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.00 – 1.13 (m, 2H), 1.30 – 1.44 (m, 2H), 1.78 – 1.89 (m, 4H), 2.38 (d, *J* = 1.4 Hz, 3H), 2.44 – 2.51 (m, 1H), 3.13 – 3.23 (m, 4H), 7.08 (d, *J* = 1.1 Hz, 1H), 7.53 (d, *J* = 8.5 Hz, 2H), 7.89 (d, *J* = 8.5 Hz, 2H), 10.05 (s, 1H). MS (APCI, *m/z*) 419 $[M+H]^+$.

(2*E*)-2-(4-Cyclopropylsulfonylphenyl)-*N*-(5-methyl-1,3,4-thiadiazol-2-yl)-2-pyrrolidin-1-ylimino-acetamide (9f)



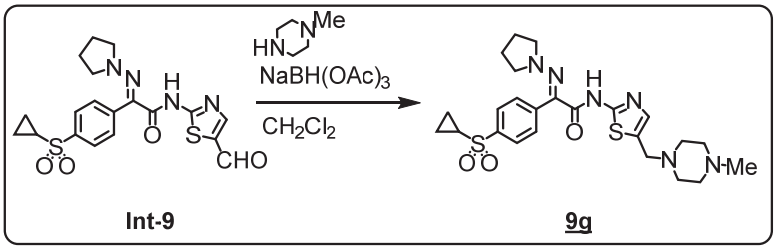
To an ice-cooling solution of **Int-8** (500 mg, 0.31 mmol), 2-amino-5-methyl-1,3,4-thiadiazole (268 mg, 2.33 mmol) and DMAP (284 mg, 2.33 mmol) in CH_2Cl_2 (10 mL) was added EDC·HCl (446 mg, 2.33 mmol) in one portion and the mixture was stirred for 30 min. After being warmed to room temperature and stirred for 24 hours, the reaction mixture was quenched with additional water and the whole was extracted with chloroform. The organic layer was dried over Na_2SO_4, treated with activated charcoal, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 100/0 – 90/10) to give **9f** (399 mg, 61% yield) as a yellow powder. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.00 – 1.12 (m, 2H), 1.33 – 1.43 (m, 2H), 1.82 – 1.92 (m, 4H), 2.45 – 2.50 (m, 1H), 2.69 (s, 3H), 3.16 – 3.26 (m, 4H), 7.51 (d, 8.4 Hz, 2H), 7.90 (d, 8.4 Hz, 2H), 10.25 (s, 1H). MS (APCI, *m/z*) 420 $[M+H]^+$. HPLC 99.9% (t_R = 9.8 min, L-column ODS (5 μM particle size, 4.6 x 150 mm), CH_3CN/20 mM phosphate buffer (pH 6.5) (35/65)).

(2*E*)-2-(4-cyclopropylsulfonylphenyl)-*N*-(5-formylthiazol-2-yl)-2-pyrrolidin-1-ylimino-acetamide (**Int-9**)



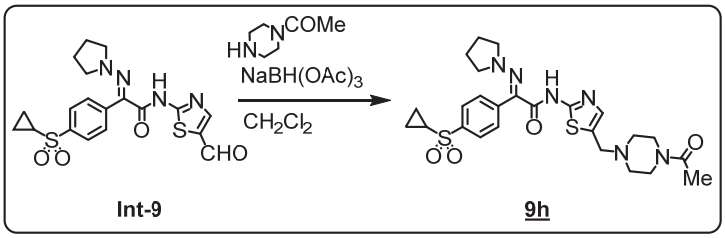
To an ice-cooling solution of **Int-8** (4.04 g, 12.53 mmol), 2-amino-5-formylthiazole (1.93 g, 15.04 mmol) and DMAP (1.84 g, 15.04 mmol) in CH_2Cl_2 (90 mL) was added EDC·HCl (3.12 g, 16.29 mmol) in one portion and the mixture was stirred for 30 min. After being warmed to room temperature and stirred for 24 hours, the reaction mixture was quenched with additional water and the whole was extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4, treated with activated charcoal, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 100/0 – 90/10), then triturated from hexane – EtOAc to give **Int-9** (3.28 g, 7.58 mmol, 61% yield) as a yellowish powder. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.03 – 1.13 (m, 2H), 1.34 – 1.43 (m, 2H), 1.82 – 1.92 (m, 4H), 2.47 – 2.54 (m, 1H), 3.17 – 3.27 (m, 4H), 7.52 (d, *J* = 8.6 Hz, 2H), 7.91 (d, *J* = 8.6 Hz, 2H), 8.11 (s, 1H), 9.95 (s, 1H), 10.37 (s, 1H). MS (APCI, *m/z*) 433 $[M+H]^+$.

(2*E*)-2-(4-Cyclopropylsulfonylphenyl)-*N*-[5-[(4-Methylpiperazin-1-yl)methyl]thiazol-2-yl]-2-pyrrolidin-1-ylimino-acetamide (**9g**)



Sodium triacetoxyborohydride (368 mg, 1.74 mmol) was added portionwise to a stirred ice-cooling solution of **Int-9** (250 mg, 0.58 mmol) and 1-methylpiperazine (154 µL, 1.39 mmol) in CH_2Cl_2 (15 mL) and stirred for 15 min. The reaction mixture was allowed to warm to room temperature and stirred for 20 hours. The reaction mixture was quenched with a saturated aq $NaHCO_3$ solution and extracted with chloroform. The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 95/5 – 90/10), then triturated from hexane – EtOAc to give **9g** (191 mg, 64% yield) as a colorless powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.00 – 1.12 (m, 2H), 1.32 – 1.42 (m, 2H), 1.80 – 1.90 (m, 4H), 2.29 (s, 3H), 2.36 – 2.65 (m, 8H), 2.45 – 2.51 (m, 1H), 3.14 – 3.25 (m, 4H), 3.67 (s, 2H), 7.22 (s, 1H), 7.52 (d, 8.5 Hz, 2H), 7.89 (d, 8.2 Hz, 2H), 10.08 (s, 1H). MS (APCI, *m/z*) 517 $[M+H]^+$. HPLC 100% (t_R = 8.3 min, L-column ODS (5 µM particle size, 4.6 x 150 mm), CH_3CN/20 mM phosphate buffer (pH 6.5) (30/70)).

(2*E*)-*N*-[5-[(4-acetylpiperazin-1-yl)methyl]thiazol-2-yl]-2-(4-cyclopropylsulfonyl-phenyl)-2-pyrrolidin-1-ylimino-acetamide (**9h**)



Sodium triacetoxyborohydride (615 mg, 2.90 mmol) was added portionwise to an ice-cooling solution of **Int-9** (500 mg, 1.16 mmol) and 1-acetylpiperazine (296 mg, 2.31 mmol) in CH_2Cl_2 (10 mL) and stirred for 15 min. The reaction mixture was allowed to warm to room temperature and stirred for 4 hours. The reaction mixture was quenched with saturated aq $NaHCO_3$ solution and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 100/0 – 95/5), then triturated from EtOAc – *i*-Pr_2O to give **9h** (537 mg, 85% yield) as a colorless powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.04 – 1.12 (m, 2H), 1.12 – 1.20 (m, 2H), 1.70 – 1.82

(m, 4H), 1.96 (s, 3H), 2.31 (t, J = 4.8 Hz, 2H), 2.38 (t, J = 4.7 Hz, 2H), 2.87 – 2.95 (m, 1H), 3.16 – 3.23 (m, 4H), 3.37 – 3.43 (m, 4H), 3.66 (s, 2H), 7.29 (s, 1H), 7.53 (d, J = 8.5 Hz, 2H), 7.87 (d, J = 8.5 Hz, 2H), 11.15 (s, 1H). MS (APCI, *m/z*) 545 $[M+H]^+$. HPLC 99.6% (t_R = 8.4 min, L-column ODS (5 μM particle size, 4.6 x 150 mm), CH_3CN/20 mM phosphate buffer (pH 6.5) (35/65)).

Pharmacology

A glucokinase activation effect was evaluated as follows:

A glucokinase activity was examined by measuring the amount of NADPH obtained in generating 6-phosphogluconic acid from glucose-6-phosphate dehydrogenase wherein glucose-6-phosphoric acid is a coupling enzyme, not by directly measuring the generated glucose-6-phosphoric acid. The glucokinase enzyme used in the examination was human-liver type GST-GK expressed by *E. Coli.* The measurement of the activity was carried out by the following procedures. Specifically, 30 mM HEPES buffer (pH7.4) containing 30 mM $MgCl_2$, 30 mM KCl, 1 mM DTT, 5 mM NADP (Nacalai), 0.7 mU/mL G6PDH (Roche 737-232 grade II from yeast) and 0.17 μL/mL GST-GK was prepared as a reaction solution. An evaluating compound dissolved in DMSO was added to the reaction solution to give final concentration of 0.01 to 100 μM (5% DMSO). Thereto was added glucose (final concentration of 5 mM) as a substrate and was added ATP (final concentration of 5 mM) to proceed the reaction. The reaction temperature was 30°C and a generation of NADPH was monitored by changes of absorbance of 340 nm. An increase of absorbance for 10 minutes after starting reaction was measured and the blank-corrected value was used as a GK activity (mOD/min). EC_{50} level was calculated by a GK activity level in an addition of the evaluating compound at each concentration.

Effect of GK activators on blood glucose levels in C57BL/6J mice was evaluated as follows:

Male 8-week-old C57BL/6J mice (JCL Japan, Inc., Tokyo, Japan) were kept in a temperature (23 ± 2 °C) and humidity (55 ± 15 %) controlled room with a 12-h light–dark cycle and allowed free access to an ordinary rodent chow diet (CRF1; Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water. Blood glucose lowering test was carried out at 11 weeks old.

Mice were fasted for 18 hours before starting and during experiment. The animals were divided into experimental groups matched for body weights. The experiment was started at 13:00. An evaluating compound dissolved in 10% gelucire, or vehicle were orally administered. New blood samples were taken from the tail tips just before and 30, 60, 120,

and 240 minutes after the administration.

Blood samples were deproteinized with 0.1 N $ZnSO_4$ and 0.075 N $Ba(OH)_2$ following the complete hemolysis with 9 volumes of pure water. After centrifugation with 1,500 × *g* at 4 °C for 10 minutes, the glucose concentration of the supernatant was determined using Glucose C-II Test Wako (Wako Pure Chemical Industries, Ltd.).

Effect of GK activators on blood glucose levels in SD rats was evaluated as follows:

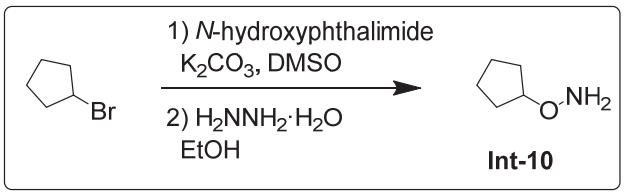
Male 5-week-old SD rats (SLC Japan, Inc., Inasa, Shizuoka, Japan) were kept in a temperature (23 ± 2 °C) and humidity (55 ± 15 %) controlled room with a 12-h light–dark cycle and allowed free access to an ordinary rodent chow diet (CRF1; Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water. Blood glucose lowering test was carried out at 6 weeks old. The Animal Ethics Committee of Tanabe Seiyaku Co., Ltd. approved all the experimental procedures.

Rats were fasted for 18 hours before starting and during experiment. The animals were divided into the experimental groups matched for body weights. The experiment starts at 13:00. Compound **8h** was dissolved in 10% gelucire, or vehicle were orally administered. New blood samples were taken from tail tips under conscious state just before and 30, 60, 120, 240 and 420 minutes after the administration.

Blood samples were deproteinized with 0.1 N $ZnSO_4$ and 0.075 N $Ba(OH)_2$ following the complete hemolysis with 9 volumes of pure water. After centrifugation with 1,500 × *g* at 4 °C for 10 minutes, the glucose concentration of the supernatant was determined using Glucose C-II Test Wako (Wako Pure Chemical Industries, Ltd.).

第３章第２節に関する実験の部

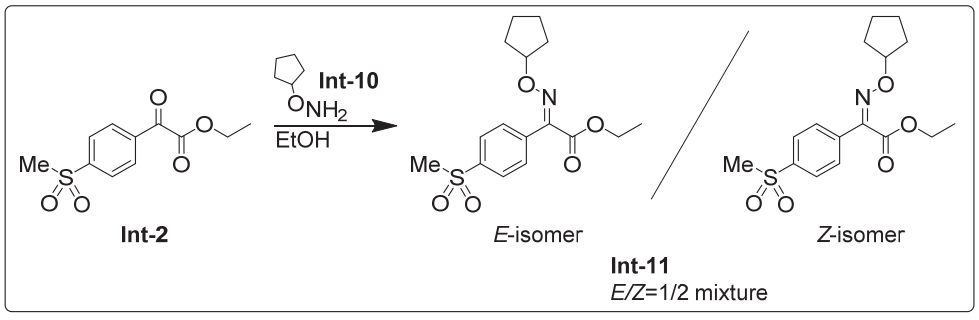
_O_-Cyclopentylhydroxylamine (Int-10)[xx]



To a solution of *N*-hydroxyphthalimide (60.0 g, 368 mmol) in DMSO were added K_2CO_3 (127.2 g, 920 mmol) and bromocyclopentane (82.2 g, 552 mmol) at room temperature. The reaction mixture was stirred at 80℃ for 3 hours. After the mixture was cooled to room temperature, ice-cooled water was added and the resulted suspension was stirred more 1 hour. The precipitates were collected by filtration and washed with water, and dried in vacuo to give 2-(cyclopentoxy)isoindoline-1,3-dione (70.8 g, 83% yield) as a powder. ^{1}H NMR ($CDCl_3$) δ 1.56 – 1.68 (m, 2H), 1.68 – 1.84 (m, 2H), 1.86 – 2.06 (m, 4H), 4.87 – 4.96 (m, 1H), 7.71 – 7.77 (m, 2H), 7.79 – 7.87 (m, 2H). MS (APCI, *m/z*) 232 $[M+H]^+$.

