博士学位論文

心血管系の副作用を軽減した
選択的β_3アドレナリン受容体アゴニストの創製研究

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序論

過活動膀胱（Overactive Bladder, OAB）は 2002 年に国際禁制学会が定義した疾患である。[1]過活動膀胱では、主に蓄尿時に不髄意的に膀胱が収縮する排尿筋過活動により正常な蓄尿が困難になり、急な尿意をもよおし、我慢できないほどの尿意切迫感、さらに頻尿（昼間頻尿、夜間頻尿）や切迫性尿失禁（尿漏れ）などの諸症状が認められる。これら過活動膀胱の症状は患者にとって高いストレスとなり、そして生活の質を大きく下げる。[2]

人口をベースとした 5 か国（カナダ、ドイツ、イタリア、スウェーデン、イギリス）における推定によると、18 歳以上の約 12%が過活動膀胱の症状を有していると考えられている。[3]また、男性と女性の割合に大きな差は無く、年齢の上昇と共に患者が増える傾向がある。また、日本における日本排尿機能学会が行った調査によると 40 歳以上の過活動膀胱の有病率は 12.4%であり、有病者は 810 万人と推定されている。[4]

現在、過活動膀胱の治療の第一選択薬である抗コリン薬（ムスカリン受容体アンタゴニスト）は、排尿回数、尿意切迫感を改善させるものの、口渇、便秘、視力障害、尿閉などの副作用が報告されている。[5]それらの副作用はムスカリン受容体アンタゴニストの作用メカニズムに関連がある。[5b]すなわち、標的分子であるムスカリン受容体は膀胱の他に唾液腺にも発現しており、そのアンタゴニストは口渇を引き起こす。また、抗コリン薬は膀胱収縮を抑える働きをするため、残尿も生じやすい。近年、膀胱選択性を高めた抗コリン薬も開発されているが、副作用、特に口渇を完全に回避するまでには至っていない。

近年の調査によると、抗コリン薬を 12 か月間継続投与できた過活動膀胱の患者は 35%以下であり、抗コリン薬の副作用は過活動膀胱患者の長期間服薬が困難であることを示唆しており、その結果として治療満足度の低下につながっている。[6]

上記治療満足度を向上させるために、過活動膀胱治療薬の新規標的分子として、アドレナリン受容体（adrenergic receptor, AR）の一つであるβ_3-AR が注目されている。[7] アドレナリン受容体はα_1-AR、α_2-AR、β-AR の 3 つのサブファミリーに分類されており、その β-AR はβ_1、β_2、β_3 の 3 つのサブタイプが知られている。[8]アドレナリン受容体の生体内リガンドはアドレナリン、ノルアドレナリンである。[8]

G タンパク共役型受容体であるβ_3-AR は、1980 年代にヒトゲノム解析により発見されたβ-AR サブタイプの 1 つである。[8-9]β_3-AR は脂肪細胞の分解や熱発生、さらに胆嚢、胃、小腸、前立腺、結腸、膀胱の弛緩に関わっていることが知られている。[10]特に、ヒト膀胱組織におけるβ-AR の mRNA を解析した結果、97%がβ_3-AR であることが報告されており[11]、ヒトの膀胱弛緩においてβ-AR のサブタイプのうち、β_3-AR が最も重要な機能を持っていることが示唆されている[12]。さらに、β_3-AR アゴニストはラットにおける蓄尿を向上させた[13]ことから、β_3-AR アゴニストは抗コリン薬に見られる副作用を示さない新規メカニズムの過活動膀胱治療薬として非常に期待されている[14]。

過活動膀胱の治療薬として臨床試験が実施されたβ_3-AR アゴニストは **1** (ミラベグロン、mirabegron、YM-178)[15]、**2** (ソラベグロン、solabegron、GW427353)[16]、 **3** (リトベグロン、ritobegron、KUC-7483)[17]、**4** (ビベグロン、vibegron、MK-4618)[14b]が知られている（Figure 1）。ミラベグロン（**1**）は過活動膀胱の治療薬として承認されており[18]、β_3-AR アゴニストという新規メカニズムであるため、抗コリン薬と比較して口渇等の副作用が少ないことが特徴である[7]。

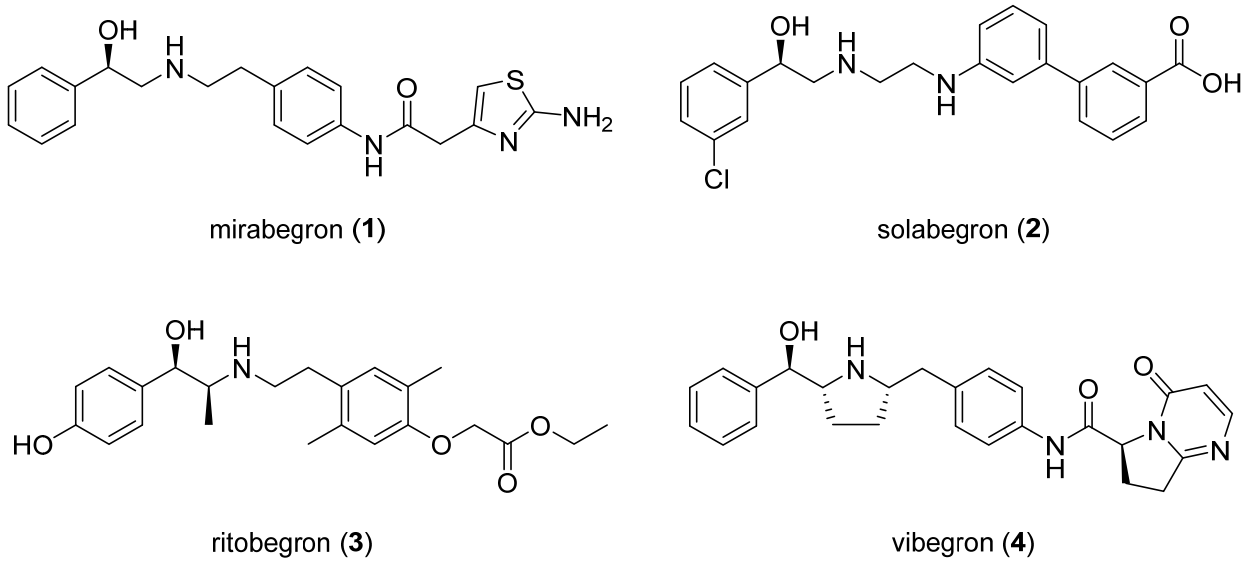

Figure 1. Chemical structures of **1**, **2**, **3**, and **4**.

しかしながら、ミラベグロンは血圧上昇や心拍数上昇など心血管系に対する副作用を引き起こすことが知られている。[7]その原因は特定されていないものの、ミラベグロンの心拍数上昇作用に関しては β_1-AR*に対するアゴニスト活性が原因の一つとして考えられる。[20]さらに、ミラベグロンは CYP2D6 に対する阻害作用があるため、CYP2D6 で代謝される医薬との薬物間相互作用が懸念される。[21]

上述の通り、現在上市されているミラベグロンにはいくつか懸念点がある。そのミラベグロンよりも優れた治療薬を目指し、β_3-AR に対する高い選択性を持ち、血圧上昇を含む心血管系の副作用を軽減した新規な化合物を創出することで、ベストインクラスの過活動膀胱治療薬になると考え、本研究に着手した。

* β_1-AR は心臓や脂肪細胞に発現している。一方、β_2-AR は血管、子宮、気道平滑筋に発現している[19]。

第１章　新規なインダゾール骨格を有するβ_3-AR アゴニスト化合物 **11** の創出

第１節　ヒット化合物取得の戦略

AR の生体内リガンドであるアドレナリン、β-AR 非選択的アゴニストであるイソプロテレノール、代表的なβ_3-AR アゴニストであるミラベグロン並びにソラベグロンに共通する部分構造はアリールエタノールアミンであり、β_3-AR アゴニスト活性を示すにはアリールエタノールアミンが必須構造であると考えた（Figure 2、赤色部分）。一方、イソプロテレノールとβ_3-AR アゴニストの構造上の違いは、アリールエタノールアミンの右側にアミノチアゾールやビアリールカルボン酸などを有することである（Figure 2、点線内）。従って、β_3-AR アゴニスト活性を有する新規なヒット化合物の取得を目的として、アリールエタノールアミンの右側にβ_3-AR と水素結合が期待できる種々の 2 環性ヘテロ環*をエーテルリンカーで結合したライブラリーに対し、β_3-AR アゴニスト活性評価を実施した（Figure 2、中段）。その評価方法はβ_3-AR を発現させた Chinese Hamster Ovary （CHO） 細胞を用いて、細胞内の cAMP を定量した。その結果、アリールエタノールアミンの右側にインダゾール骨格を有する化合物 **5** をヒット化合物として見出した（Figure 2、下段）。Table 1 に化合物 **5** の AR アゴニスト活性 EC_{50}†とその内在活性（intrinsic activity, IA）‡示す。化合物 **5** は強いβ_3-AR アゴニスト活性（EC_{50} = 21 nM）、並びにβ_1-AR とβ_2-AR§に対して高いβ_3-AR 選択性（β_1/β_3、β_2/β_3 >476-fold）を有していた。化合物 **5** は文献等で報告されている他のヒット化合物[22]（Figure 3）と比較し、β_1 とβ_2-AR アゴニスト活性が非常に弱く、β_3-AR に対して極めて高選択的な特徴を有しているため、さらに詳細な薬理プロファイルを検討した。

β_1-AR とβ_2-AR に無効な高選択的β_3-AR アゴニストが血圧心拍に与える影響を確認するために、麻酔下のラットに対して化合物 **5** を静脈内投与した。その結果を Table 2 に示す。化合物 **5** 投与後、一過的な二相性の平均血圧（mean blood pressure, MBP）の変化（明らかな上昇と若干の低下）が観測された。その変化は投与後 5 分以内で起き、その後ベースラインに戻った。MBP の最大上昇率は 24.2%、最大低下率は 8.5%であった。また、血圧（heart rate, HR）は投与後 1-5 分の間で 7.9%上昇した。なぜ、高選択的なβ_3-AR アゴニストである化合物 **5** が血圧上昇を引き起こしたのかを考察するために、β-AR のサブファミリーであるα_1-AR**とα_2-AR†† のアゴニスト活性を評価した。その結果を Table 1 に示す。化合物 **5** はα_{1A}-AR に対して強いアゴニスト活性（EC_{50} = 219 nM）を有することが分かった。α_1-AR は広く全身に分布しており、特に心血管系組織、平滑筋、脳に発現している。[23]また、α_1-AR アゴニストは末梢の血管収縮を介して血圧を上昇させることが知られている。[24]さらに、化合物 **5** の血圧上昇はα_{1A}-AR アンタゴニストであるシロドシン[25]を事前投与することにより、完全に抑制された。ゆえに、化合物 **5** の血圧上昇はα_1-AR アゴニスト活性に由来すると示唆された。したがって、筆者はα_{1A}-AR アゴニスト活性を減弱させたβ_3-AR に高選択的な化合物創出を目指し、ヒット化合物 **5** の構造をベースに化合物最適化研究を実施した。