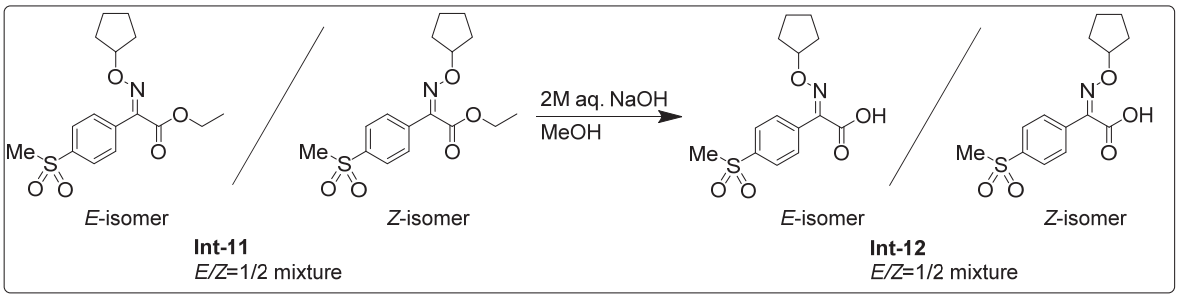
To a solution of 2-(cyclopentoxy)isoindoline-1,3-dione (10.4 g, 44.9 mmol) in CH_2Cl_2 (56 mL) and MeOH (5.5 mL) was added hydrazine monohydrate (4.36 mL, 89.9 mmol) at room temperature. The resulted suspension was stirred at room temperature for 4 hours and insolubles were removed by filtration. The filtrate was washed with 5 M ammonia solution and the aqueous layer was extracted with CH_2Cl_2 twice. The combined organic layers were washed with brine, dried over anhydrous MgSO4, filtered, and concentrated in vacuo to give **Int-10** (3.8 g, 84% yield) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44 – 1.58 (m, 2H), 1.58 – 1.78 (m, 6H), 4.13 – 4.22 (m, 1H), 5.22 (br s, 2H). MS (APCI, *m/z*) 102 $[M+H]^+$.

Ethyl (2*E*)-2-(cyclopentoxyimino)-2-(4-methylsulfonylphenyl)acetate and ethyl (2*Z*)-2-(cyclopentoxyimino)-2-(4-methylsulfonylphenyl)acetate (Int-11)[21]



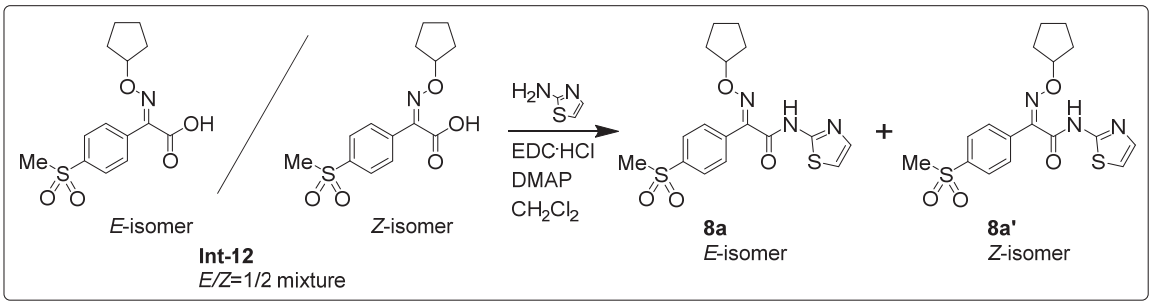
A mixture of **Int-2** (1.00 g, 3.90 mmol) and **Int-10** (592 mg, 5.85 mmol) in EtOH (20 mL) was heated at 60℃ and stirred for 24 hours. The reaction mixture was concentrated in vacuo. The residue oil was purified by NH-silica gel column chromatography (Hexane/EtOAc = 4/1 – 1/1) to give **Int-11** (603 mg, 46% yield) as *E/Z* = 1/2 mixture. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.36 (t, *J* = 7.0 Hz, 0.9H), 1.38 (t, *J* = 7.1 Hz, 2.1 H), 1.55 – 2.00 (m, 8H), 3.05 (s, 2.1H), 3.09 (s, 0.9H), 4.36 (q, *J* = 7.0 Hz, 0.6H), 4.43 (q, *J* = 7.2 Hz, 1.4H), 4.85 – 4.93 (m, 0.7H), 4.93 – 4.98 (m, 0.3H), 7.58 (d, *J* = 8.6 Hz, 0.6H), 7.77 (d, *J* = 8.6 Hz, 1.4H), 7.94 (d, *J* = 8.6 Hz, 1.4H), 7.97 (d, *J* = 0.6H).

(2*E*)-2-(cyclopentoxyimino)-2-(4-methylsulfonylphenyl)acetic acid and (2*Z*)-2-(cyclopentoxyimino)-2-(4-methylsulfonylphenyl)acetic acid (Int-12)



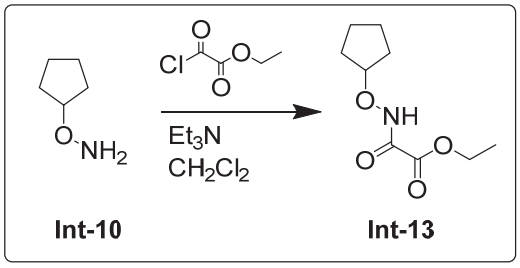
To a solution of **Int-11** (588 mg, 1.73 mmol) in MeOH (1.8 mL) was added 2 M aq NaOH (0.95 mL, 1.90 mmol) and the mixture was stirred at room temperature for 16 hour. Additional 2 M aq NaOH (0.35 mL, 0.70 mmol) and the mixture was stirred at room temperature for 4 hours. The reaction mixture was added 1 M aq HCl (2.6 mL) and stirred for 30 minutes, then insolubles were collected by solvent decantation. The resulted caramel was washed with THF and then concentrated in vacuo to give **Int-12** (564mg, quantitative yield) as a white paste. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.44 – 1.83 (m, 8H), 3.22 (s, 2.1H), 3.27 (s, 0.9H), 4.71 – 4.78 (m, 0.7H), 4.78 – 4.84 (m, 0.3H), 7.61 (d, *J* = 8.6 Hz, 0.6H), 7.76 (d, *J* = 8.8 Hz, 1.4H), 7.95 (d, *J* = 8.6 Hz, 2H). MS (ESI, *m/z*) 643 $[2M-2H+Na]^-$.

(2*E*)-2-(cyclopentoxyimino)-2-(4-methylsulfonylphenyl)-*N*-thiazol-2-yl-acetamide (8a) and (2*Z*)-2-(cyclopentoxyimino)-2-(4-methylsulfonylphenyl)-N-thiazol-2-yl-acetamide (8a')



To an ice-cooling solution of **Int-12** (278 mg, 0.86 mmol), 2-aminothiazole (347 mg, 3.47 mmol) and DMAP (106 mg, 0.87 mmol) in CH_2Cl_2 (4.4 mL) was added EDC·HCl (332 mg, 1.73 mmol) in one portion. After being warmed to room temperature and stirred for 4 hours, the reaction mixture was quenched with additional water and the whole was extracted with EtOAc. The organic layer was dried over Na_2SO_4, filtered, concentrated under reduced pressure, and purified by silica gel column chromatography (Hexane/EtOAc = 70/30 – 40/60) to give less polar isomer **8a'** (43 mg, 13% yield) as a colorless solid and more polar isomer **8a** (34 mg, 10% yield) as a colorless solid. (**8a**) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.58 – 1.70 (m, 4H), 1.82 – 1.93 (m, 4H), 3.08 (s, 3H), 4.93 (m, 1H), 7.09 (d, *J* = 3.6 Hz, 1H), 7.51 (d, *J* = 3.6 Hz, 1H), 7.70 (d, *J* = 8.5 Hz, 2H), 8.02 (d, *J* = 8.8 Hz, 2H), 10.13 (br s, 1H). MS (APCI, *m/z*) 394 $[M+H]^+$. (**8a'**) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.55 – 1.75 (m, 4H), 1.80 – 1.96 (m, 4H), 3.06 (s, 3H), 4.93 (m, 1H), 7.05 (d, *J* = 3.6 Hz, 1H), 7.40 (d, *J* = 3.6 Hz, 1H), 7.89 (d, *J* = 8.8 Hz, 2H), 7.97 (d, *J* = 8.5 Hz, 2H), 11.60 (br s, 1H). MS (APCI, *m/z*) 394 $[M+H]^+$.

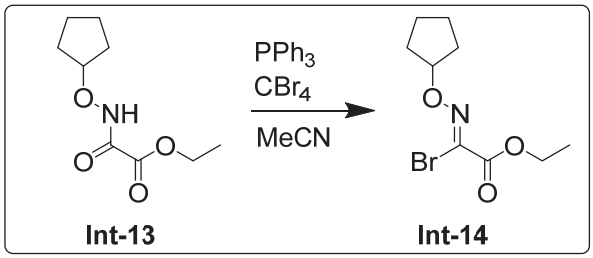
Ethyl 2-(cyclopentoxyamino)-2-oxo-acetate (**Int-13**)



To an ice-cooling solution of **Int-10** (5.00 g, 49.4 mmol) and triethylamine (8.15 mL, 59.0 mmol) in THF (50 mL) was added a solution of chloroglyoxylic acid ethyl ester (5.0 mL, 45.0 mL) dropwise for 30 minutes. After being stirred for 30 minutes, the reaction mixture was warmed to room temperature and stirred for 1 hour. The insolubles were removed by filtration and the filtrate was concentrated to dryness. The residue was purified by silica gel column chromatography (Hexane/EtOAc = 3/1) to give **Int-13** (7.97

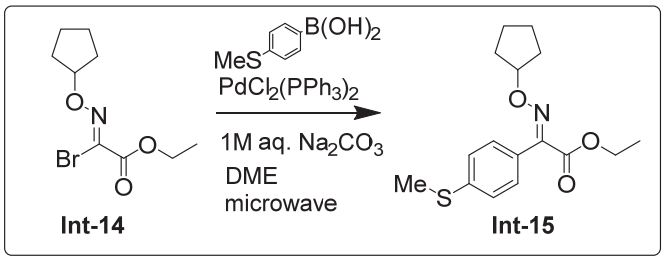
g, 88% yield) as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39 (t, *J* = 7.1 Hz, 3H), 1.52 – 1.64 (m, 2H), 1.66 – 1.96 (m, 6H), 4.35 (q, *J* = 7.2 Hz, 2H), 4.56 – 4.65 (m, 1H), 9.33 (s, 1H). MS (APCI, *m/z*) 202 $[M+H]^+$. IR (nujol, cm^{-1}) 1693, 1757.

Ethyl (2*Z*)-2-bromo-2-(cyclopentoxyimino)acetate (Int-14)[32]



To a stirring solution of **Int-13** (2.00 g, 10.0 mmol) and PPh_3 (3.93 g, 15.0 mmol) in CH_3CN (80 mL) was added CBr_4 (4.97 g, 15.0 mmol) at room temperature. Then, the reaction mixture was stirred at reflux temperature for 3 hours. After the mixture was cooled to room temperature, the solvent was removed in vacuo. The residue caramel was diluted with hexane (50 mL) – Et_2O (50 mL) – CH_3CN (10 mL) and insolubles were removed by filtration. The filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (Hexane/EtOAc = 30/1) to give **Int-14** (1.87 g, 87% yield) as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38 (t, *J* = 7.0 Hz, 3H), 1.58 – 1.66 (m, 2H), 1.66 – 1.82 (m, 2H), 1.82 – 2.02 (m, 4H), 4.38 (q, *J* = 7.1 Hz, 2H), 5.02 (tt, *J* = 5.4 Hz, 2.9 Hz, 1H). MS (APCI, *m/z*) not ionized. IR (neat, cm^{-1}) 1566, 1738. Anal. calcd for $C_9H_{14}NO_3Br$ C: 40.93, H: 5.34, N: 5.30, Br: 30.25; found C: 40.79, H: 5.39, N: 5.25, Br: 30.26.

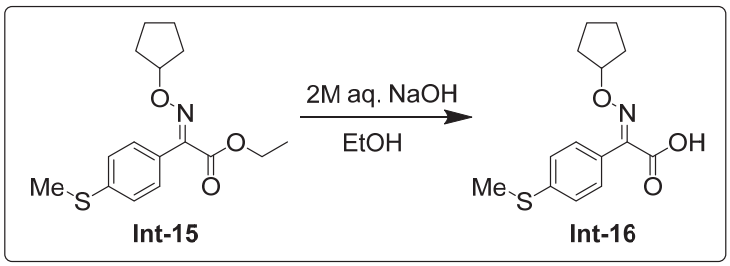
Ethyl (2*E*)-2-(cyclopentoxyimino)-2-(4-methylsulfanylphenyl)acetate (Int-15)[33]



To a solution of **Int-14** (5.28 g, 20 mmol) and 4-(methylthio)phenylboronic acid (5.04 g, 30 mmol) in 1,2-dimethoxyethane (120 mL) were added 1 M aq Na_2CO_3 (60 mL) and

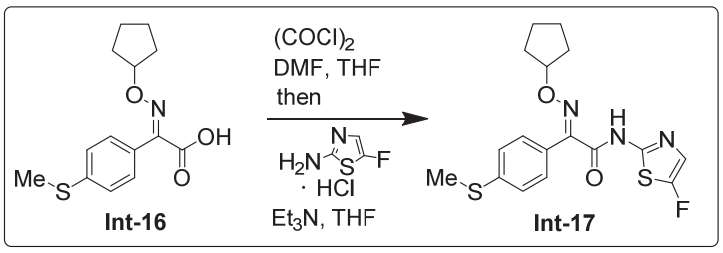
bis(triphenylphosphine)palladium(II) dichloride (1.40 g, 2.0 mmol) under argon atmosphere. The reaction mixture was warmed to 80℃ with microwave irradiation and stirred for 30 minutes. After cooled to room temperature, the reaction mixture was diluted with EtOAc and the whole was washed with water, saturated aq $NaHCO_3$, brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (Hexane/EtOAc = 50/1 – 30/1) to give **Int-15** (4.20 g, 68% yield) as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35 (t, *J* = 7.1 Hz, 3H), 1.48 – 1.72 (m, 4H), 1.76 – 1.92 (m, 4H), 2.50 (s, 3H), 4.34 (q, *J* = 7.1 Hz, 2H), 4.86 – 4.93 (m, 1H), 7.23 (d, *J* = 8.8 Hz, 2H), 7.39 (d, *J* = 8.8 Hz, 2H). MS (APCI, *m/z*) 308 $[M+H]^+$.

(2*E*)-2-(cyclopentoxyimino)-2-(4-methylsulfanylphenyl)acetic acid (**Int-16**)



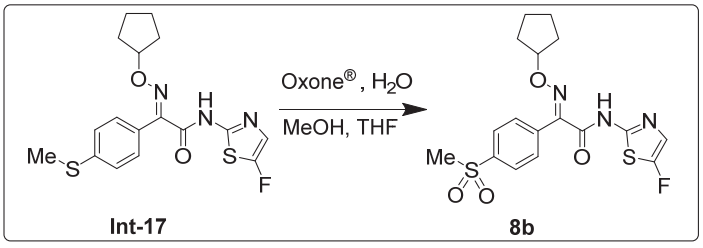
To a solution of **Int-15** (4.00 g, 13.0 mmol) in EtOH (26 mL) was added 2 M aq NaOH (13 mL, 26.0 mmol) and the mixture was stirred at room temperature for 2 hours. The mixture was concentrated in vacuo to remove EtOH, and then the residue was diluted in $CHCl_3$ and water, then acidified with 2 M aq HCl (13 mL) to pH 3. The whole was extracted with $CHCl_3$ 3 times. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo to give **Int-16** (3.58 g, 99% yield) as a yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.56 – 1.76 (m, 4H), 1.83 – 1.93 (m, 4H), 2.50 (s, 3H), 4.85 – 4.95 (m, 1H), 7.26 (d, *J* = 8.8 Hz, 2H), 7.52 (d, *J* = 8.8 Hz, 2H). MS (ESI, *m/z*) 278 $[M-H]^-$.

(2*E*)-2-(cyclopentoxyimino)-*N*-(5-fluorothiazol-2-yl)-2-(4-methylsulfanylphenyl)acetamide (**Int-17**)



A solution of DMF (143 μL, 1.85 mmol) in dry THF (6 mL) was cooled to –25℃ under argon atmosphere and then treated with oxalyl chloride (154 μL, 1.76 mmol). The reaction mixture was allowed to warm to room temperature, sirred for 15 minutes, and re-cooled to –25℃. A solution of **Int-16** (320 mg, 1.14 mmol) in THF (3 mL) was added via syringe. The reaction mixture was stirred at 0℃ for 1 hour and at room temperature for 1 hour. After the reaction mixture was cooled to –45℃, a mixture of 2-amino-5-fluorothiazole hydrochloride (1.13 g, 7.30 mmol) and triethylamine (1.02 mL, 7.30 mmol) in dry THF (3.0 mL) was added via pipet. The reaction mixture was then allowed to warn to 0℃ and stirred for 1 hour. The reaction was quenched with additional water and saturated aq $NaHCO_3$. The whole was extracted with EtOAc 3 times. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (Hexane/EtOAc = 90/10 – 75/25) to give **Int-17** (59 mg, 14% yield) as colorless caramel. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.53 – 1.67 (m, 4H), 1.75 – 1.91 (m, 4H), 2.51 (s, 3H), 4.85 – 4.90 (m, 1H), 7.32 (d, *J* = 8.6 Hz, 2H), 7.39 (d, *J* = 2.6 Hz, 1H), 7.49 (d, *J* = 8.5 Hz, 2H), 12.49 (s, 1H). MS (APCI, *m/z*) 380 $[M+H]^+$.

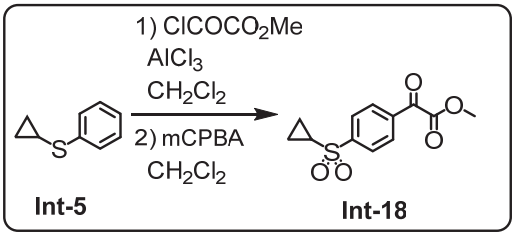
(2*E*)-2-(cyclopentoxyimino)-*N*-(5-fluorothiazol-2-yl)-2-(4-methylsulfonylphenyl)acetamide (8b)



To a solution of **Int-17** (46 mg, 0.12 mmol) in THF (3 mL) and MeOH (3 mL) was added Oxone® (335 mg, 0.54 mmol) in water (2 mL) dropwise in ice-cooling temperature.

The mixture was allowed to warm to room temperature and stirred for 2 hours. The reaction was quenched with additional 10% aq Na_2SO_3 (4 mL) and saturated aq $NaHCO_3$ (3 mL), then the whole was extracted with EtOAc 3 times. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (Hexane/EtOAc = 80/20 – 50/50) to give **8b** (36 mg, 74% yield) as a colorless powder. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.52 – 1.64 (m, 4H), 1.78 – 1.91 (m, 4H), 3.28 (s, 3H), 4.91 – 4.96 (m, 1H), 7.40 (d, *J* = 2.4 Hz, 1H), 7.74 (d, *J* = 8.5 Hz, 2H), 8.01 (d, *J* = 8.5 Hz, 2H), 12.57 (s, 1H). MS (APCI, *m/z*) 412 $[M+H]^+$.

Methyl 2-(4-cyclopropylsulfonylphenyl)-2-oxo-acetate (**Int-18**)

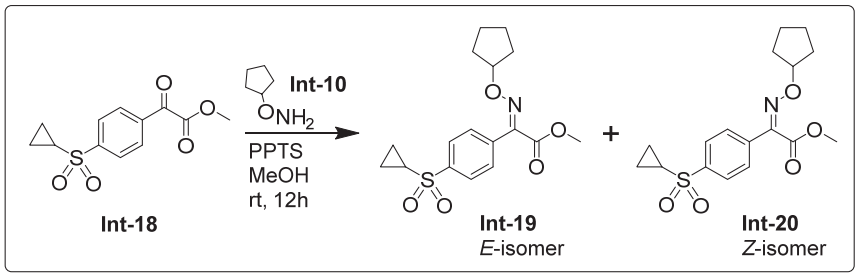


To a solution of aluminum chloride (52.70 g, 395.4 mmol) in CH_2Cl_2 (300 mL) was added chloroglyoxylic acid methyl ester (26.7 mL, 290 mmol) dropwise over 10 minutes under ice-cooling bath temperature, and the mixture was stirred for 30 minutes at the same temperature. Then, a solution of cyclopropyl phenyl sulfide (**Int-5**: 39.60 g, 263.5 mmol) in CH_2Cl_2 (10 ml) was added dropwise to the above mixture over 15 minutes. After the ice-water bath was removed, the reaction mixture was stirred at room temperature for 2 hours. Water was carefully added to the reaction mixture under ice-cooling bath temperature, then the whole was extracted with EtOAc 3 times. The combined organic layers were washed with water, saturated aq $NaHCO_3$, brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was triturated in EtOAc to give methyl 2-(4-cyclopropylsulfanylphenyl)-2-oxo-acetate as a yellow powder (43.51 g, 70% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.65-0.83 (m, 2H), 1.07-1.29 (m, 2H), 2.20 (tt, *J* = 7.4 Hz and 4.4 Hz, 1H), 3.97 (s, 3H), 7.44 (d, *J* = 8.8 Hz, 2H), 7.93 (d, *J* = 9.0 Hz, 2H). MS (APCI, *m/z*) 237 $[M+H]^+$.

To a solution of methyl 2-(4-cyclopropylsulfanylphenyl)-2-oxo-acetate (5.00 g, 21.16 mmol) in THF (65 mL) and MeOH (65 mL) was added a solution of Oxone® (15.6 g, 25.39 mmol) in water (45 mL) under ice-cooling bath temperature. The mixture was

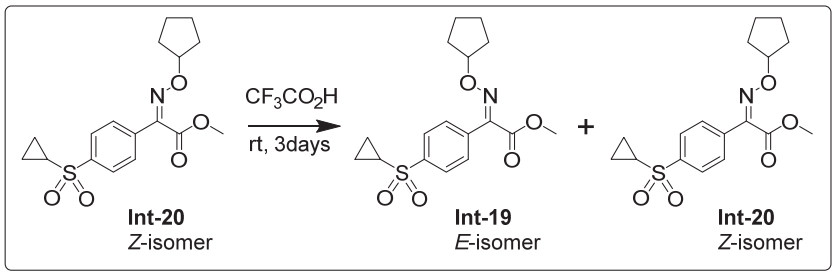
allowed to warm to room temperature and stirred for 20 hours. An insoluble material was removed by filtration and the filtrate was extracted with EtOAc 3 times. The combined organic layers were washed with saturated aq $NaHCO_3$, brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was recrystallized from hexane – EtOAc to give **Int-18** as a colorless powder (3.86 g, 68% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.05 – 1.15 (m, 2H), 1.35 – 1.45 (m, 2H), 2.49 (tt, *J* = 7.9 Hz, 4.8 Hz, 1H), 4.02 (s, 3H), 8.05 (d, *J* = 8.8 Hz, 2H), 8.23 (d, *J* = 8.8 Hz, 2H). MS (APCI, *m/z*) 286 $[M+NH_4]^+$. IR (nujol, cm^{-1}) 1693, 1735. Anal. calcd for $C_{12}H_{12}O_5S$ C: 53.72, H: 4.51, S: 11.95; found C: 53.72, H: 4.51, S: 11.95.

Methyl (2*E*)-2-(cyclopentoxyimino)-2-(4-cyclopropylsulfonylphenyl)acetate (**Int-19**) and **Methyl (2*Z*)-2-(cyclopentoxyimino)-2-(4-cyclopropylsulfonylphenyl)acetate** (**Int-20**)



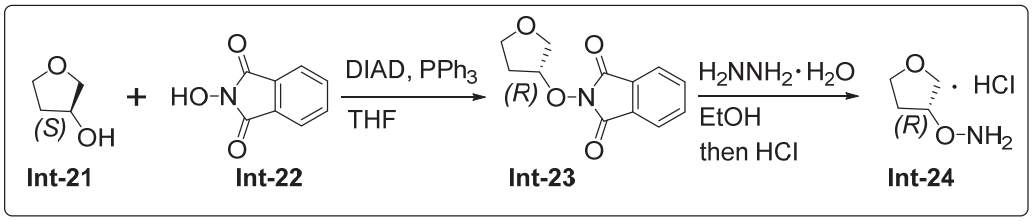
To a mixture of **Int-18** (10.0 g, 37.2 mmol) and **Int-10** (4.86 g, 48.0 mmol) in MeOH (100 mL) was added pyridinium *p*-toluenesulfonate (PPTS, 10 mg, 0.04 mmol) and the mixture was stirred at room temperature for 12 hours. Further addition of **Int-10** (3.54 g, 35.0 mmol), the mixture was stirred for 24 hours and concentrated in vacuo. The residual oil was purified by silica gel column chromatography (Hexane/EtOAc = 65/35) to give less polar isomer **Int-20** (6.17 g, 47% yield) as yellow oil and more polar isomer **Int-19** (3.05 g, 23% yield) as a colorless powder. (**Int-19**) ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98 – 1.16 (m, 2H), 1.34 – 1.43 (m, 2H), 1.50 – 1.68 (m, 4H), 1.76 – 1.90 (m, 4H), 2.49 (tt, *J* = 5.8 Hz, 4.7 Hz, 1H), 3.90 (s, 3H), 4.94 (tt, *J* = 4.2 Hz, 4.0 Hz, 1H), 7.57 (d, *J* = 8.6 Hz, 2H), 7.92 (d, *J* = 8.8 Hz, 2H). MS (APCI, *m/z*) 352 $[M+H]^+$. (**Int-20**) ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98 – 1.14 (m, 2H), 1.30 – 1.44 (m, 2H), 1.52 – 1.76 (m, 4H), 1.76 – 2.02 (m, 4H), 2.49 (tt, *J* = 4.9 Hz, 4.7 Hz, 1H), 3.94 (s, 3H), 4.85 – 4.92 (m, 1H), 7.74 (d, *J* = 8.8 Hz, 2H), 7.90 (d, *J* = 8.8 Hz, 2H). MS (APCI, *m/z*) 352 $[M+H]^+$.