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* インダゾール、イソキノリン、1H-ピラゾロ[3,4-b]ピリジン、ベンゾイソキサゾール、ベンゾチアゾール、ベンゾイミダゾールなど

† 内在活性の 50%の cAMP を上昇させるときの化合物濃度

‡ 細胞内の cAMP を上昇させるアゴニスト作用の程度を示している。陽性対象として非選択的β-AR アゴニストのイソプロテレノール（isoproterenol）が示す cAMP 濃度を 100%とし、化合物の内在活性を算出した。

§ β_1-AR もしくはβ_2-AR アゴニスト活性の評価はβ_3-AR アゴニスト活性評価と同様に β_1-AR もしくはβ_2-AR を発現させた CHO 細胞を用いて、細胞内の cAMP を定量し、化合物のβ_1-AR もしくはβ_2-AR アゴニスト活性を評価した。

** α_1-AR はα_{1A}、α_{1B}、α_{1D} の 3 つのサブタイプが知られている[8]

†† α_2-AR はα_{2A}、α_{2B}、α_{2c} の 3 つのサブタイプが知られている[8]

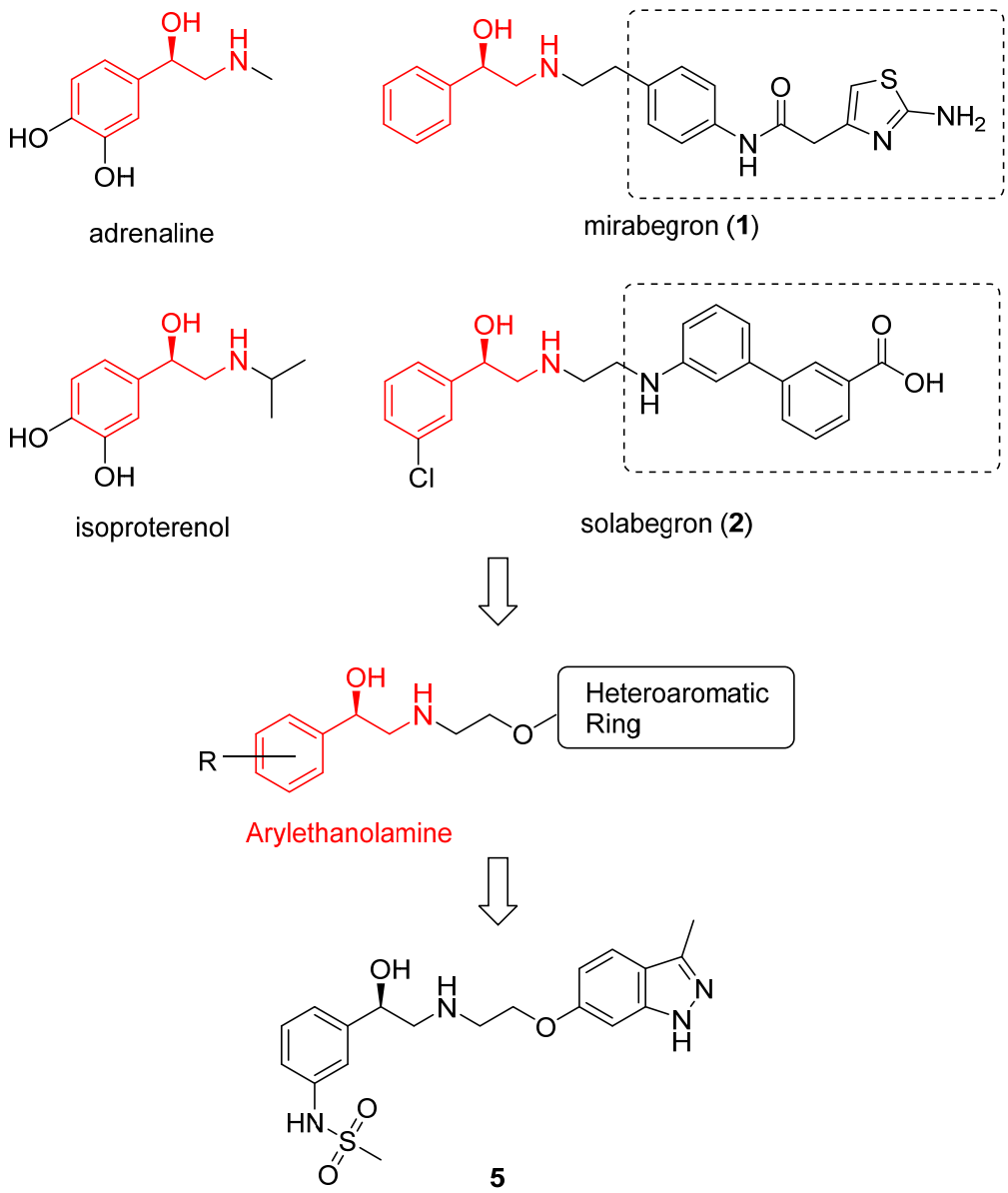
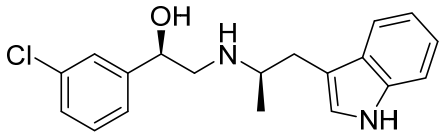


Figure 2. Hit identification strategy

Table 1. Human adrenergic receptor agonist activity of 5[a]

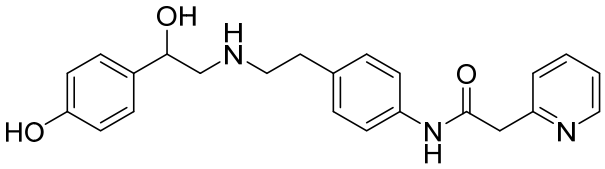
AR	EC_{50} (nM)[a]	IA (%)	Selectivity
β_3	21 ± 0.67	82[b]	
β_1	>10000	6.0[b]	$\beta_1 / \beta_3 > 476$
β_2	>10000	4.0[b]	$\beta_2 / \beta_3 > 476$
α_{1A}	219 ± 6.9	71[c]	$\alpha_{1A} / \beta_3 = 10$

[a]Data are shown as means ± SEM (n=3). Compound **5** exhibited insignificant agonistic activity for α_{1B}, α_{1D}, α_{2A}, α_{2B} or α_{2C} (EC_{50} >10000 nM). [b]IA (intrinsic activity): maximum response induced by isoproterenol was defined as 100%. [c]IA: maximum response induced by noradrenaline was defined as 100%.



Compound A[22a]

β_3-AR EC_{50} = 5.4 nM
β_1-AR EC_{50} = 1.9 nM
β_2-AR EC_{50} = 25 nM
Selectivity β_1/β_3 = 0.35
Selectivity β_2/β_3 = 4.6



Compound B[22b]

β_3-AR EC_{50} = 160 nM
β_1-AR EC_{50} = 810 nM
β_2-AR EC_{50} = 10000 nM
Selectivity β_1/β_3 = 5
Selectivity β_2/β_3 = 63

Figure 3. Chemical structure of phenylethanolamine-based hit compounds

Table 2. Effect of 5 on HR and MBP in anesthetized rats[a]

		HR		MBP	
Dose (mg/kg)	n	Increase (%)	Decrease (%)	Increase (%)	Decrease (%)
Saline	5	1.8 ± 0.9	4.5 ± 0.8	1.0 ± 0.5	5.4 ± 1.9
1	3	4.2 ± 2.3	1.3 ± 0.7	7.5 ± 0.2	9.9 ± 0.2
3	4	7.9 ± 2.9	8.8 ± 4.5	24.2 ± 7.7	8.5 ± 1.1
1[b]	1	1.7	ND	ND	20.6

[a]Compound **5** was administered intravenously to rats, and, thereafter, HR and MBP were recorded for 30 min. Maximum increase or decrease of HR or MBP is shown as a value (%) relative to the baseline. Data are shown as means ± SEM. [b]Silodosin, a selective α_{1A}-AR antagonist, was administered intravenously at a dose of 0.01 mg/kg before administration of compound **5**. n: number, ND: not detected.

第２節　α_{1A}-AR に対する選択性向上のための化合物設計方針

上述の通り、化合物 **5** はラットにおいて血圧上昇を示したことから、化合物 **5** のα-AR に対する選択性評価に加え、化合物 **5** のラットによる代謝物が血圧上昇を引き起こす可能性を考慮し、肝ミクロソームによる代謝安定性評価と代謝物構造解析も行った。化合物 **5** はヒトミクロソームに対して *in vitro* 代謝安定性（$CL_{int,u}$）*は良好であったが、ラットミクロソームに対して代謝安定性は低かった（human $CL_{int,u}$ < 10 mL/min/kg、rat $CL_{int,u}$ = 103 mL/min/kg）。さらに、ラットミクロソームによる代謝物の構造解析を行った結果、ラットにおける主代謝物としてインダゾール 3 位のメチル基が水酸化された化合物 **6** を同定した（Figure 4）。その水酸化体 **6** はβ_3-AR とα_{1A}-AR のアゴニスト活性を示し（β_3-AR EC_{50} = 111 nM、α_{1A}-AR EC_{50} = 33 nM）、特に、α_{1A}-AR アゴニスト活性は化合物 **5** よりも強かった。一方、化合物 **5** はヒトミクロソームの代謝安定性が良好なため、ヒトの代謝において水酸化体 **6** はほとんど生成しないと考えられる。

ラット主代謝物解析から同定された水酸化体 **6** のβ_3-AR とα_{1A}-AR のアゴニスト活性評価より、化合物 **5** のインダゾール 3 位のメチル基をヒドロキシメチル基に変換することは大きくα_{1A}-AR アゴニスト活性を変化させるという構造最適化のきっかけをつかむことができた。すなわち、インダゾール 3 位の置換