Oxime *E/Z* isomerization of Methyl (2*Z*)-2-(cyclopentoxyimino)-2-(4-cyclopropylsulfonylphenyl)acetate (Int-20)



A solution of **Int-20** (6.17 g, 17.56 mmol) was dissolved in trifluoroacetic acid (60 mL) and the mixture was stirred at room temperature for 3 days. The solvent was removed in vacuo and the residual oil was purified by silica gel column chromatography (Hexane/EtOAc = 3/1) to give **Int-19** (3.72 g, 60% yield) and **Int-20** (1.69 g, 27% yield).

***O*-[(3*R*)-tetrahydrofuran-3-yl]hydroxylamine;hydrochloride (Int-24)**

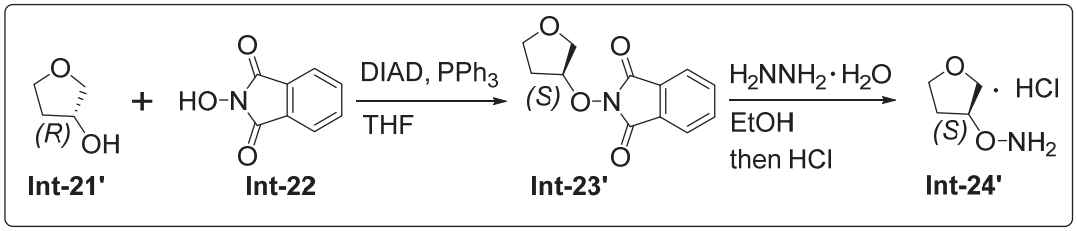


To a solution of *N*-hydroxyphthalimide (**Int-22**: 17.99 g, 110.3 mmol) and triphenylphosphine (PPh_3: 32.15 g, 122.58 mmol) in THF (360 mL) was added (*S*)-(+)-3-hydroxytetrahydrofurane (**Int-21**: 9.0 g, 102.15 mmol), then cooled in ice-water bath temperature. A solution of diisopropylazodicarboxylate (DIAD, 24.78 g, 122.58 mmol) was added dropwise over 30 minutes. After being stirred at the same temperature for 1 hour, the reaction mixture was allowed to warm to room temperature and stirred for 15 hours. The reaction mixture was concentrated in vacuo and the residue oil was purified by silica gel column chlomatography (Hexane/EtOAc = 65/35), then recrystallized from EtOAc to give **Int-23** (14.53 g, 61% yield) as a colorless powder. MS (APCI, *m/z*) 234 $[M+H]^+$. $[\alpha]_D^{23}$ –10.02 (*c* = 1.00, MeOH).

To a solution of **Int-23** in EtOH (31 mL) was added hydrazine monohydrate (1.54 mL, 31.87 mmol) and the mixture was stirred at reflux temperature for 30 minutes. To the mixture was added concentrated HCl (3.4 mL), then the mixture was stirred more 5 minutes, diluted with water (10.8 mL) and cooled to room temperature. Insolubles were removed by filtration and washed with EtOH (15 mL). The filtrate was concentrated in

vacuo. The resulted solid was recrystallized from THF – Et_2O to give **Int-24** (2.28 g, 53% yield) as a colorless powder. ^{1}H NMR (500MHz, DMSO-d_6) δ 2.00 – 2.15 (m, 2H), 3.63 – 3.70 (m, 2H), 3.76 (q, *J* = 7.7 Hz, 1H), 3.92 (d, *J* = 10.9 Hz, 1H), 4.75 – 4.90 (m, 1H), 10.77 (br s, 3H). MS (APCI, *m/z*) 104 $[M+H]^+$. mp 144 – 145°C. $[\alpha]_D^{23}$ –0.99 (*c* = 1.01, MeOH). Anal calcd for $C_4H_9NO_2$·HCl C: 34.42, H: 7.22, N: 10.03, Cl: 25.40; found C: 34.28, H: 7.35, N: 10.11, Cl: 25.35.

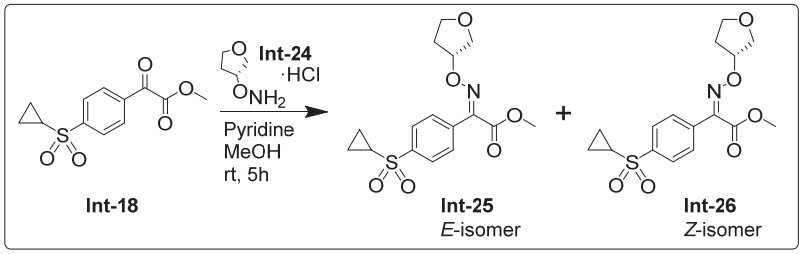
***O*-[(3*S*)-tetrahydrofuran-3-yl]hydroxylamine;hydrochloride (Int-24')**



The intermediate **Int-23'** was prepared in the same manner as described for **Int-23** using **Int-21'** in 94% yield as a colorless powder. MS (APCI, *m/z*) 234 $[M+H]^+$. $[\alpha]_D^{25}$ +13.82 (*c* = 1.00, MeOH).

The title compound was prepared in the same manner as described for **Int-24** using **Int-23'** in 92% yield as a colorless powder. ^{1}H NMR (500MHz, DMSO-d_6) δ 2.00 – 2.15 (m, 2H), 3.63 – 3.70 (m, 2H), 3.76 (q, *J* = 7.7 Hz, 1H), 3.92 (d, *J* = 10.9 Hz, 1H), 4.77 – 4.88 (m, 1H), 10.77 (br s, 3H). MS (APCI, *m/z*) 104 $[M+H]^+$. $[\alpha]_D^{23}$ +1.19 (*c* = 1.01, MeOH). mp 145 – 146°C. Anal calcd for $C_4H_9NO_2$·HCl C: 34.42, H: 7.22, N: 10.03, Cl: 25.40; found C: 34.14, H: 7.30, N: 10.26, Cl: 25.23.

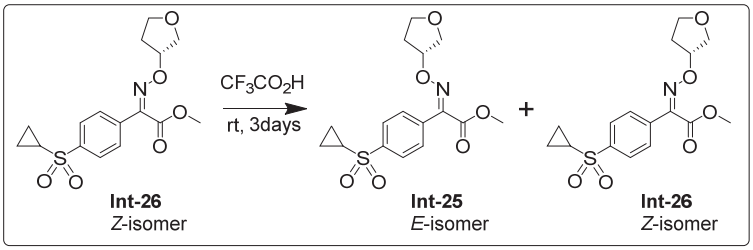
Methyl (2*E*)-2-(4-cyclopropylsulfonylphenyl)-2-[(3*R*)-tetrahydrofuran-3-yl]oxyimino-acetate (Int-25) and **Methyl (2*Z*)-2-(4-cyclopropylsulfonylphenyl)-2-[(3*R*)-tetrahydrofuran-3-yl]oxyimino-acetate (Int-26)**



To a mixture of **Int-18** (5.0 g, 18.64 mmol) and **Int-24** (3.12 g, 22.36 mmol) in MeOH

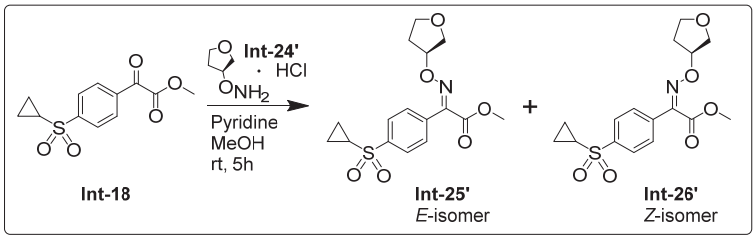
(50 mL) was added pyridine (2.36 mL, 27.96 mmol) and the mixture was stirred at room temperature for 5 hours. The mixture was concentrated in vacuo. The residual oil was acidified with 1 M aq HCl and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The redidue oil was purified by silica gel column chromatography (Hexane/EtOAc = 75/25 – 0/100) to give less polar isomer **Int-26** (4.68 g, 64% yield) as a colorless powder and more polar isomer **Int-25** (2.21 g, 34% yield) as colorless oil. (**Int-25**) ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.04 – 1.13 (m, 2H), 1.35 – 1.44 (m, 2H), 2.05 – 2.26 (m, 2H), 2.49 (tt, *J* = 8.0 Hz, 4.8 Hz, 1H), 3.78 – 3.92 (m, 3H), 3.91 (s, 3H), 3.98 – 4.03 (m, 1H), 5.08 – 5.14 (m, 1H), 7.59 (d, *J* = 8.8 Hz, 2H), 7.94 (d, *J* = 8.8 Hz, 2H). MS (APCI, *m/z*) 354 $[M+H]^+$. $[\alpha]_D^{25}$ +13.97 (*c* = 1.00, MeOH). (**Int-26**) ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95 – 1.15 (m, 2H), 1.32 – 1.41 (m, 2H), 2.13 – 2.55 (m, 2H), 2.45 (tt, *J* = 7.9 Hz, 4.8 Hz, 1H), 3.82 – 3.97 (m, 3H), 3.96 (s, 3H), 3.99 – 4.06 (m, 1H), 5.03 – 5.09 (m, 1H), 7.74 (d, *J* = 8.8 Hz, 2H), 7.91 (d, *J* = 8.8 Hz, 2H). MS (APCI, *m/z*) 354 $[M+H]^+$.

Oxime *E/Z* isomerization of Methyl (2*Z*)-2-(4-cyclopropylsulfonylphenyl)-2-[(3*R*)-tetrahydrofuran-3-yl]oxyimino-acetate (Int-26)



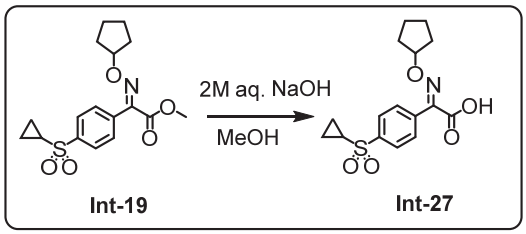
In the same manner as described for **Int-20** using **Int-26**, **Int-25** was obtained in 64% yield and **Int-26** was obtained in 32% yield.

Methyl (2*E*)-2-(4-cyclopropylsulfonylphenyl)-2-[(3*S*)-tetrahydrofuran-3-yl]oxyimino-acetate (Int-25') and **Methyl (2*Z*)-2-(4-cyclopropylsulfonylphenyl)-2-[(3*S*)-tetrahydrofuran-3-yl]oxyimino-acetate (Int-26')**



To a mixture of **Int-18** (5.0 g, 18.64 mmol) and **Int-24'** (3.12 g, 22.36 mmol) in MeOH (50 mL) was added pyridine (2.36 mL, 27.96 mmol) and the mixture was stirred at room temperature for 17 hours. The mixture was concentrated in vacuo. The residual oil was acidified with 1 M aq HCl and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The redidue oil was purified by silica gel column chromatography (Hexane/EtOAc = 75/25 – 0/100) to give less polar isomer **Int-26'** (4.31 g, 65% yield) as a colorless powder and more polar isomer **Int-25'** (2.54 g, 39% yield) as colorless oil. (**Int-25'**) ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.04 – 1.13 (m, 2H), 1.35 – 1.44 (m, 2H), 2.05 – 2.26 (m, 2H), 2.49 (tt, *J* = 8.0 Hz, 4.8 Hz, 1H), 3.78 – 3.92 (m, 3H), 3.91 (s, 3H), 3.98 – 4.03 (m, 1H), 5.08 – 5.14 (m, 1H), 7.59 (d, *J* = 8.8 Hz, 2H), 7.94 (d, *J* = 8.8 Hz, 2H). MS (APCI, *m/z*) 354 $[M+H]^+$. $[\alpha]_D^{25}$ –13.37 (*c* = 1.00, MeOH). (**Int-26'**) ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95 – 1.15 (m, 2H), 1.32 – 1.41 (m, 2H), 2.13 – 2.55 (m, 2H), 2.45 (tt, *J* = 7.9 Hz, 4.8 Hz, 1H), 3.82 – 3.97 (m, 3H), 3.96 (s, 3H), 3.99 – 4.06 (m, 1H), 5.03 – 5.09 (m, 1H), 7.74 (d, *J* = 8.8 Hz, 2H), 7.91 (d, *J* = 8.8 Hz, 2H). MS (APCI, *m/z*) 354 $[M+H]^+$.

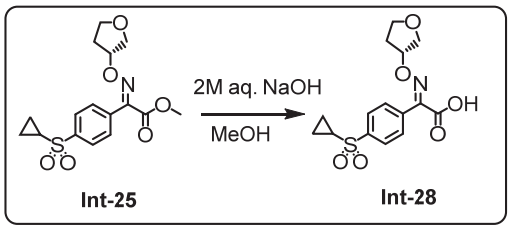
(2*E*)-2-(cyclopentoxyimino)-2-(4-cyclopropylsulfonylphenyl)acetic acid (**Int-27**)



To a solution of **Int-19** (6.7 g, 19.1 mmol) in MeOH (70 mL) was added 2 M aq NaOH (14.3 mL, 28.6 mmol) and the mixture was stirred at ice-cooling temperature for 2 hours. The mixture was diluted with CH_2Cl_2 and acidified with 2 M aq HCl to pH 3. The whole was extracted with CH_2Cl_2 three times. The combined organic layers were washed with water, brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The residue solid was triturated in Et_2O to give **Int-27** (5.8 g, 90% yield) as a colorless powder.

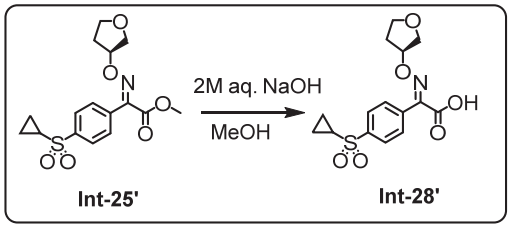
^{1}H NMR (500 MHz, DMSO-d$_6$) δ 1.02 – 1.11 (m, 2H), 1.11 – 1.22 (m, 2H), 1.49 – 1.58 (m, 4H), 1.66 – 1.86 (m, 4H), 2.89 – 2.97 (m, 1H), 4.80 – 4.86 (m, 1H), 7.62 (d, *J* = 8.2 Hz, 2H), 7.93 (d, *J* = 8.4 Hz, 2H), 13.54 (br s, 1H). MS (APCI, *m/z*) 673 $[2M-H]^-$.

(2*E*)-2-(4-cyclopropylsulfonylphenyl)-2-[(3*R*)-tetrahydrofuran-3-yl]oxyimino-acetic acid (Int-28)



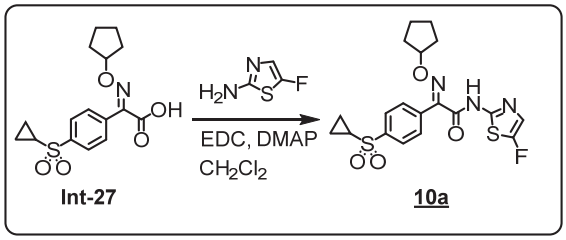
The title compound was prepared in the same manner as described for **Int-27** using **Int-25** in 90% yield as a colorless powder. ^{1}H NMR (500 MHz, DMSO-d$_6$) δ 1.01 – 1.12 (m, 2H), 1.12 – 1.21 (m, 2H), 1.93 – 2.03 (m, 1H), 2.07 – 2.18 (m, 1H), 2.86 – 2.99 (m, 1H), 3.63 – 3.73 (m, 2H), 3.73 – 3.87 (m, 2H), 5.02 – 5.08 (m, 1H), 7.66 (d, *J* = 8.5 Hz, 2H), 7.95 (d, *J* = 8.3 Hz, 2H), 13.62 (br s, 1H). MS (APCI, *m/z*) 354 $[M+H]^+$. $[\alpha]_D^{25}$ +14.71 (*c* = 1.01, MeOH).

(2*E*)-2-(4-cyclopropylsulfonylphenyl)-2-[(3*S*)-tetrahydrofuran-3-yl]oxyimino-acetic acid (Int-28')



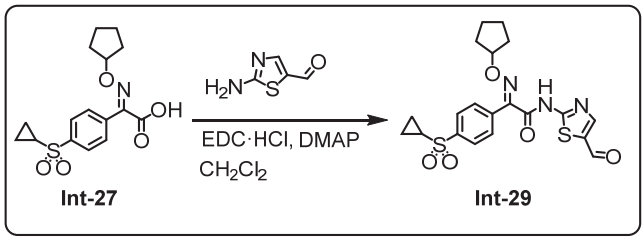
The title compound was prepared in the same manner as described for **Int-27** using **Int-25'** in 87% yield as a colorless powder. ^{1}H NMR (500 MHz, DMSO-d$_6$) δ 1.01 – 1.12 (m, 2H), 1.12 – 1.21 (m, 2H), 1.93 – 2.03 (m, 1H), 2.07 – 2.18 (m, 1H), 2.86 – 2.99 (m, 1H), 3.63 – 3.73 (m, 2H), 3.73 – 3.87 (m, 2H), 5.02 – 5.08 (m, 1H), 7.66 (d, *J* = 8.5 Hz, 2H), 7.95 (d, *J* = 8.3 Hz, 2H), 13.62 (br s, 1H). MS (APCI, *m/z*) 354 $[M+H]^+$. $[\alpha]_D^{25}$ –14.14 (*c* = 1.00, MeOH).

(2*E*)-2-(cyclopentoxyimino)-2-(4-cyclopropylsulfonylphenyl)-*N*-(5-fluorothiazol-2-yl)acetamide (10a)



To an ice-cooling solution of **Int-27** (100 mg, 0.296 mmol), 2-amino-5-fluorothiazole (137 mg, 0.888 mmol) and DMAP (54 mg, 0.444 mmol) in CH_2Cl_2 (3 mL) was added EDC (80 μL, 0.444 mmol) in one portion. Then the mixture was stirred at room temperature for 24 hours and concentrated to dryness. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 100/0 – 95/5) to give **10a** (51 mg, 39% yield) as a colorless powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.02 – 1.12 (m, 2H), 1.34 – 1.42 (m, 2H), 1.56 – 1.70 (m, 4H), 1.78 – 1.93 (m, 4H), 2.44 – 2.55 (m, 1H), 4.87 – 4.97 (m, 1H), 7.09 (d, *J* = 2.7 Hz, 1H), 7.65 (d, *J* = 8.8 Hz, 2H), 7.97 (d, *J* = 8.8 Hz, 2H), 9.86 (s, 1H). MS (APCI, *m/z*) 438 $[M+H]^+$.

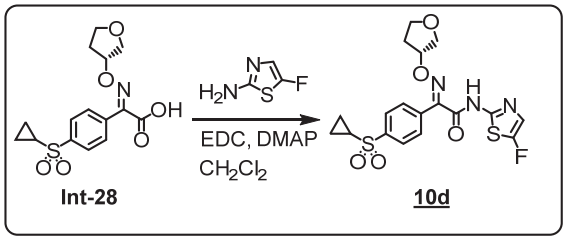
(2*E*)-2-(cyclopentoxyimino)-2-(4-cyclopropylsulfonylphenyl)-*N*-(5-formylthiazol-2-yl)acetamide (**Int-29**)



To an ice-cooling solution of **Int-27** (2.40 g, 7.11 mmol), 2-amino-5-formylthiazole (1.18 g, 9.25 mmol) and DMAP (1.04 g, 8.53 mmol) in CH_2Cl_2 (60 mL) was added EDC·HCl (1.68 g, 8.53 mmol) in one portion. Then the mixture was stirred at room temperature for 18 hours and concentrated to dryness. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 100/0 – 98/2) and then triturated in Et_2O to give **Int-29** (1.66 g, 52% yield) as a yellowish powder. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.05 – 1.12 (m, 2H), 1.13 – 1.22 (m, 2H), 1.50 – 1.65 (m, 4H), 1.75 – 1.95 (m, 4H), 2.92 – 2.98 (m, 1H), 4.90 – 5.00 (m, 1H), 7.76 (d, *J* = 8.5 Hz, 2H), 7.99 (d, *J* = 8.4 Hz, 2H), 8.52 (s, 1H), 10.00 (s, 1H), 13.13 (br s, 1H). MS (APCI, *m/z*) 448 $[M+H]^+$.

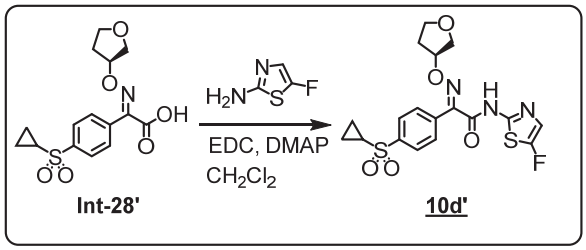
(2*E*)-2-(4-cyclopropylsulfonylphenyl)-*N*-(5-fluorothiazol-2-yl)-2-[(3*R*)-

tetrahydrofuran-3-yl]oxyimino-acetamide (10d)



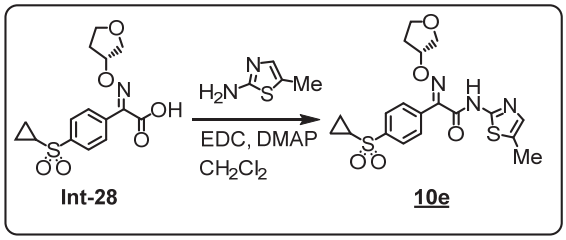
The title compound was prepared in the same manner as described for **10a** using **Int-28** instead of **Int-27** in 32% yield as a colorless powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.00-1.18 (m, 2H), 1.32-1.43 (m, 2H), 2.08-2.32 (m, 2H), 2.49 (tt, *J* = 8.0 Hz, 4.9 Hz, 1H), 3.80-3.95 (m, 3H), 4.04 (d, *J* = 10.8 Hz, 1H), 5.07-5.13 (m, 1H), 7.10 (d, *J* = 2.9 Hz, 1H), 7.67 (d, *J* = 8.6 Hz, 2H), 7.98 (d, *J* = 8.6 Hz, 2H), 9.84 (s, 1H). MS (APCI, *m/z*) 440 $[M+H]^+$. HPLC 99.34% (t_R = 11.6 min, L-column ODS (5 μM particle size, 4.6 x 150 mm), CH_3CN/20 mM phosphate buffer (pH 6.5) (40/60)).

(2*E*)-2-(4-cyclopropylsulfonylphenyl)-*N*-(5-fluorothiazol-2-yl)-2-[(3*S*)-tetrahydrofuran-3-yl]oxyimino-acetamide (10d')



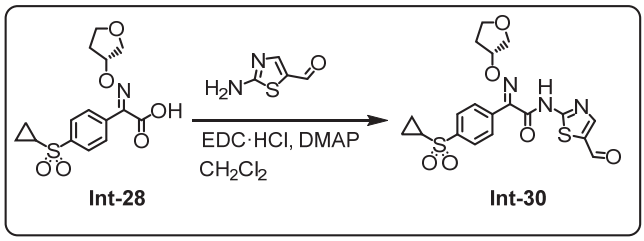
The title compound was prepared in the same manner as described for **10a** using **Int-28'** in 32% yield as a colorless powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.00-1.18 (m, 2H), 1.32-1.43 (m, 2H), 2.08-2.32 (m, 2H), 2.49 (tt, *J* = 8.0 Hz, 4.9 Hz, 1H), 3.80-3.95 (m, 3H), 4.04 (d, *J* = 10.8 Hz, 1H), 5.07-5.13 (m, 1H), 7.10 (d, *J* = 2.9 Hz, 1H), 7.67 (d, *J* = 8.6 Hz, 2H), 7.98 (d, *J* = 8.6 Hz, 2H), 9.84 (s, 1H). MS (APCI, *m/z*) 440 $[M+H]^+$.

(2*E*)-2-(4-cyclopropylsulfonylphenyl)-*N*-(5-methylthiazol-2-yl)-2-[(3*R*)-tetrahydrofuran-3-yl]oxyimino-acetamide (10e)



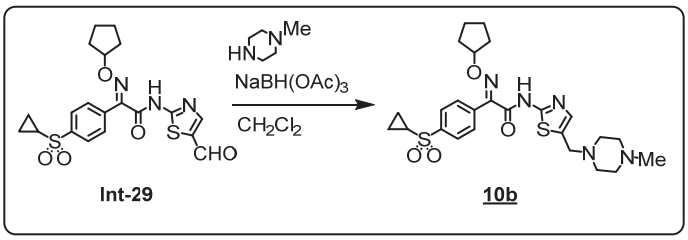
The title compound was prepared in the same manner as described for **10a** using **Int-28** and 2-amino-5-methylthiazole in 70% yield as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.03 – 1.13 (m, 2H), 1.35 – 1.45 (m, 2H), 2.07 – 2.31 (m, 2H), 2.43 (d, *J* = 1.1 Hz, 3H), 2.44-2.55 (m, 1H), 3.80 – 3.95 (m, 3H), 4.04 (d, *J* = 10.8 Hz, 1H), 5.04 – 5.20 (m, 1H), 7.15 (q, *J* = 1.3 Hz, 1H), 7.69 (d, *J* = 8.8 Hz, 2H), 7.97 (d, *J* = 8.6 Hz, 2H), 9.98 (br s, 1H). MS (APCI, *m/z*) 436 $[M+H]^+$.

(2*E*)-2-(4-cyclopropylsulfonylphenyl)-*N*-(5-formylthiazol-2-yl)-2-[(3*R*)-tetrahydrofuran-3-yl]oxyimino-acetamide (**Int-30**)



The title compound was prepared in the same manner as described for **Int-29** using **Int-28** instead of **Int-27** in 32% yield as a yellowish powder. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.06 – 1.13 (m, 2H), 1.13 – 1.20 (m, 2H), 2.09 – 2.23 (m, 2H), 2.91 – 2.99 (m, 1H), 3.67 – 3.81 (m, 3H), 4.03 (d, *J* = 10.8 Hz, 1H), 5.12 – 5.17 (m, 1H), 7.79 (d, *J* = 8.3 Hz, 2H), 7.99 (d, *J* = 8.5 Hz, 2H), 8.51 (s, 1H), 9.99 (s, 1H), 13.22 (br s, 1H). MS (APCI, *m/z*) 450 $[M+H]^+$.

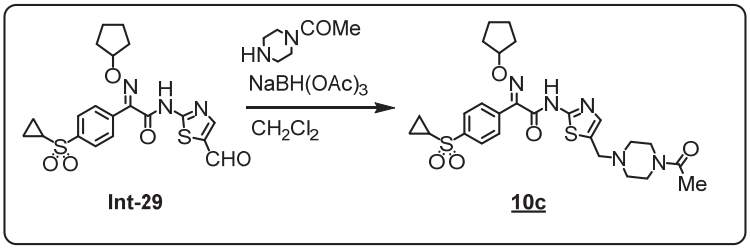
(2*E*)-2-(cyclopentoxyimino)-2-(4-cyclopropylsulfonylphenyl)-*N*-[5-[(4-methylpiperazin-1-yl)methyl]thiazol-2-yl]acetamide (**10b**)



To a stirred solution of **Int-29** (100 mg, 0.22 mmol), 1-methylpiperazine (33.6 mg, 0.34

mmol) in CH_2Cl_2 (3 mL) was added sodium triacetoxyborohydride (72 mg, 0.34 mmol) in one portion. Then the mixture was stirred at room temperature for 24 hours. The reaction was diluted with water and extracted with $CHCl_3$. The organic extract was dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by NH-silica gel column chromatography ($CHCl_3$/MeOH = 100/0 – 95/5) to give **10b** (94.7 mg, 80% yield) as a colorless powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.03 – 1.11 (m, 2H), 1.35 – 1.43 (m, 2H), 1.57 – 1.71 (m, 4H), 1.82 – 1.92 (m, 4H), 2.27 (s, 3H), 2.20 – 2.70 (m, 8H), 2.43 – 2.55 (m, 1H), 3.69 (d, *J* = 0.9 Hz, 2H), 4.88 – 4.96 (m, 1H), 7.29 (s, 1H), 7.68 (d, *J* = 8.6 Hz, 2H), 7.95 (d, *J* = 8.8 Hz, 2H), 10.00 (br s, 1H). MS (APCI, *m/z*) 532 $[M+H]^+$.