* 数字が大きいほど代謝に対して不安定であることを示している

基はα_{1A}-AR アゴニスト活性に重要な変化を及ぼすと予想した。そこで、筆者はインダゾール 3 位の置換基変換を行うことで、α_{1A}-AR アゴニスト活性に対して選択性を向上させたβ_3-AR アゴニストを創出できると考え、合成を行った。

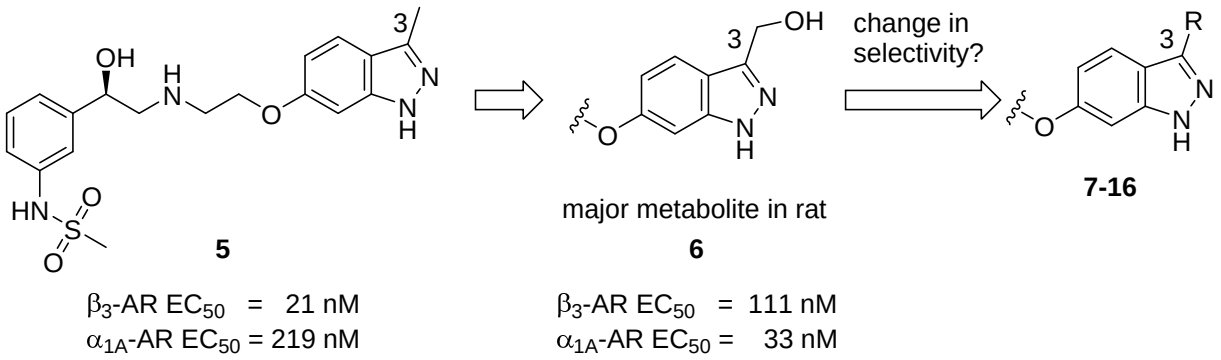


Figure 4. Design of indazole derivatives

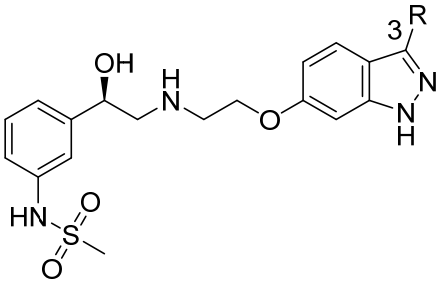
第３節　インダゾール 3 位の変換による構造－β_3-AR 並びにα_{1A}-AR アゴニスト活性相関

（１）構造－β_3-AR アゴニスト活性相関

Table 3 にインダゾール 3 位を変換した化合物のβ_3-AR アゴニスト活性を示す。メチル基 **5** をメトキシ基 **7** に変換した結果、β_3-AR アゴニスト活性は向上しなかった（**5**, EC_{50} = 21 nM vs. **7**, EC_{50} = 40 nM）。一方、メチル基 **5** をエトキシ基 **8** に変換した結果、活性が減弱した（**5**, EC_{50} = 21 nM vs. **8**, EC_{50} = 71 nM）。また、メチル基 **5** をクロロ基 **9**、エチル基 **10**、イソプロピル基 **11**、シクロプロピル基 **13** に変換した結果、活性にほとんど変化はなかった（**5**, EC_{50} = 21 nM vs. **9**, EC_{50} = 43 nM; **10**, EC_{50} = 14 nM; **11**, EC_{50} = 13 nM; **13**, EC_{50} = 17 nM）。しかし、メチル基 **5** を *t*－ブチル基 **12** もしくはフェニル基 **16** に変換した結果、活性が大きく減弱した（**5**, EC_{50} = 21 nM vs. **12**, EC_{50} = 338 nM, **16**, EC_{50} = >10000 nM）。シクロブチル基 **14**、トリフルオロメチル基 **15** はメチル基 **5** よりも活性がやや減弱した（**14**, EC_{50} = 66 nM, **15**, EC_{50} = 64 nM vs. **5**, EC_{50} = 21 nM）。以上をまとめると、β_3-AR アゴニスト活性の強さの順は **5**, **7**, **9**, **10**, **11**, **13** > **8**, **14**, **15** > **12** > **16** であった。

イソプロピル基 **11** よりも大きい置換基である *t*－ブチル基 **12** やフェニル基 **16** はβ_3-AR アゴニスト活性が減弱することから、イソプロピル基以下のサイズがβ_3-AR との結合に適していると考えられる。その原因は置換基がイソプロピル基よりも大きい場合、β_3-AR のアミノ酸残基と反発することが推定される。

Table 3. Human β_3- and α_{1A}-AR agonistic activity of indazole derivatives[a]



Compound	R	β_3		α_{1A}		Selectivity
		EC_{50} (nM)	IA (%)[b]	EC_{50} (nM)	IA (%)[c]	α_{1A} / β_3
5	Me	21 ± 0.67	82	219 ± 6.9	71	10
7	OMe	40 ± 1.9	70	252	60	6.3
8	OEt	71 ± 6.1	69	277	59	3.9
9	Cl	43 ± 2.9	89	61	91	1.4
10	Et	14 ± 0.58	78	857 ± 164	31	61
11	*i*-Pr	13 ± 1.5	69	>10000	9.1	>769
12	*t*-Bu	338 ± 159	62	>10000	19	>30
13		17 ± 3.8	72	1548 ± 419	19	91
14		66	68	>10000	4	>151
15	CF_3	64 ± 11	86	812 ± 179	31	13
16	Ph	>10000	29	>10000	6	
isoproterenol[*]		86 ± 3.7	100	NT		
noradrenaline[†]		NT		9.1 ± 0.52	100	

[a]Data are shown as means ± SEM (n≧3) or are presented as the average of two experiments. [b]IA (intrinsic activity): maximum response induced by isoproterenol was defined as 100%. [c]IA: maximum response induced by noradrenaline was defined as 100%. NT: not tested.

[*]非選択的β-AR アゴニストであり、β-AR アゴニスト評価系の陽性対象として一般的に用いられる。[15]
[†]生体内のリガンドであり、α-AR アゴニスト評価系の陽性対象として一般的に用いられる。

（２）構造－α_{1A}-AR アゴニスト活性相関

Table 3 にインダゾール 3 位を変換した化合物のα_{1A}-AR アゴニスト活性を示す。まず、メトキシ体 **7** あるいはエトキシ体 **8** は、α_{1A}-AR アゴニスト活性に変化は無かった（**5**, EC_{50} = 219 nM vs. **7**, EC_{50} = 252 nM, **8**, EC_{50} = 277 nM）。また、クロロ体 **9** はメチル体 **5** よりもα_{1A}-AR アゴニスト活性が向上した（**9**, EC_{50} = 61 nM vs. **5**, EC_{50} = 219 nM）。一方、エチル体 **10**、シクロプロピル体 **13**、トリフルオロメチル体 **15** へと変換した結果、大きくα_{1A}-AR アゴニスト活性が減弱した（**5**, EC_{50} = 219 nM vs. **10**, EC_{50} = 857 nM; **13**, EC_{50} = 1548 nM; **15**, EC_{50} = 812 nM）。さらに、イソプロピル体 **11**、 *t* - ブチル体 **12**、シクロブチル体 **14** あるいはフェニル体 **16** は、α_{1A}-AR アゴニスト活性を示さなかった。以上をまとめると、α_{1A}-AR アゴニスト活性の強さの順は **9** > **5**, **7**, **8** > **10**, **13**, **15** >> **11**, **12**, **14**, **16** であった。

メトキシ体 **7** 並びに Figure 4 のヒドロキシメチル体 **6** が強いα_{1A}-AR アゴニスト活性（α_{1A}-AR EC_{50} = 33 nM）を示したことからインダゾール 3 位ヒドロキシメチル基やメトキシ基はα_{1A}-AR のアミノ酸残基と水素結合等の相互作用をしている可能性が考えられる。一方、イソプロピル体 **11** のような分岐アルキル基は嵩高く、疎水性が高いために上記水素結合を形成することができず、α_{1A}-AR アゴニスト活性を示さないことが推測される。

（３）α_{1A}/β_3 選択性の構造相関

β_3-AR アゴニスト活性とα_{1A}-AR アゴニスト活性を基に、α_{1A}-AR に対してβ_3-AR の選択性の順は **11** > **14** > **13** > **10** > **12** > **15** > **5** > **7** > **8** > **9** であった。ゆえに、β_3-AR アゴニスト活性の強さとα_{1A}-AR に対する選択性から化合物 **10**、化合物 **11** 並びに化合物 **13** を選択し、更なる薬理評価を実施した。

（４）β_1-AR とβ_2-AR のアゴニスト活性

Table 4 にβ_3、β_2、β_1、並びにα_{1A}-AR に対するアゴニスト活性を示す。化合物 **10**、化合物 **11**、並びに化合物 **13** はβ_1-AR とβ_2-AR に対してアゴニスト活性を示さなかった（β_1-AR EC_{50} = >10000 nM; β_2-AR EC_{50} = >10000 nM）。したがって、これら 3 化合物はβ_3-AR 高選択的アゴニストであることが示唆された。

Table 4. Summary of human β and α_{1A}-AR agonistic activity of indazole derivatives 10, 11 and 13[a]

Compound	β_3		β_1		β_2		α_{1A}	
	EC_{50} (nM)	IA (%)[b]	EC_{50} (nM)	IA (%)[b]	EC_{50} (nM)	IA (%)[b]	EC_{50} (nM)	IA (%)[c]
10	14 ± 0.58	78	>10000	5.4	>10000	1.9	857 ± 164	31
11	13 ± 1.5	69	>10000	11.9	>10000	5.6	>10000	9.1
13	17 ± 3.8	72	>10000	10.3	>10000	6.3	1548 ± 419	19
isoproterenol	86 ± 3.7	100	3.2 ± 0.29	100	13 ± 3.0	100	NT	
noradrenaline	NT		NT		NT		9.1 ± 0.52	100

[a]Data are shown as means ± SEM (n≧3). [b]IA (intrinsic activity): maximum response induced by isoproterenol was defined as 100%. [c]IA: maximum response induced by noradrenaline was defined as 100%. NT: not tested.