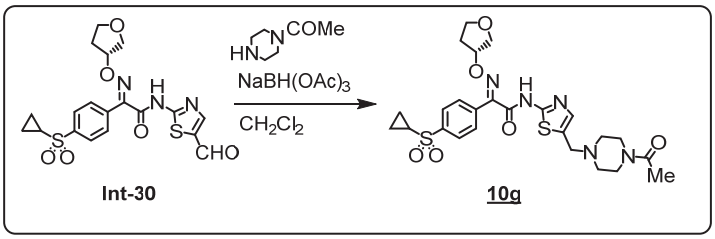
(2*E*)-*N*-[5-[(4-acetylpiperazin-1-yl)methyl]thiazol-2-yl]-2-(cyclopentoxyimino)-2-(4-cyclopropylsulfonylphenyl)acetamide (10c)



Int-29 **10c**

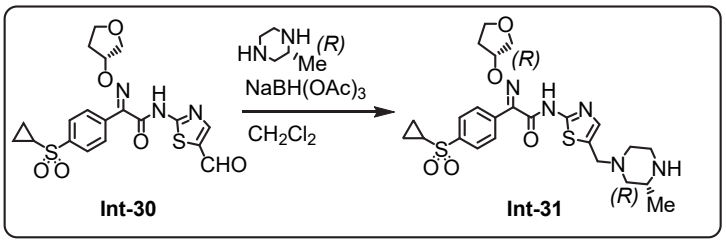
The title compound was prepared in the same manner as described for **10b** using 1-acetylpiperadine in 86% yield as a colorless powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.02 – 1.12 (m, 2H), 1.34 – 1.43 (m, 2H), 1.56 – 1.72 (m, 4H), 1.78 – 1.94 (m, 4H), 2.08 (s, 3H), 2.41 – 2.53 (m, 5H), 3.45 (t, *J* = 5.4 Hz, 1H), 3.61 (t, *J* = 5.4 Hz, 1H), 3.71 (s, 2H), 4.88 – 4.96 (m, 1H), 7.29 (s, 1H), 7.67 (d, *J* = 8.6 Hz, 2H), 7.96 (d, *J* = 8.6 Hz, 2H), 10.10 (s, 1H). MS (APCI, *m/z*) 560 $[M+H]^+$.

(2*E*)-*N*-[5-[(4-acetylpiperazin-1-yl)methyl]thiazol-2-yl]-2-(4-cyclopropylsulfonylphenyl)-2-[(3*R*)-tetrahydrofuran-3-yl]oxyimino-acetamide (10g)



To a stirred solution of **Int-30** (500 mg, 1.11 mmol), 1-acetylpiperazine (428 mg, 3.34 mmol) in $CHCl_3$ (20 mL) was added sodium triacetoxyborohydride (707 mg, 3.34 mmol) in one portion. Then the mixture was stirred at room temperature for 20 hours. The reaction was diluted with saturated aq $NaHCO_3$ and extracted with EtOAc 3 times. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 100/0 – 95/5) and then recrystallization from EtOAc – hexane to give **10g** (551 mg, 89% yield) as a colorless powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.03 – 1.13 (m, 2H), 1.34 – 1.44 (m, 2H), 2.07 (s, 3H), 2.10 – 2.32 (m, 2H), 2.42 – 2.55 (m, 5H), 3.45 (t, *J* = 5.0 Hz, 2H), 3.61 (t, *J* = 5.0 Hz, 1H), 3.71 (d, *J* = 0.7 Hz, 2H), 3.80 – 3.96 (m, 2H), 4.05 (d, *J* = 11.0 Hz, 1H), 5.06 – 5.12 (m, 1H), 7.29 (s, 1H), 7.69 (d, *J* = 8.8 Hz, 2H), 7.97 (d, *J* = 8.8 Hz, 2H), 10.00 (s, 1H). MS (APCI, *m/z*) 562 $[M+H]^+$. Anal calcd for $C_{25}H_{31}N_5O_6S_2$ C: 53.39, H: 5.56, N: 12.47, S: 11.42; found C: 53.39, H: 5.44, N: 12.44, S: 11.35. mp 159 – 161℃.

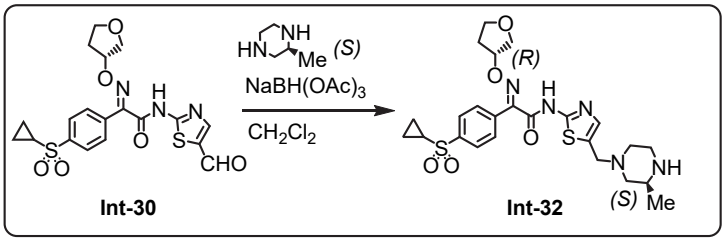
(2*E*)-2-(4-cyclopropylsulfonylphenyl)-*N*-[5-[[(3*R*)-3-methylpiperazin-1-yl]methyl]thiazol-2-yl]-2-[(3*R*)-tetrahydrofuran-3-yl]oxyimino-acetamide (**Int-31**)



The title compound was prepared in the same manner as described for **10g** using (*R*)-2-methylpiperazine in 49% yield as a yellowish powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.02 (d, *J* = 6.4 Hz, 3H), 1.03 – 1.13 (m, 2H), 1.35 – 1.45 (m, 2H), 1.74 (t, *J* = 10.4 Hz, 1H), 2.00 – 2.30 (m, 3H), 2.49 (tt, J = 7.9 Hz, 4.8 Hz, 1H), 2.75 – 3.02 (m, 5H), 3.68 (s, 2H), 3.80 – 3.95 (m, 3H), 4.05 (d, *J* = 10.8 Hz, 1H), 5.05 – 5.15 (m, 1H), 7.28 (s, 1H),

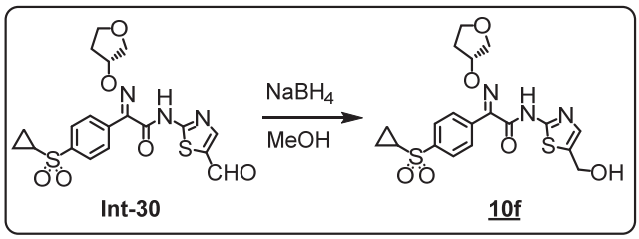
7.68 (d, *J* = 8.8 Hz, 2H), 7.97 (d, *J* = 8.8 Hz, 2H), 9.20 – 9.50 (br s, 1H). MS (APCI, *m/z*) 534 $[M+H]^+$.

(2*E*)-2-(4-cyclopropylsulfonylphenyl)-N-[5-[[(3*S*)-3-methylpiperazin-1-yl]methyl]thiazol-2-yl]-2-[(3*R*)-tetrahydrofuran-3-yl]oxyimino-acetamide (**Int-32**)



The title compound was prepared in the same manner as described for **10g** using (*S*)-2-methylpiperazine in 73% yield as a yellowish powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.01 (d, *J* = 6.2 Hz, 3H), 1.03 – 1.13 (m, 2H), 1.35 – 1.45 (m, 2H), 1.72 (t, *J* = 10.4 Hz, 1H), 2.00 – 2.32 (m, 3H), 2.49 (tt, J = 7.9 Hz, 4.8 Hz, 1H), 2.74 – 3.00 (m, 5H), 3.68 (s, 2H), 3.80 – 3.95 (m, 3H), 4.04 (d, *J* = 10.8 Hz, 1H), 5.05 – 5.12 (m, 1H), 7.29 (s, 1H), 7.69 (d, *J* = 8.8 Hz, 2H), 7.97 (d, *J* = 8.6 Hz, 2H). MS (APCI, *m/z*) 534 $[M+H]^+$.

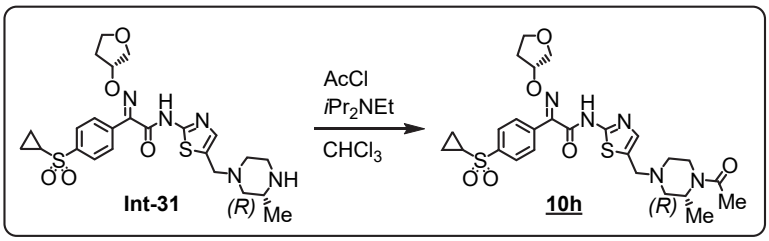
(2*E*)-2-(4-cyclopropylsulfonylphenyl)-*N*-[5-(hydroxymethyl)thiazol-2-yl]-2-[(3*R*)-tetrahydrofuran-3-yl]oxyimino-acetamide (**10f**)



To an ice-cooling suspension of **Int-30** (20.0 g, 44.5 mmol) in MeOH (400 mL) was added sodium borohydride (3.37 g, 89.1 mmol) in one portion. The reaction mixture was stirred at the same temperature for 3 hours and quenched with additional 1 M aq HCl to pH 5. The mixture was extracted with $CHCl_3$ 3 times. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue oil was purified by silica gel column chromatography ($CHCl_3$/MeOH = 95/5 – 85/15) to give **10f** (21.03 g, quantitative yield) as a yellowish powder. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.02 – 1.14 (m, 2H), 1.35 – 1.42 (m, 2H), 1.98 (t, *J* = 5.1 Hz, 1H), 2.08 – 2.32 (m, 2H), 2.49 (tt, *J* = 7.9 Hz, 4.8 Hz, 1H), 3.81 – 3.95 (m, 3H), 4.04 (dt, *J* = 10.8 Hz, 1.1 Hz, 1H), 4.84 (d, *J* =

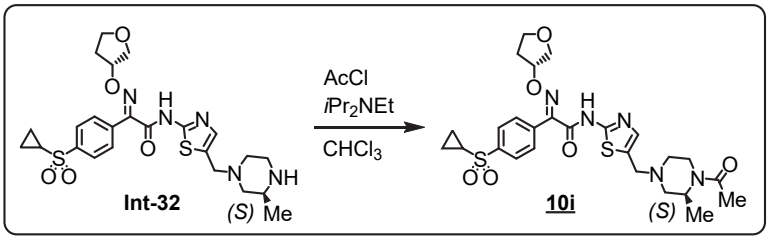
5.1 Hz, 2H), 5.06 – 5.12 (m, 1H), 7.39 (t, *J* = 0.8 Hz, 1H), 7.69 (d, *J* = 8.8 Hz, 2H), 7.98 (d, *J* = 8.3 Hz, 2H), 10.18 (br s, 1H). MS (APCI, *m/z*) 452 $[M+H]^+$. HPLC 99.40% (t_R = 7.4 min, L-column ODS (5 μM particle size, 4.6 x 150 mm), CH_3CN/20 mM phosphate buffer (pH 6.5) (30/70)).

(2*E*)-*N*-[5-[[(3*R*)-4-acetyl-3-methyl-piperazin-1-yl]methyl]thiazol-2-yl]-2-(4-cyclopropylsulfonylphenyl)-2-[(3*R*)-tetrahydrofuran-3-yl]oxyimino-acetamide (10h)



To an ice-cooling mixture of **Int-31** (30.0 mg, 0.037 mmol) and diisopropyl ethyl amine (20 μL, 0.112 mmol) in $CHCl_3$ (1.5 mL) was added acetyl chloride (4 μL, 0.055 mmol). The reaction mixture was stirred at room temperature for 12 hours and quenched with additional saturated aq $NaHCO_3$. The mixture was extracted with $CHCl_3$ three times. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue oil was purified by silica gel column chromatography ($CHCl_3$/MeOH = 97/3) to give **10h** (20.0 mg, 67% yield) as a colorless powder. ^1H NMR (300 MHz, $CDCl_3$) δ 1.03 – 1.13 (m, 2H), 1.22 (d, *J* = 7.0 Hz, 1.5H), 1.32 (d, *J* = 6.4 Hz, 1.5H), 1.35 – 1.45 (m, 2H), 2.00 – 2.30 (m, 4H), 2.06 (s, 1.5H), 2.08 (s, 1.5H), 2.49 (tt, J = 7.9 Hz, 4.8 Hz, 1H), 2.72 (t, *J* = 9.7 Hz, 1H), 2.82 – 3.02 (m, 1.5H), 3.30 – 3.60 (m, 1H), 3.60 – 3.80 (m, 2H), 3.80 – 4.00 (m, 3.5H), 4.04 (d, *J* = 10.8 Hz, 1H), 4.32-4.45 (m, 0.5H), 4.65-4.82 (m, 0.5H), 5.09 (ddt, *J* = 5.9 Hz, 4.3 Hz, 1.7 Hz, 1H), 7.29 (s, 1H), 7.69 (d, *J* = 8.8 Hz, 2H), 7.97 (d, *J* = 8.6 Hz, 2H), 10.02 (br s, 1H). MS (APCI, *m/z*) 576 $[M+H]^+$.