第４節　ラットの心血管系に与える影響

上述の通り、化合物 **10**、化合物 **11** 並びに化合物 **13** はβ_3-AR 高選択的であることが示されたので、ラットを用いて心血管系に与える影響を評価し、ヒット化合物 **5** 並びにミラベグロン（**1**）と比較した。Table 5 に結果を示すように、インダゾール 3 位が最適化された 3 化合物はすべて MBP の上昇を引き起こさなかった。一方、ヒット化合物 **5** は大きく MBP の上昇（24.2%）を引き起こした。同様の評価にて、ミラベグロン（**1**）は MBP の大きな低下（38.3%）を引き起こした。また、化合物 **11** は HR に関して影響を与えなかった。一方、ミラベグロン（**1**）は HR の上昇（10.0%）を引き起こした。MBP と HR の結果より、化合物 **10**、化合物 **11** 並びに化合物 **13** はミラベグロン（**1**）並びにヒット化合物 **5** と比較し、MBP や HR に与える影響は少なく、その要因はβ_3-AR に高選択的であることが示唆された。

Table 5. Effects of intravenous administration of indazole derivatives on HR and MBP in anaesthetized rats[a]

		HR		MBP	
Compound	N	Increase (%)	Decrease (%)	Increase (%)	Decrease (%)
Saline	5	1.8 ± 0.9	4.5 ± 0.8	1.0 ± 0.5	5.4 ± 1.9
mirabegron (**1**)	3	10.0 ± 2.5	ND	3.5 ± 3.5	38.3 ± 3.4
5	4	7.9 ± 2.9	8.8 ± 4.5	24.2 ± 7.7	8.5 ± 1.1
10	6	9.6 ± 1.3	1.6 ± 2.0	3.1 ± 0.2	10.8 ± 1.1
11	6	3.3 ± 0.6	1.8 ± 0.5	4.7 ± 0.9	7.6 ± 1.7
13	3	5.7 ± 1.5	3.3 ± 0.7	2.7 ± 0.3	11.0 ± 3.0

[a]Compounds were administered intravenously to rats (3 mg/kg), and, thereafter, HR and MBP were recorded for 30 min. Maximum increase or decrease of HR or MBP is shown as a value (%) relative to the baseline. Data are shown as means ± SEM. N: Numbers of rats used. ND: Not detected.

第５節　化合物 **11** の排尿筋弛緩作用に関して

次に、化合物 **11** の膀胱平滑筋に対する弛緩作用を評価した。これまでにラットを用いた膀胱平滑筋に対する評価法が知られている[15]が、化合物 **11** はヒトとラットでβ_3-AR に対する活性に種差があり、ラットのβ_3-AR アゴニスト活性が弱かった（EC_{50} = 535 nM, IA = 95%）。種々の動物に対するβ_3-AR アゴニスト活性を評価した結果、化合物 **11** はマーモセットに対して強いβ_3-AR アゴニスト活性を有することが分かった（EC_{50} = 15 nM, IA = 75%）。ゆえに、化合物 **11** はマーモセットを用いて膀胱平滑筋に対する弛緩作用を確認した。本評価では、マーモセットの膀胱を摘出し、事前に塩化カリウムを用いて排尿筋を収縮させた後、化合物を添加して膀胱平滑筋の弛緩を測定した。Figure 5 に示すように、化合物 **11** は用量依存的に弛緩作用を示し、その最大弛緩率は陽性対象の非選択的β-AR アゴニストであるイソプロテレノールと同等であった。この結果からマーモセットの膀胱弛緩は高選択的β_3-AR アゴニスト **11** の作用で十分に引き起こすことができると考えられ、その弛緩作用は蓄尿時の膀胱用量の増大につながると推定される。

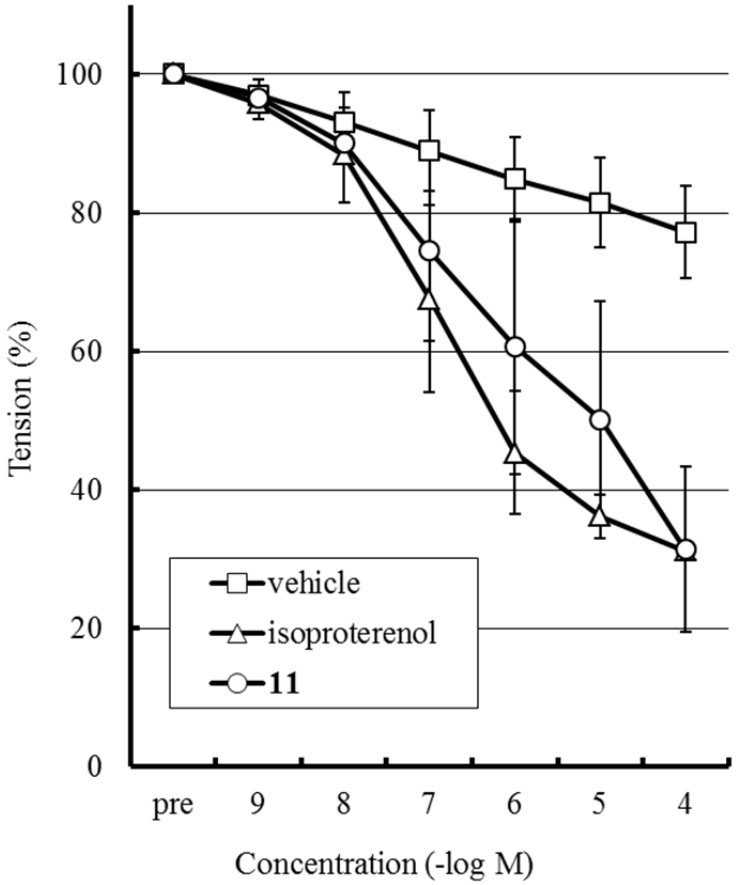


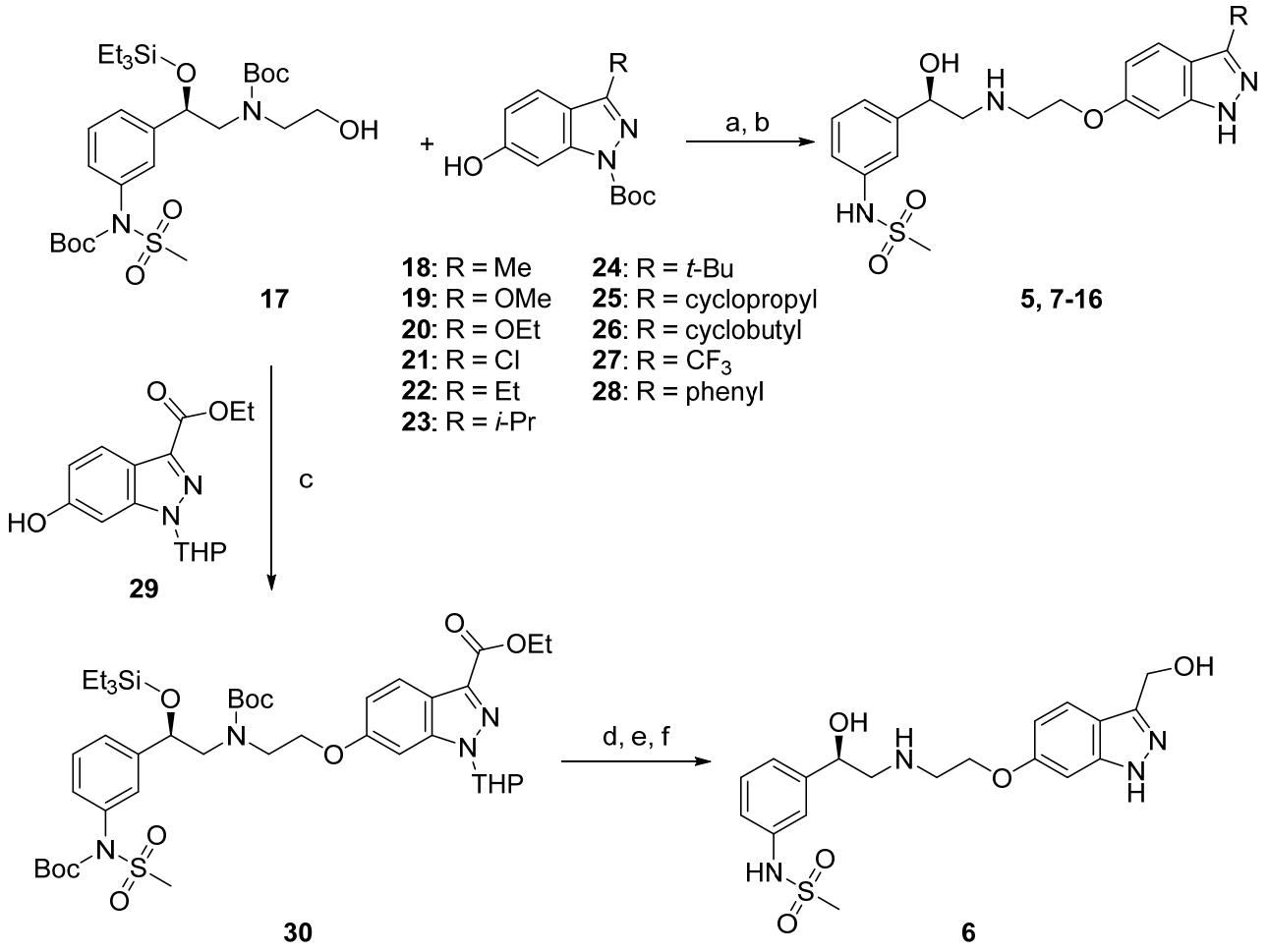
Figure 5. Relaxation of marmoset urinary bladder smooth muscle by **11**. Each point represents the mean ± SD (n = 3). The marmoset urinary bladder was cut by a midline incision and 6 muscle strips of the bladder body were prepared. The strips were pre-contracted with 40 mmol/L KCl, and, after the tension had stabilized, test compounds were cumulatively added. When the relaxation response at the maximum concentration of the test compound was completed, papaverine was added at a final concentration of 10^{-4} mol/L, and the maximum relaxation response of each strip was determined. See Experimental Section for further details.