(2*E*)-*N*-[5-[[(3*S*)-4-acetyl-3-methyl-piperazin-1-yl]methyl]thiazol-2-yl]-2-(4-cyclopropylsulfonylphenyl)-2-[(3*R*)-tetrahydrofuran-3-yl]oxyimino-acetamide (10i)



The title compound was prepared in the same manner as described for **10h** using **Int-32** in 57% yield as a colorless powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.03 – 1.13 (m, 2H), 1.22 (d, *J* = 7.0 Hz, 1.5H), 1.32 (d, *J* = 6.4 Hz, 1.5H), 1.35 – 1.44 (m, 2H), 2.00 – 2.32 (m, 4H), 2.05 (s, 1.5H), 2.08 (s, 1.5H), 2.48 (tt, *J* = 7.9 Hz, 4.8 Hz, 1H), 2.71 (t, *J* = 9.7 Hz, 1H), 2.82 – 3.00 (m, 1.5H), 3.30 – 3.58 (m, 1H), 3.60 – 3.80 (m, 2H), 3.80 – 4.00 (m, 3.5H), 4.04 (d, *J* = 10.8 Hz, 1H), 4.33 – 4.43 (m, 0.5H), 4.65 – 4.85 (m, 0.5H), 5.05 – 5.13 (m, 1H), 7.29 (s, 1H), 7.69 (d, *J* = 8.6 Hz, 2H), 7.97 (d, *J* = 8.6 Hz, 2H), 10.00 (br s, 1H). MS (APCI, *m/z*) 576 $[M+H]^+$. HPLC 99.95% (t_R = 9.2 min, L-column ODS (5 μM particle size, 4.6 x 150 mm), CH_3CN/20 mM phosphate buffer (pH 6.5) (35/65)). Anal. calcd for $C_{26}H_{33}N_5O_6S_2$ C: 54.24, H: 5.78, N:12.17, S: 11.14; found C: 54.13, H: 5.78, N: 12.10, S: 11.07. $[\alpha]_D^{22}$ +71.45 (*c* = 1.01, MeOH). mp 163.5 – 164.5℃.

Pharmacology

Effects of 10i (TA-2395) on insulin secretion in MIN6 cells.

Materials and methods

Cell culture

MIN6 cells were cultured for 2 days in 24-well plates at the density of 2×10^5 cells/mL/well with Dulbecco's Modified Eagle's Medium (DMEM containing 25 mM glucose, Invitrogen, UK) supplemented with 15% fetal bovine serum, 50 U/mL penicillin and 50 μg/mL streptomycin under a humidified condition of 5% CO_2-95% air at 37 °C.

Assay for insulin secretion

MIN6 cells were preincubated for 0.5 hours in HEPES-balanced Krebs-Ringer bicarbonate (HEPES-KRB) buffer (119 mM NaCl, 4.74 mM KCl, 2.54 mM $CaCl_2$, 1.19 mM $MgCl_2$, 1.19 mM KH_2PO_4, 25 mM $NaHCO_3$, 10 mM HEPES, pH 7.4) containing 0.5% BSA and 5 mM glucose at 37 °C. For determination of insulin secretion, cells were incubated for 1 hour in HEPES-KRB buffer containing 0.5% BSA, various concentrations

(0 to 25 mM) of glucose and test compounds at 37 °C. Test compounds were dissolved in dimethyl sulfoxide (DMSO) and added to the buffer with the volume of 0.5% (v/v). The supernatant was collected after the incubation to measure the amount of secreted insulin. Insulin concentration was determined by a radioimmunoassay using Rat Insulin [^{125}I] Biotrak Assay System (Amersham Biosciences). Results were expressed as ng of insulin per hour and mg protein.

Determination of cellular protein

After the release experiment, cells were washed three times with saline and solubilized by 1% SDS solution. The cellular protein content was determined using a BCA protein assay kit (PIERCE).

Analysis of EC_{50} values

For construction of concentration-response curves, effects of **10i** (TA-2395) was determined at a glucose concentration of 5 mM. The response was expressed as percent of the maximum increase induced by the compounds. The EC_{50} value in each experiment was analyzed by GraphPad Prism3.02. Mean EC_{50} values and 95% confidence intervals (95% CI) were estimated from the results of 3 experiments.

Statistical analysis

Statistical analysis for the effects of **10i** (TA-2395) was performed using Student's *t*-test with EXSAS 7.5.3.1. *P* values less than 0.05 were considered statistically significant. The concentration-dependency of the effects of TA-2395 on insulin secretion were assessed by the nonlinear regression analysis method using SAS 8.2. *P* values less than 0.05 were considered concentration-dependent. The concentration-dependency of the effects of glucose on insulin secretion were assessed by the linear regression analysis method using EXSAS 7.5.3.1. *P* values of test for slope less than 0.05 were considered concentration-dependent.

Effects of 10i (TA-2395) on glucose phosphorylation in rat primary hepatocytes

Materials and methods

Hepatocyte isolation and culture

Hepatocytes were isolated by perfusion of rat liver with collagenase in the Department of Exploratory Toxicology, Exploratory Toxicology and DMPK Research Laboratories of TANABE Seiyaku Co., Ltd.. They were suspended in William's E medium containing 5% FBS, 1 μM insulin, 1 μM dexamethasone, 6.25 μg/mL transferrin, 6.25 ng/mL selenium, 100 U/mL penicillin and 100 μg/mL streptomycin, and seeded in collagen-coated 24-well plates at the density of 2×10^5 cells/mL/well. After 2-hour incubation in a 5% CO_2/air incubator, the medium was replaced by serum-free α-MEM

containing 10 nM dexamethasone, 6.25 μg/mL transferrin, 6.25 ng/mL selenium, 100 U/mL penicillin and 100 μg/mL streptomycin, and the cells were cultured for 16 ~ 20 hours before the experiments.

Assay for glucose phosphorylation

For determination of glucose phosphorylation, cells were incubated for 3 hours in Hanks' balanced salt solution containing 1.5 μCi/mL of [2-^{3}H] glucose and test compounds. Test compounds were dissolved in dimethyl sulfoxide (DMSO) and added to the buffer with the volume of 0.5% (v/v). The supernatant was collected after the incubation for determination of ^3H_2O converted from [2-^{3}H] glucose. ^3H_2O was separated with AG1x8 resin (borate form). Radioactivity was measured by a liquid scintillation counter (2200CA TRI-CARB®, Packard). Glucose phosphorylation was expressed by nmol of ^3H_2O formation per hour and mg protein.

Determination of cellular protein

Cells were washed twice with saline and solubilized by 1% SDS solution. Cellular protein was determined by using a BCA protein assay kit (PIERCE).

Analysis of EC_{50} values

For construction of concentration-response curves, increased phosphorylation by **10i** (TA-2395) was determined at glucose concentrations of 5 and 15 mM. The response was expressed as the percent of the maximum increase induced by **10i** (TA-2395). The EC_{50} value in each experiment was analyzed by GraphPad Prism3.02. Mean EC_{50} values and 95% confidence intervals (95% CI) were estimated from the results of 3 experiments.

Statistical analysis

Statistical analysis for the effects of **10i** (TA-2395) was performed using Student's *t*-test with EXSAS 7.5.3.1. *P* values less than 0.05 were considered statistically significant. Nonlinear regression analysis method was used to assess the concentration-dependency of the effects of TA-2395 on increase of phosphorylation with SAS 8.2. *P* values less than 0.05 were considered concentration-dependent. Linear regression analysis method was used to assess the concentration-dependency of the effect of glucose on glucose phosphorylation with EXSAS 7.5.3.1. *P* values of test for slope less than 0.05 were considered concentration-dependent.

Hypoglycemic and insulinotropic effects of 10i (TA-2395), glimepiride and metformin in Sprague-Dawley (SD) rats.

Materials and methods

Animals

Male 5-week-old SD rats (Charles River Japan Inc., Tokyo, Japan) were kept in an air-conditioned room with controlled temperature (23 ± 2°C), humidity (55 ± 15%) and 12 hour light–dark cycle to acclimatize for one week. They were allowed free access to an ordinary rodent chow diet (CRF1; Oriental Yeast Co., Ltd., Tokyo, Japan) and water. The Animal Ethics Committee of TANABE Seiyaku Co., Ltd. approved all the experimental procedures.

Grouping

Rats were weighed at 9:00. Blood samples of 10 μL were taken from tail tips under conscious state to measure the blood glucose levels. Then, rats were divided into experimental groups matched for blood glucose levels and body weights (CV < 10%).

Compound administration and blood sampling

Compounds were dissolved or suspended in 0.1% Nikkol HCO-40 (polyoxyethylene hydrogenated castor oil 40) solution (vehicle) and administered by oral gavage with the volume of 10 mL/kg at 13:00. Blood samples (210 μL) were taken from tail tips under conscious state just before and 0.5, 1, 2, 4 and 7 hours after the administration. Blood sample of 10 μL was used for measuring the blood glucose level, and the plasma was separated from the rest of the blood in 1.5 mL test tubes containing 4 μL heparin. All rats were euthanized by excess CO_2 gas at the end of experiment.

Analytical methods

[Blood glucose] Blood samples were deproteinized with 0.1 N $ZnSO_4$ and 0.075 N $Ba(OH)_2$ following the complete hemolysis with 9 volumes of pure water. After centrifugation with 1,500×*g* at 4°C for 10 minutes, the glucose concentration of the supernatant was determined using commercially available colorimetric enzymatic assay kits (Glucose CII-test Wako, Wako Pure Chemical Industries, Ltd.).

[Plasma insulin] Plasma samples were obtained by centrifugation with 1,500×*g* at 4 °C for 10 minutes of heparin-treated blood. Plasma insulin levels were measured by radioimmunoassay using Rat Insulin [^{125}I] Biotrak Assay System (GE Healthcare Biosciences).

Statistical analysis

Significant differences between groups were evaluated by repeated measurement ANOVA followed by Dunnett's method (EXSAS Version 7.5.0, Arm). *P* values less than 0.05 were considered statistically significant.

Oral Glucose Tolelance Test (OGTT) of 10i (TA-2395) in GK rats

Male 8-week-old GK and Wistar rats (GK/slc, Wistar/slc) (SLC Japan, Inc., Inasa, Shizuoka, Japan) were kept in a temperature (23 ± 2°C) and humidity (55 ± 15%)

controlled room with a 12-h light–dark cycle and allowed free access to an ordinary rodent chow diet (CRF1; Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water. Oral glucose tolerance test was carried out at 12 weeks old. The Animal Ethics Committee of Tanabe Seiyaku Co., Ltd. approved all the experimental procedures.

Rats were divided into the experimental groups matched for blood glucose levels and body weights.

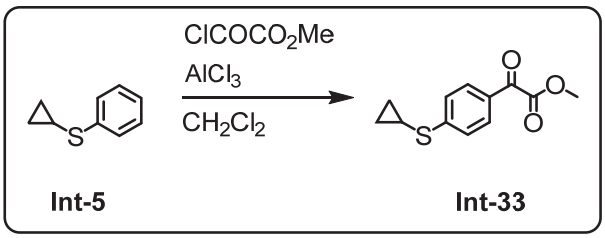
Rats were fasted from 16:00 on the day before the experiment. TA-2395 was dissolved or suspended in 0.1% NIKKOL (HCO-40) (vehicle) and administered by oral gavage with the volume of 10 mL/kg at 13:00 (-1 hour). Wistar and control GK rats received the same volume of vehicle. D-(+)-glucose was orally given by a stomach tube (1 g/10 mL/kg) 1 hour after the TA-2395 administration. Blood samples were taken from tail tips under conscious state just before and 0.25, 0.5, 1 and 2 hours after the glucose challenge.

Blood samples were deproteinized with 0.1 N $ZnSO_4$ and 0.075 N $Ba(OH)_2$ following the complete hemolysis with 9 volumes of pure water. After centrifugation with 1,500 × *g* at 4°C for 10 minutes, the glucose concentration of the supernatant was determined using Glucose C-II Test Wako (Wako Pure Chemical Industries, Ltd.).

Significant differences between groups were evaluated by repeated- measurement ANOVA followed by Dunnett's method (EXSAS Ver 7.5.0 and 7.5.2). *P* values less than 0.05 were considered statistically significant.

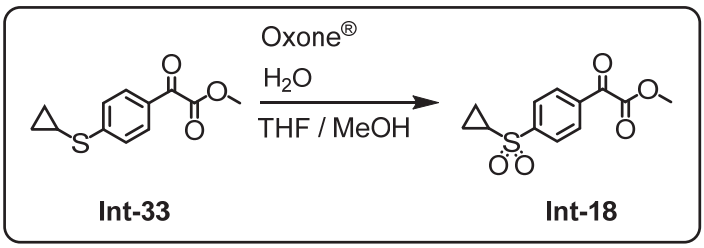
第４章に関する実験の部

Step1. Preparation of methyl 2-(4-cyclopropylsulfanylphenyl)-2-oxo-acetate (Int-33).



A solution of aluminum chloride (2.7 kg, 20.0 mol) in CH_2Cl_2 (18 L) was cooled to 5℃ and chloroglyoxylic acid methyl ester (2.0 kg, 16.0 mol) was added dropwise under the same temperature. The mixture was stirred for 30 minutes at the same temperature. Then, a solution of cyclopropyl phenyl sulfide (**Int-5**, 2.0 kg, 13.3 mol) in CH_2Cl_2 (2.8 L) was added dropwise to the above mixture. The reaction mixture was allowed to warm to room temperature and stirred for 1.5 hours, then poured onto ice-water carefully. The whole was extracted with CH_2Cl_2. The organic layer was concentrated in vacuo. The residue was diluted with EtOAc and then washed with brine, saturated aq $NaHCO_3$, brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was triturated in hexane to give **Int-33** as a yellow powder (2.17 kg, 69% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.65-0.83 (m, 2H), 1.07-1.29 (m, 2H), 2.20 (tt, *J* = 7.4 Hz and 4.4 Hz, 1H), 3.97 (s, 3H), 7.44 (d, *J* = 8.8 Hz, 2H), 7.93 (d, *J* = 9.0 Hz, 2H).