第６節　インダゾール誘導体 **5**～**16** の合成

（１）ターゲット化合物 **5**～**16** の合成ルート

インダゾール誘導体である化合物 **5**～**16** の合成に関して Scheme 1 に示す。アルコール中間体 **17** とインダゾール中間体 **18**～**28** に対して光延反応を用いてエーテル結合を形成し、続いて塩酸を用いて保護基を除去することにより化合物 **5** 並びに化合物 **7**～**16** を合成した。同様の方法にて、アルコール中間体 **17** とインダゾールエステル中間体 **29** を光延反応にて結合させることで **30** を得た。**30** のエチルエステルを加水分解により、カルボン酸へと誘導後、そのカルボン酸をボラン－ＴＨＦ錯体を用いてアルコールへと還元、続く塩酸による脱保護にて、化合物 **6** を得た。

Scheme 1. Synthesis of targets **5-16**[a]

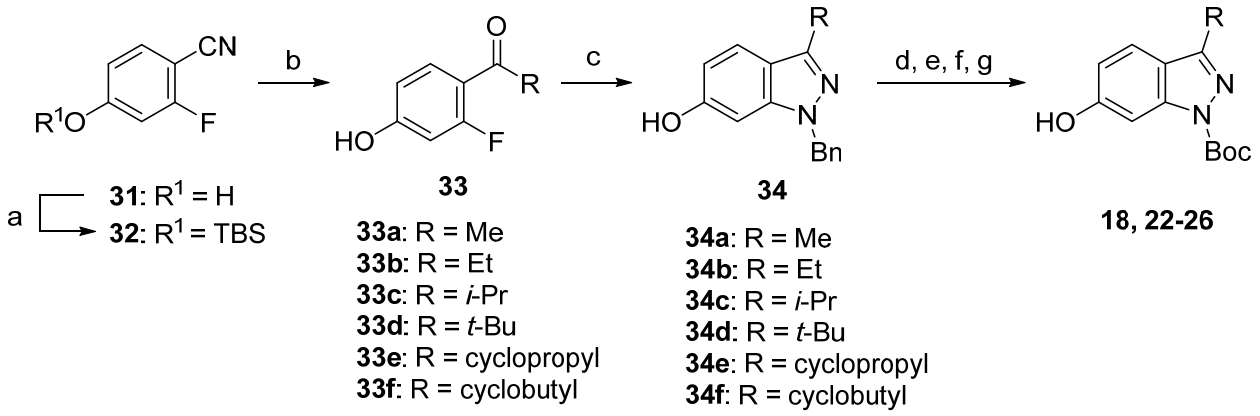


[a]Reagents and conditions: (a) TMAD, PPh_3, toluene, rt; (b) 4 M HCl in EtOAc or 4 M HCl in 1,4-dioxane, rt; (c) TMAD, PPh_3, THF, rt; (d) 2 M NaOH, MeOH, 40 °C; (e) BH_3·THF, THF, rt; (f) conc. HCl aq, EtOH, rt.

（２）インダゾール中間体 **18-28** の合成

インダゾール 3 位にアルキル基を有する中間体 **18**、**22-26** の合成ルートを Scheme 2 に示す。フェノール **31** の水酸基を TBS 基で保護し、続いて Grignard 試薬を用いてシアノ基への求核付加反応と続く加水分解によりケトン **33a-33f** へと変換した。イソプロピル基や *t* ーブチル基のような嵩高い Grignard 試薬の場合には CuBr の添加[26] が効果的であった。得られたケトン **33a-33f** に対し、ベンジルヒドラジンを用いてインダゾール環を構築（**34a-34f**）した。続いて、保護基を変換し、インダゾール中間体 **18**、**22-26** を合成した。

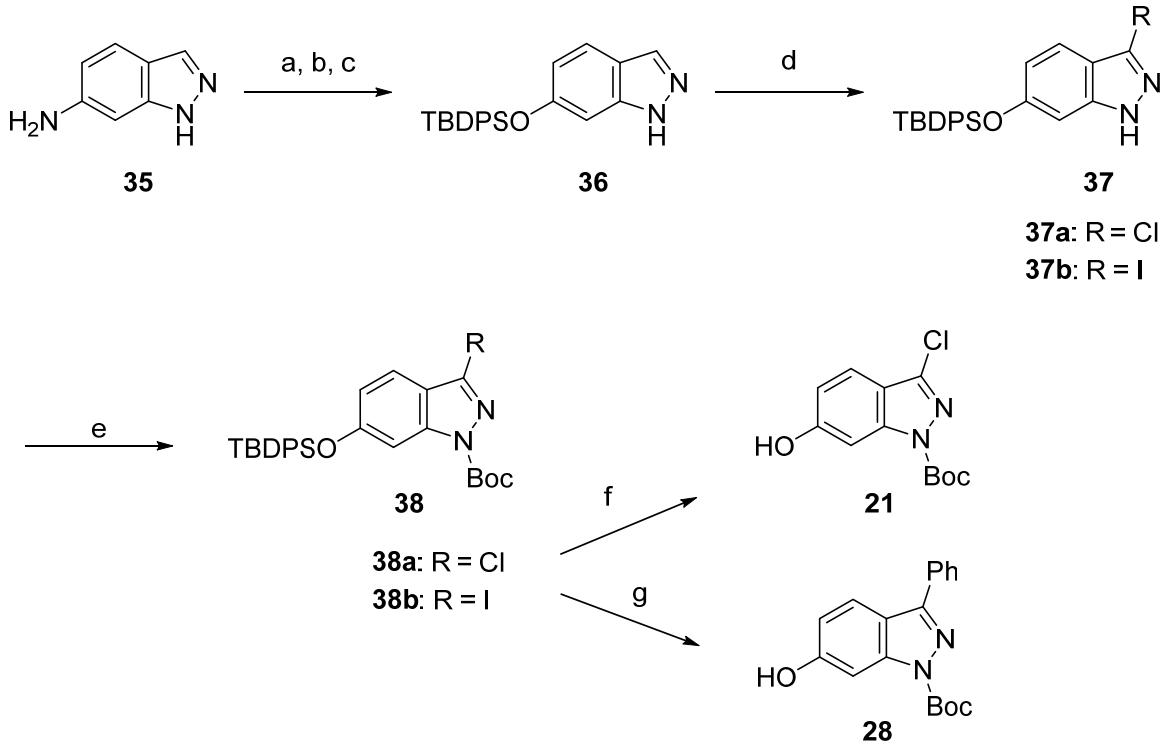
Scheme 2. Preparation of indazole intermediates (**18** and **22-26**)[a]



[a]Reagents and conditions: (a) TBSCl, imidazole, DMF, rt; (b) **33b,e**: RMgBr, THF, reflux, then 5 M HCl aq, reflux; **34c,d,f**: RMgBr or RMgCl, CuBr, THF, reflux, then 5 M HCl aq, reflux; (c) NH_2-NHBn·2HCl, AcONa, xylene, reflux; (d) H_2, Pd/C, conc. HCl, EtOH, 60 °C; (e)TBDPSCl, imidazole, DMF, rt; (f) Boc_2O, Et_3N, DMAP, THF or CH_3CN, rt; (g) TBAF, THF, rt.

インダゾール 3 位にクロロ基もしくはフェニル基を持つ中間体 **21**、**28** の合成ルートを Scheme 3 に示す。６－アミノインダゾール **35** は亜硝酸ナトリウムにてジアゾニウム塩とし、酢酸にてアセチル保護されたフェノール体へ導いた。続いて、アセチル基を変換し、TBDPS 基で保護された６－ヒドロキシインダゾール **36** を得た。塩基性条件下、インダゾール 3 位のハロゲン化にて、クロロ体 **37a**、ヨウ素体 **37b** へと変換した。インダゾールの NH を Boc 基で保護し、TBDPS 基の脱保護を行うことで中間体 **21** を得た。また、**38b** をパラジウム触媒による鈴木 - 宮浦クロスカップリングを行うことで中間体 **28** を得た。なお、本クロスカップリング条件下にて TBDPS 基の除去も反応系中で進行した。

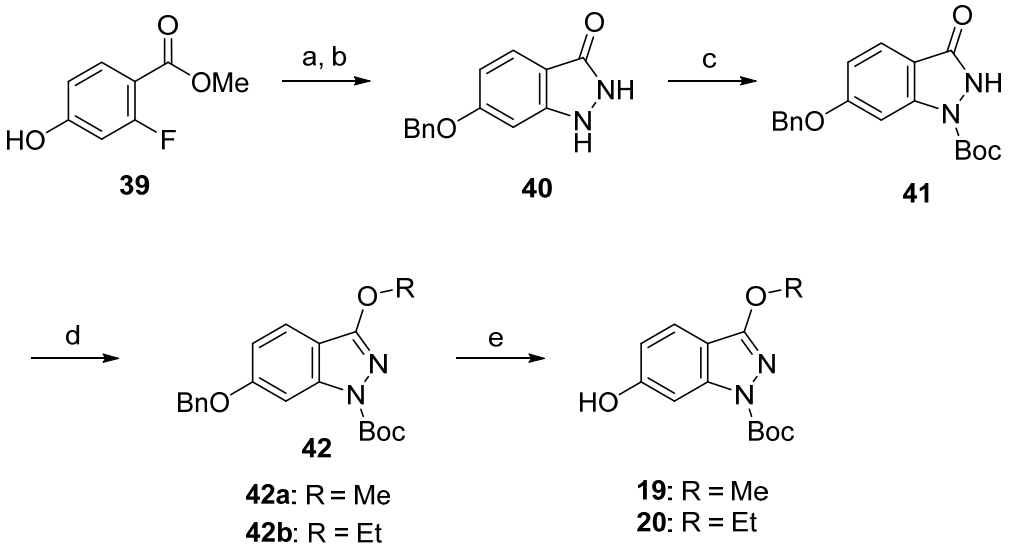
Scheme 3. Preparation of **21** and **28**[a]



[a]Reagents and conditions: (a) $NaNO_2$, HBF_4 aq, H_2O, 0 °C, then AcOH, 130 °C; (b) 2 M NaOH aq, EtOH; (c) TBDPSCl, imidazole, DMF, rt; (d) *tert*-BuOK, THF, then NCS or I_2; (e) Boc_2O, Et_3N, DMAP, THF, rt; (f) TBAF, THF, rt; (g) $PhB(OH)_2$, $(o\text{-Tol})_3P$, $Pd_2(dba)_3$, K_3PO_4, DMF/H_2O, 80 °C.