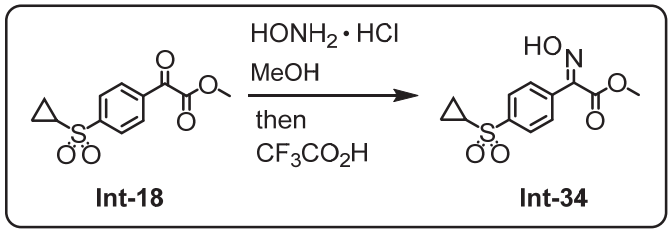
Step2. Preparation of methyl 2-(4-cyclopropylsulfonylphenyl)-2-oxo-acetate (Int-18).



A solution of **Int-33** (2.17 kg, 9.18 mol) in THF (60 L) and MeOH (60 mL) was cooled to 0℃ and then a solution of Oxone® (6.78 kg, 11.0 mol) in water (20 L) was added dropwise over 2 hours under the same temperature. The mixture was allowed to warm to

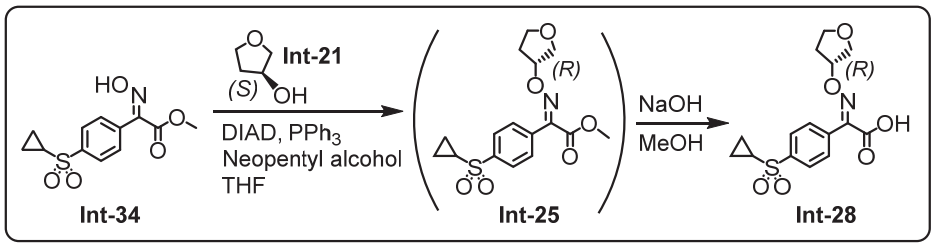
room temperature and stirred overnight. An insoluble material was removed by filtration and washed with MeOH. The filtrate was treated with 20% aqueous HClO (1.4 L) and $NaHCO_3$ (2.78 kg) to pH 4, then the insolubles were removed by filtration. The filtrate was partially concentrated in vacuo. The resulted aqueous residue was saturated with NaCl and then extracted with EtOAc. The organic layer was washed with saturated aq $NaHCO_3$, brine, dried over Na_2SO_4, and concentrated in vacuo. The residue solid was triturated in Et_2O to give **Int-18** as a yellow powder (2.00 kg, 81% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.05 – 1.15 (m, 2H), 1.35 – 1.45 (m, 2H), 2.49 (tt, *J* = 7.9 Hz, 4.8 Hz, 1H), 4.02 (s, 3H), 8.05 (d, *J* = 8.8 Hz, 2H), 8.23 (d, *J* = 8.8 Hz, 2H). MS (APCI, *m/z*) 286 $[M+NH_4]^+$.

Step3. Preparation of methyl (2*E*)-2-(4-cyclopropylsulfonylphenyl)-2-hydroxyimino-acetate (Int-34).



A mixture of **Int-18** (1.49 kg, 5.5 mol) and hydroxylamine hydrochloride (539 g, 7.76 mol) in MeOH (10.3 L) was stirred at refluxed temperature for 3 hours, then the reaction mixture was concentrated in vacuo. The residue was diluted with water and extracted with EtOAc twice. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude material was dissolved in trifluoroacetic acid (6.70 kg), then the mixture was stirred at room temperature overnight and concentrated in vacuo. The residue was azeotroped with toluene 3 times. The crude material was recrystallized from hexane – EtOAc to give **Int-34** (1.30 kg, 82% yield) as a colorless powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.03 – 1.12 (m, 2H), 1.12 – 1.20 (m, 2H), 2.88 – 2.99 (m, 1H), 3.78 (s, 3H), 7.65 (d, *J* = 8.3 Hz, 2H), 7.94 (d, *J* = 8.5 Hz, 2H), 12.81 (s, 1H). MS (APCI, *m/z*) 301 $[M+H]^+$.

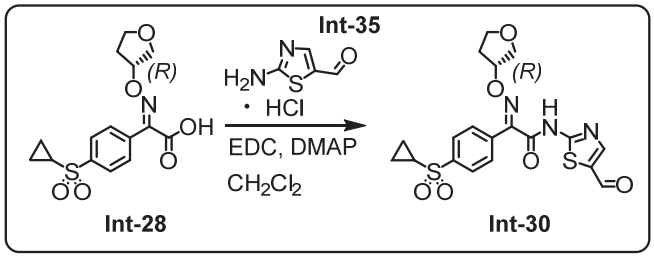
Step4 and Step5. One pot preparation of (2*E*)-2-(4-cyclopropylsulfonylphenyl)-2-[(3*R*)-tetrahydrofuran-3-yl]oxyimino-acetic acid (Int-28) from Int-34.



Step4. A mixture of **Int-34** (1.30 kg, 4.6 mol), **Int-21** (484 g, 5.5 mol), PPh_3 (1.68 kg, 6.4 mol), and neopentyl alcohol (202 g, 2.3 mol) in dry THF (19.4 L) was cooled to 0℃ under nitrogen atmosphere, then DIAD (1.30 kg, 6.4 mol) was added dropwise over 30 minutes. After being stirred at the same temperature for 3 hours, the reaction mixture was allowed to warm to room temperature and stirred overnight. **Int-25** was not isolated and directly used to the next step.

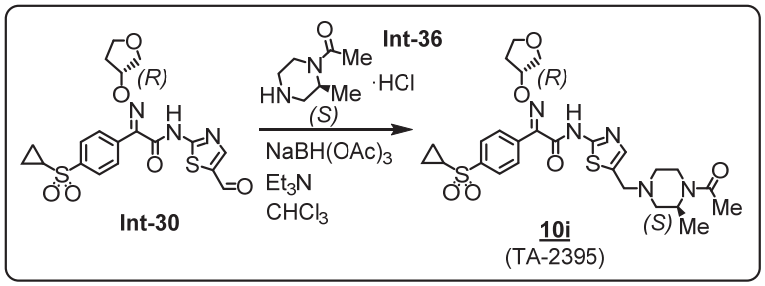
Step5. The above mixture was cooled to 0℃, then MeOH (6.5 L), water (2.0 L), and 48% aq NaOH (1.0 L) was added succesively. The reaction mixture was stirred for 1 hour at the same temperature. The organic solvent was removed in vacuo. The aqueous layer was extracted with EtOAc (9 L) 4 times to remove the impurities. The resulted aqueous layer was cooled to 6℃ and acidified with 10% aq HCl (2.1 L) to pH 3. The whole was extracted with $CHCl_3$ (12 L) twice. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue solid was dissolved in hot EtOAc (17 L) and treated with activated charcoal (170 g). After being cooled to room temperature, the insolubles were removed by filtration and washed with EtOAc. After the filtrate was partially concentrated in vacuo to remain the solvent (5.5 L), the residue was diluted with hexane (4.2 L) at 40℃ and then cooled to room temperature to give **Int-28** (1.17 kg, 75% yield) as a colorless powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.01 – 1.12 (m, 2H), 1.12 – 1.21 (m, 2H), 1.93 – 2.03 (m, 1H), 2.07 – 2.18 (m, 1H), 2.86 – 2.99 (m, 1H), 3.63 – 3.73 (m, 2H), 3.73 – 3.87 (m, 2H), 5.02 – 5.08 (m, 1H), 7.66 (d, J = 8.5 Hz, 2H), 7.95 (d, J = 8.3 Hz, 2H), 13.62 (br s, 1H). MS (ESI, *m/z*) 338 [M-H]$^-$. HPLC 96.51% (t_R = 3.9 min, L-column ODS (5 μM particle size, 4.6 x 150 mm), CH_3CN/20 mM phosphate buffer (pH 6.5) (35/65)).

Step6. Preparation of (2*E*)-2-(4-cyclopropylsulfonylphenyl)-*N*-(5-formylthiazol-2-yl)-2-[(3*R*)-tetrahydrofuran-3-yl]oxyimino-acetamide (Int-30).



To a stirred suspension of **Int-28** (967 g, 2.85 mol) and 2-amino-5-formylthiazole hydrochloride (**Int-35**, 704 g, 4.28 mol) was added DMAP (820 g, 4.28 mol) in dry CH_2Cl_2 (30 L). After being stirred at room temperature for 1 hour, EDC was added, then the reaction mixture was stirred at room temperature overnight. The reaction mixture was extracted with 10% citric acid (8 L) 3 times. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was dissolved in EtOAc (12 L) – CH_3CN (12 L), and the solution was treated with activated charcoal (300 g). After being stirred at room temperature for 1 hour, the insolubles were removed by filtration and the filtrate was concentrated in vacuo. The residue was recrystallized from EtOAc (7 L) to give **Int-30** (813 g, 63% yield) as a yellowish powder. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.06 – 1.13 (m, 2H), 1.13 – 1.20 (m, 2H), 2.09 – 2.23 (m, 2H), 2.91 – 2.99 (m, 1H), 3.67 – 3.81 (m, 3H), 4.03 (d, J = 10.8 Hz, 1H), 5.12 – 5.17 (m, 1H), 7.79 (d, J = 8.3 Hz, 2H), 7.99 (d, J = 8.5 Hz, 2H), 8.51 (s, 1H), 9.99 (s, 1H), 13.22 (br s, 1H). MS (APCI, *m/z*) 450 $[M+H]^+$. HPLC 97.03% (t_R = 8.8 min, L-column ODS (5 μM particle size, 4.6 x 150 mm), CH_3CN/20 mM phosphate buffer (pH 6.5) (35/65)).

Step 7. Preparation of (2*E*)-*N*-[5-[[(3*S*)-4-acetyl-3-methyl-piperazin-1-yl]methyl]thiazol-2-yl]-2-(4-cyclopropylsulfonylphenyl)-2-[(3*R*)-tetrahydrofuran-3-yl]oxyimino-acetamide (TA-2395, 10i).



Under argon atmosphere, **Int-30** (412 g, 0.92 mol) and 1-[(2*S*)-2-methylpiperazin-1-yl]ethan-1-one hydrochloride (**Int-36**: 282 g, 1.38 mol) were dissolved in $CHCl_3$ (8.7 L), then triethylamine (204 g, 2.02 mol) and sodium triacetoxyborohydride (584 g, 2.76 mol) were added successively. After being stirred at room temperature overnight, the reaction

was cooled to 5℃ and quenched with additional saturated aq $NaHCO_3$ (3.5 L). An insoluble material was removed by filtration. The two layers were separated, and the aqueous layer was extracted with $CHCl_3$ twice. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was dissolved in $CHCl_3$ (3 L) – MeOH (0.17 L) and the solution was passed through a short silica gel pad (700 g) with the assist of $CHCl_3$/MeOH = 20/1 solution (4.5 L). The filtrate was concentrated in vacuo. The resulted solid was recrystallized from EtOAc (1.9 L) to give **10i** (TA-2395, 380 g, 72% yield) as a colorless powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.03 – 1.13 (m, 2H), 1.22 (d, *J* = 7.0 Hz, 1.5H), 1.32 (d, *J* = 6.4 Hz, 1.5H), 1.35 – 1.44 (m, 2H), 2.00 – 2.32 (m, 4H), 2.05 (s, 1.5H), 2.08 (s, 1.5H), 2.48 (tt, *J* = 7.9 Hz, 4.8 Hz, 1H), 2.71 (t, *J* = 9.7 Hz, 1H), 2.82 – 3.00 (m, 1.5H), 3.30 – 3.58 (m, 1H), 3.60 – 3.80 (m, 2H), 3.80 – 4.00 (m, 3.5H), 4.04 (d, *J* = 10.8 Hz, 1H), 4.33 – 4.43 (m, 0.5H), 4.65 – 4.85 (m, 0.5H), 5.05 – 5.13 (m, 1H), 7.29 (s, 1H), 7.69 (d, *J* = 8.6 Hz, 2H), 7.97 (d, *J* = 8.6 Hz, 2H), 10.00 (br s, 1H). MS (APCI, *m/z*) 576 $[M+H]^+$. HPLC 98.20% (t_R = 10.5 min, L-column ODS (5 μM particle size, 4.6 x 150 mm), CH_3CN/20 mM phosphate buffer (pH 6.5) (35/65)).

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35. (a) オキシム水酸基の pKa 値は文献値で約 11 であり、光延反応の進行に必要とされている pKa 値 15 以下を満たしている。(b) Bell R.P.; Higginson W. C. E. *Pro. Royal Soc.* **1949**, *197*, 141. (c) For a comprehensive compilation of Bordwell pKa data see: http://www.chem.wisc.edu/areas/reich/pkatable/index.htm

主論文目録

本学位論文内容は下記の発表論文による

1. Sugama H.; Matsudaira T.; Yanagisawa H.; Ohashi R.; Nawano M.; Yasuda K.; Takayama H. Design, synthesis, and pharmacological evaluation of 2-(4-sulfonylphenyl)-2-[(*E*)-pyrrolidin-1-ylimino]-*N*-thiazoleacetamides as glucokinase activators. *Bioorganic & Medicinal Chemistry Letters*, **2020**, 30, 127249.

主査および副査名

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