インダゾール 3 位にアルコキシ基を持つ中間体 **19**、**20** の合成ルートを Scheme 4 に示す。フェノール **39** の水酸基をベンジル基にて保護し、ヒドラジン水和物存在下、マイクロウェーブを用いた加熱反応を実施し、インダゾール体 **40** を得た。Patel らの方法[27]を参考に、**40** に対して di-Boc 保護を行った後、アンモニアーメタノールで処理することで *O*-Boc 基を除去し、*N*-Boc 体 **41** を得た。続いて銀塩を用いた酸素原子選択的なアルキル化[28]にて、**42a**、**42b** を得た後、ベンジル基を除去することで中間体 **19**、**20** を得た。

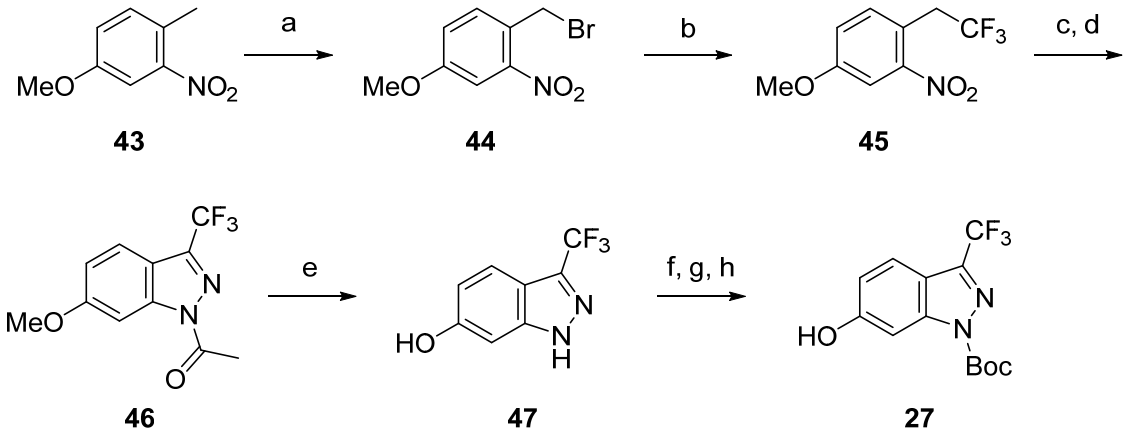
Scheme 4. Preparation of **19** and **20**[a]



[a]Reagents and conditions: (a) BnBr, K_2CO_3, DMF, 50 °C; (b) NH_2-NH_2・H_2O, *n*-BuOH, MW, 160 °C; (c) Boc_2O, Et_3N, DMAP, CH_2Cl_2, rt, then 7 M NH_3/MeOH, MeOH, rt; (d) R-I , Ag_2CO_3, toluene, MW, 60 °C or 90 °C; (e) H_2, Pd/C, THF, rt.

インダゾール 3 位にトリフルオロメチル基を持つ中間体 **27** の合成ルートを Scheme 5 に示す。ニトロトルエン **43** に対し、ラジカル条件下でベンジル位を臭素化することで化合物 **44** を得た。銅触媒存在下、FSO_2CF_2COOMe を用いたトリフルオロメチル化[29]にて、化合物 **45** を得た。**45** のニトロ基を還元後、アミノ基のアセチル化と亜硝酸アミルにおけるインダゾール環構築によりトリフルオロメチルインダゾール **46** を得た。**46** は臭化水素酸水溶液を用いてメトキシ基とアセチル基の脱保護を行い、**47** へと導いた後、保護基を変換し、中間体 **27** を得た。

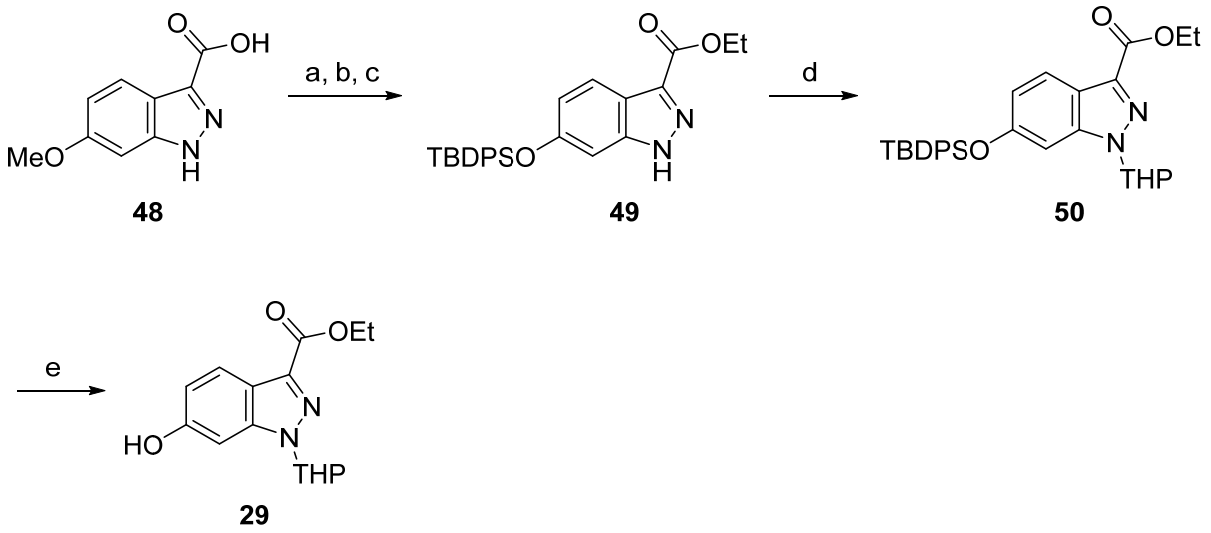
Scheme 5. Preparation of **27**[a]



[a]Reagents and conditions: (a) NBS, $(PhCOO)_2$, CCl_4, reflux; (b) FSO_2CF_2COOMe, CuI, DMF, 100 °C; (c) H_2, Pd/C, MeOH, rt; (d) Ac_2O, AcOK, PhCl, rt, then isoamyl nitrite, 80 °C, (e) 48% HBr aq, 110 °C; (f) TBDPSCl, imidazole, DMF, rt; (g) Boc_2O, Et_3N, DMAP, THF, rt; (h) TBAF, THF, rt.

インダゾール 3 位にエチルエステル基を持つ中間体 **29** の合成ルートを Scheme 6 に示す。インダゾールカルボン酸 **48** の保護基を変換し、エステル体 **49** を得た。**49** のインダゾールの NH を THP 基で保護して **50** へと導いた後、TBDPS 基を除去し、中間体 **29** を得た。

Scheme 6. Preparation of **29**[a]

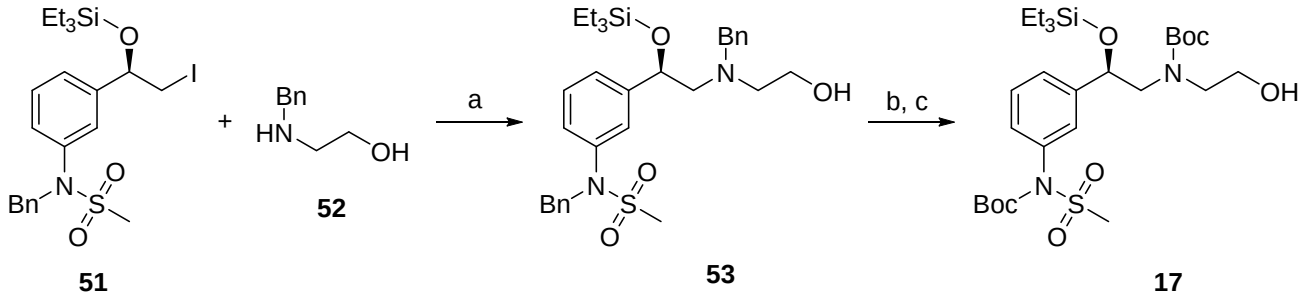


[a]Reagents and conditions: (a) 48% HBr aq, reflux; (b) EtOH, $SOCl_2$, 60 °C; (c) TBDPSCl, imidazole, DMF, rt; (d) DHP, TsOH H_2O, toluene, 60 °C; (e) TBAF, THF, rt.

（３）アルコール中間体 **17** の合成

ベンジルアミン **52** のアミノ基を化合物 **51**[30]でアルキル化を行い、化合物 **53** へと誘導した。続いて水素添加によりベンジル基を除去後、Boc 基へと変換し、アルコール中間体 **17** を得た（Scheme 7）。

Scheme 7. Preparation of the alcohol intermediate **17**[a]



[a]Reagents and conditions: (a) neat. 100 °C; (b) H_2, 20% $Pd(OH)_2$/C, MeOH/THF, 50 °C; (c) Boc_2O, Et_3N, DMAP, THF, rt.

第７節　第１章まとめ

アリールエタノールアミンライブラリーから見出したヒット化合物 **5** のインダゾール 3 位を変換することにより、β_3-AR に高選択的な化合物 **11** を見出した。化合物 **11** はマーモセットに対して用量依存的に排尿筋弛緩作用を示した。さらに、化合物 **11** はミラベグロン（**1**）と比較し、ラットを用いた血圧心拍の評価において影響を与えなかった。したがって、化合物 **11** はβ_3-AR 高選択的であり、心血管系に対する副作用が少ない特徴を有していることが示唆された。

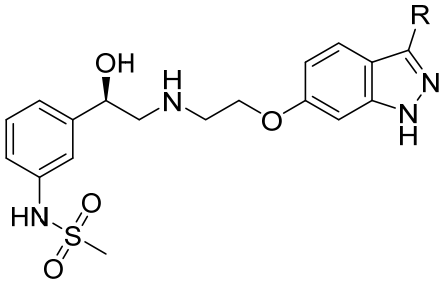
第２章　スルホンアミド変換による薬物動態プロファイルの最適化

第１節　化合物 **11** 並びにその類縁体の薬物動態プロファイル

過活動膀胱の治療薬は患者が容易に服用できる経口剤が望ましいため、第 1 章で述べた代表的な 4 化合物が経口投与可能かどうか薬物動態プロファイルの評価を実施した。*in vitro* 薬物動態の結果を Table 6 に示す。第１章の第２節で述べたように、化合物 **5** は *in vitro* ヒトミクロソーム代謝安定性（$CL_{int,u}$）*が良好であったが、化合物 **11** 並びに化合物 **14** は化合物 **5** と比較し、ヒトミクロソーム並びにラットミクロソームに対する代謝安定性が低かった。また、*in vivo* の薬物動態プロファイル並びに膜透過性、水溶性のプロファイルを Table 7 に示す。化合物 **11** は *in vivo* の薬物動態プロファイル（C_{max} and AUC）が化合物 **5** と比較して悪かった。一方、化合物 **11** は中程度の膜透過性、良好な水溶性を示したことから、化合物 **11** の低い C_{max} と AUC は代謝安定性が低いことに由来すると示唆された。

第 1 章で述べたように、インダゾールの 3 位の置換基がβ_3-AR 選択性に重要であり、β_3-AR に対する選択性の高い順番はイソプロピル体 **8** ＞シクロブチル体 **7** ＞エチル体 **6** ＞メチル体 **5** である。一方、ヒト代謝安定性が高い順番はメチル体 **5** ＞エチル体 **6** ＞イソプロピル体 **8** ＞シクロブチル体 **7** であった。ゆえに、β_3-AR 選択性が高い化合物ほど、代謝的に不安定であり、経口剤として適さないことが示唆された。すなわち、経口投与可能な過活動膀胱治療薬に仕上げるには、化合物 **11** の代謝的不安定性を改善する必要がある。したがって、この課題を克服するためにさらなる化合物最適化を実施した。

Table 6. AR agonist activity and *in vitro* metabolic stability

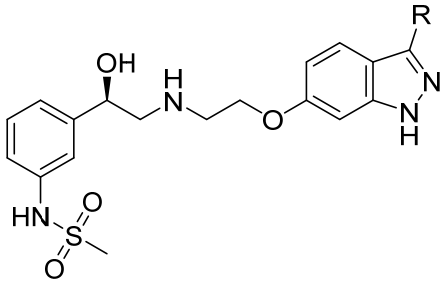


Compound	R	$h\beta_3$[a]		$h\alpha_{1A}$[a]		Selectivity	Metabolic Stability[a]	
		EC_{50} (nM)	IA[b] (%)	EC_{50} (nM)	IA[c] (%)	α_{1A} / β_3	$hCL_{int,u}$ (mL/min/kg)	$rCL_{int,u}$ (mL/min/kg)
5	Me	21 ± 0.67	82	219 ± 6.9	71	10	<10	103
10	Et	14 ± 0.58	78	857 ± 164	31	61	42	113
11	*i*-Pr	13 ± 1.5	69	>10000	9.1	>769	193	211
14		66	68	>10000	4	>151	463	641

[a]The results are shown as the mean ± SEM (n = 3) or are presented as the average of two experiments. [b]IA (intrinsic activity): maximum response induced by isoproterenol was defined as 100%. [c]IA: maximum response induced by noradrenaline was defined as 100%.

* 数字が大きいほど代謝が不安定であることを示している

Table 7. *In vitro* ADME and *in vivo* pharmacokinetics in rats



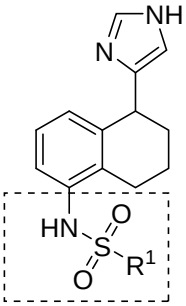
Compound	**5**	**11**
R	Me	*i*-Pr
Physicochemical properties and *in vitro* ADME profiles		
Metabolic stability[a]		
$hCL_{int,u}$ (mL/min/kg)	<10	193
$rCL_{int,u}$ (mL/min/kg)	103	211
Permeability[a]		
MDCK ($x10^{-6}$ cm/s)	2.4	4.1
Solubility[a]		
pH 1.2 solution (μM)	285	295
pH 6.8 solution (μM)	273	267
Pharmacokinetics parameters in rats[a]		
5 mg/kg[b]		
C_{max} (μg/mL)	0.107 ± 0.034	0.0452
AUC (μg·hr/mL)	0.542 ± 0.065	0.200

[a]The results are shown as the mean ± SD (n = 3) or are presented as the average of two experiments. [b]Compounds were administered orally to rats in solution with saline or water.

第２節　高いβ_3-AR 選択性とヒト代謝安定性を両立する最適化の方針

Altenbach らは、スルホンアミドを持つα_{1A}-AR アゴニスト **54** の構造活性相関を報告している。[31]すなわち、Figure 6 に示すように、化合物 **54** のスルホンアミド基のアルキルが大きくなるにつれ、α_{1A}-AR アゴニスト活性が減弱し、さらに、芳香環を持つスルホンアミド基に変換すると、α_{1A}-AR アゴニスト活性が無くなったと述べている。一方、Sawa らは、アルキルスルホンアミド **55a** と芳香族スルホンアミド **55b** を比較し、**55a** よりも **55b** の方がβ_3-AR アゴニスト活性が強いことを報告している。[32]これら 2 つの文献報告を参考にして、筆者はα_{1A}/β_3-AR 選択性は低いが、ヒト代謝安定性が高い化合物 **5** のスルホンアミド基の置換基の大きさを変換するターゲット化合物 **56** を設計した。なお、第 1 章でインダゾールメチル体 **5** のラット代謝物解析から水酸化体 **6** を同定したが、化合物 **5** 自体のヒト代謝安定性は良好であることから、ターゲット化合物 **56** はヒト代謝安定性とα_{1A}/β_3-AR 選択性の両立が期待される。

Altenbach *et al.*

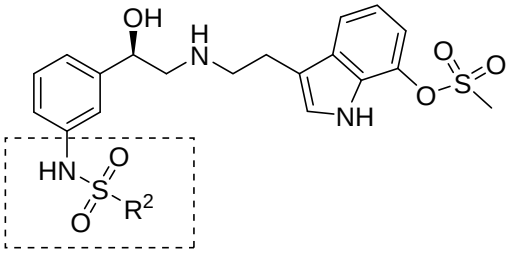


54

α_{1A}-AR agonistic activity
R^1 = Me > *i*-Pr >> 2-naphtyl (inactive)

β_3-AR agonistic activity
not reported

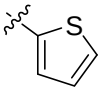
Sawa *et al.*

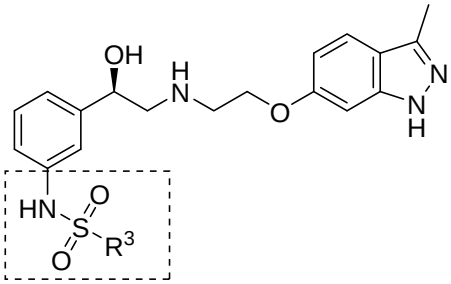


55

α_{1A}-AR agonistic activity
not reported

β_3-AR agonistic activity
55a; R^2 = ethyl, EC_{50} = 18 nM

55b; R^2 =  , EC_{50} = 1.7 nM



56

R^3 = branched alkyl, cycloalkyl, and aryl

Selective for β_3-AR over α_{1A}-AR and metabolically stable?

Figure 6. Design strategy of the sulfonamide moiety modified analogues.

第３節　スルホンアミド部分の変換による構造－β_3-AR 並びにα_{1A}-AR アゴニスト活性相関

（１）構造－β_3-AR アゴニスト活性相関

Table 8 にスルホンアミド基を変換した化合物のβ_3-AR アゴニスト活性を示す。メチル基 **5** を *n*－プロピル基 **56a** に変換した結果、活性が若干向上した（**5**, EC_{50} = 21 nM vs. **56a**, EC_{50} = 6.5 nM）。しかし、イソプロピル基 **56b** への変換はメチル基 **5** と比較し､活性が低下した（**5**, EC_{50} = 21 nM vs. **56b**, EC_{50} = 70 nM）。次にシクロアルキル基へと変換した結果、シクロプロピル基 **56c** とシクロブチル基 **56d** はイソプロピル基 **56b** と比較して活性が向上した（**56b**, EC_{50} = 70 nM vs. **56c**, EC_{50} = 43 nM; **56d**, EC_{50} = 18 nM）。しかし、シクロペンチル基 **56e** はイソプロピル基 **56b** よりも大きく活性が低下した（**56b**, EC_{50} = 70 nM vs. **56e**, EC_{50} = 309 nM）。一方、フェニル基 **56f** への変換は EC_{50} = 3.9 nM を示し、アルキル基よりも活性が向上した。

以上をまとめると、β_3-AR アゴニスト活性の強さの順は **56f** > **56a** > **56d**, **5** > **56c** > **56b** > **56e** であった。

スルホンアミドのアルキル基の変換にて *n*－プロピル基 **56a** とシクロブチル基 **56d** はイソプロピル基 **56c** やシクロペンチル基 **56e** と比較し、β_3-AR アゴニスト活性が強いことから、*n*－プロピル基やシクロブチル基のサイズがβ_3-AR との結合に良い効果を示していると考察した。一方、フェニル基 **56f** がアルキル基と比較して大幅に活性向上した結果はβ_3-AR のアミノ酸残基と CH-π もしくはπ-π のような相互作用をしていると推定した。

（２）構造－α_{1A}-AR アゴニスト活性相関

Table 8 にスルホンアミド基を変換した化合物のα_{1A}-AR アゴニスト活性を示す。*n*－プロピル体 **56a** はメチル体 **5** と比較し、α_{1A}-AR アゴニスト活性に変化は無かった（**5**, EC_{50} = 219 nM vs. **56a**, EC_{50} = 163 nM）。一方、イソプロピル体 **56b** もしくはシクロプロピル体 **56c** は、メチル体 **5** と比較し、α_{1A}-AR アゴニスト活性が減弱した（**5**, EC_{50} = 219 nM vs. **56b**, EC_{50} = 1757 nM; **56c**, EC_{50} = 1250 nM）。さらに、シクロブチル体 **56d**、シクロペンチル体 **56e** もしくはフェニル体 **56f** はα_{1A}-AR アゴニスト活性を示さなかった（EC_{50} >10000 nM）。以上をまとめると、α_{1A} -AR アゴニスト活性の強さの順は **5**, **56a** > **56c**, **56b** >> **56d**, **56e**, **56f** であった。

n－プロピル体 **56a** はイソプロピル体 **56b** よりも 10 倍α_{1A}-AR アゴニスト活性が強いので、直鎖のアルキル基はα_{1A}-AR との結合に適していると考察した。また、シクロブチル基 **56d**、シクロペンチル基 **56e** もしくはフェニル基 **56f** は全くα_{1A}-AR アゴニスト活性を示さなかったことから、嵩高い置換基はα_{1A}-AR との結合に不利であると推定した。

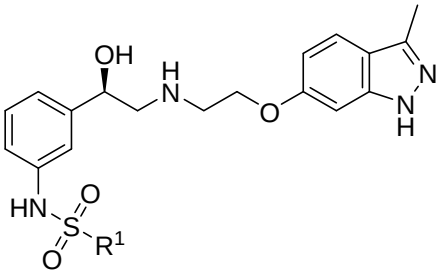
（３）α_{1A}/β_3 選択性の構造相関

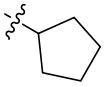
β_3-AR アゴニスト活性とα_{1A}-AR アゴニスト活性を基に、α_{1A}-AR に対してβ_3-AR の選択性の順は **56f** > **56d** >> **56a**, **56b**, **56c**, **56e** > **5** であった。ゆえに、α_{1A}-AR に対するβ_3-AR 選択性を考慮し、化合物 **56f** と化合物 **56d** を選択し、β_1 とβ_2-AR の選択性評価を実施した。

（４）β_1-AR とβ_2-AR のアゴニスト活性

Table 9 に示すように、化合物 **56d** と化合物 **56f** はβ_1 とβ_2-AR に対してアゴニスト活性を示さなかった（EC_{50} >10000 nM）。ゆえに、化合物 **56d** と化合物 **56f** はβ_3-AR に高選択的な化合物であることが示唆された。

Table 8. Human β_3- and α_{1A}-AR agonistic activities of sulfonamide derivatives[a]



Compound	R^1	β_3[b]		α_{1A}[b]		Selectivity
		EC_{50} (nM)	IA (%)[c]	EC_{50} (nM)	IA (%)[d]	α_{1A} / β_3
5	-Me	21 ± 0.67	82	219 ± 6.9	71	10
56a	-*n*-Pr	6.5 ± 0.94	79	163	84	25
56b	-*i*-Pr	70 ± 6.4	90	1757	27	25
56c		43 ± 4.3	72	1250	26	29
56d		18 ± 2.6	105	>10000	0	>556
56e		309 ± 69	58	>10000	0	>32
56f	-Ph	3.9 ± 0.067	85	>10000	0	>2564
isoproterenol		86 ± 3.7	100	NT		
noradrenaline		NT		9.1 ± 0.52	100	

[a]The results are shown as the mean ± SEM (n = 3) or are presented as the average of two experiments. [b]Human β_3- or α_{1A}-AR agonist assay, see Experimental Section for further details. [c]IA (intrinsic activity): maximum response induced by isoproterenol was defined as 100%. [d]IA: maximum response induced by noradrenaline was defined as 100%. NT: not tested.

Table 9. Summary of human β- and α_1-ARs agonistic activities[a]

Compound	β_3[b]		β_1[b]		β_2[b]		α_{1A}[b]	
	EC_{50} (nM)	IA (%)[c]	EC_{50} (nM)	IA (%)[c]	EC_{50} (nM)	IA (%)[c]	EC_{50} (nM)	IA (%)[d]
11	13 ± 1.5	69	>10000	11.9	>10000	5.6	>10000	9.1
56d[e]	18 ± 2.6	105	>10000	4	>10000	4	>10000	0
56f	3.9 ± 0.067	85	>10000	10	>10000	0	>10000	0
isoproterenol	86 ± 3.7	100	3.2 ± 0.29	100	13 ± 3.0	100	NT	
noradrenaline	NT		NT		NT		9.1 ± 0.52	100

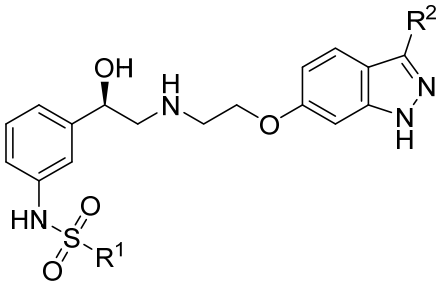
[a]The results are shown as the mean ± SEM (n = 3) or are presented as the average of two experiments. [b]Human β_1-, β_2-, β_3- or α_{1A}-AR agonist assay, see Experimental Section for further details.. [c]IA (intrinsic activity): maximum response induced by isoproterenol was defined as 100%. [d]IA: maximum response induced by noradrenaline was defined as 100%. [e]Compound **56d** exhibited insignificant agonistic activity for α_{1B}-AR and α_{1D}-AR (EC_{50} >10000 nM). NT: not tested.

第４節　化合物 **56d** と化合物 **56f** の薬物動態プロファイル

Table 10 に *in vitro* 薬物動態プロファイル並びにラットに経口投与した際の *in vivo* 血漿中濃度を測定した結果を示す。化合物 **56d** は化合物 **11** と比較し、ヒトミクロソーム代謝安定性が向上した（**11**, $hCL_{int,u}$ = 193 mL/min/kg vs. **56d**, $hCL_{int,u}$ = 57 mL/min/kg）。一方、化合物 **56f** は化合物 **11** よりもヒトミクロソーム代謝安定性が低下した（**11**, $hCL_{int,u}$ = 193 mL/min/kg vs. **56f**, $hCL_{int,u}$ = 464 mL/min/kg）。ラットミクロソーム代謝安定性に関しても同様の傾向を示し、化合物 **56d** は化合物 **11** と比較して 4 倍優れていた（**11**, $rCL_{int,u}$ = 211 mL/min/kg vs. **56d**, $rCL_{int,u}$ = 52 mL/min/kg）。また、化合物 **56d** と化合物 **56f** は中程度の膜透過性を示し、pH 1.2 と pH 6.8 の溶液に対する溶解度は優れていた。さらに、代謝安定性が向上した化合物 **56d** は化合物 **11** と比較してラットにおける経口吸収性（C_{max}、AUC 並びに半減期）が向上した。

化合物 **56d** は化合物濃度 10 μM における CYP 阻害作用を評価した結果、7 種類の CYP（CYP1A2、2B6、2C19、2C8、2C9、2D6、3A4）に対して阻害作用を示さなかった（34%未満）。一方、同濃度におけるミラベグロン（**1**）の CYP 阻害作用評価を実施した結果、CYP2D6 に対して 87%の阻害作用を示した。ゆえに、化合物 **56d** はミラベグロン（**1**）よりも CYP2D6 にて代謝される薬に対して、薬物間相互作用のリスクが低いことが示唆された。

Table 10. *In vitro* ADME and *in vivo* pharmacokinetics of 11, 56d, and 56f in rats



Compound	**11**	**56d**	**56f**
R^1	Me		Ph
R^2	*i*-Pr	Me	Me
Physicochemical and *in vitro* ADMET profiles			
Metabolic stability[a]			
$hCL_{int,u}$ (mL/min/kg)	193	57	464
$rCL_{int,u}$ (mL/min/kg)	211	52	NT
Permeability[a]			
MDCK ($x10^{-6}$ cm/s)	4.1	5.0	5.1
Solubility[a]			
pH 1.2 solution (μM)	295	299	300
pH 6.8 solution (μM)	267	278	229
Pharmacokinetics parameters in rat[a]			
5 mg/kg[b]			
C_{max} (μg/mL)	0.0452	0.119 ± 0.077	NT
AUC (μg·hr/mL)	0.200	0.312 ± 0.100	NT
Half-life (hr)	1.62	3.01 ± 1.51	NT

[a]The results are shown as the mean ± SD (n = 3) or are presented as the average of two experiments. [b]Compounds were administered orally to rats in solution with saline or water. NT: not tested.

第５節　化合物 **56d** のラット心血管系への作用と排尿筋弛緩作用

β_3-AR に高選択的で薬物動態プロファイルが改善した化合物 **56d** とミラベグロン（**1**）の心血管系の安全性プロファイルの比較を行った（Table 6）。ミラベグロン（**1**）を投与したラットは心拍数が増加し、平均血圧が大きく低下した。一方、化合物 **56d** を投与したラットはミラベグロン（**1**）と比べて心拍数や平均血圧の変動が少なかった。このデータは化合物 **56d** がミラベグロン（**1**）よりも心血管系への副作用が少なく、β_3-AR に高選択的であることがその要因であることを示唆している。

次に、マーモセットを用いて化合物 **56d** の膀胱平滑筋に対する弛緩作用評価を実施した。Figure 7 に示すように、化合物 **56d** は事前に塩化カリウムで収縮させた膀胱平滑筋を用量依存的に弛緩させ、その最大薬効は陽性対象の非選択的β-AR アゴニストであるイソプロテレノールと同等であった。この結果から Figure 5 で示した化合物 **11** と同様にマーモセットの膀胱弛緩は高選択的β_3-AR アゴニスト **56d** の作用で十分に引き起こすことができると考えられ、その弛緩作用は蓄尿時の膀胱用量の増大につながると推定される。

以上のように、β_3-AR に高選択的であり、経口投与可能で、薬物間相互作用のリスクが少なく、用量依存的に膀胱平滑筋の弛緩作用を示したので、化合物 **56d** は心血管系への副作用を軽減した過活動膀胱の治療薬になりえる有望化合物である。

Table 11. Effects of intravenous administration of 56d on HR and MBP in anaesthetized rats[a]

		HR		MBP	
Compound	N	Increase (%)	Decrease (%)	Increase (%)	Decrease (%)
Saline	5	1.8 ± 0.9	4.5 ± 0.8	1.0 ± 0.5	5.4 ± 1.9
1	3	10.0 ± 2.5	ND	3.5 ± 3.5	38.3 ± 3.4
56d	3	5.8 ± 0.4	0.9 ± 0.4	2.0 ± 0.9	12.9 ± 0.9

[a]Compounds were administered intravenously to rats (3 mg/kg) and HR and MBP recorded for 30 min. Maximum increase or decrease of HR or MBP is shown as the percentage change relative to baseline. The results are shown as the mean ± SEM. See Experimental Section for further details. N: Number of rats used. ND: Not detected.

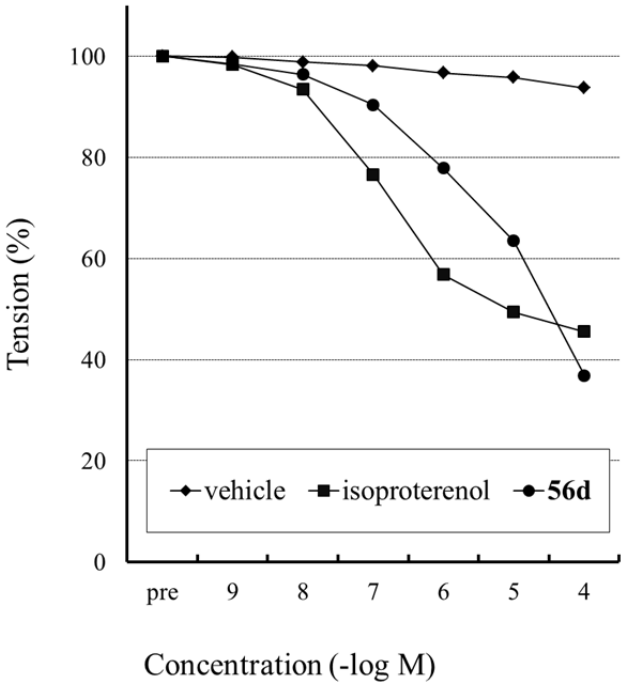


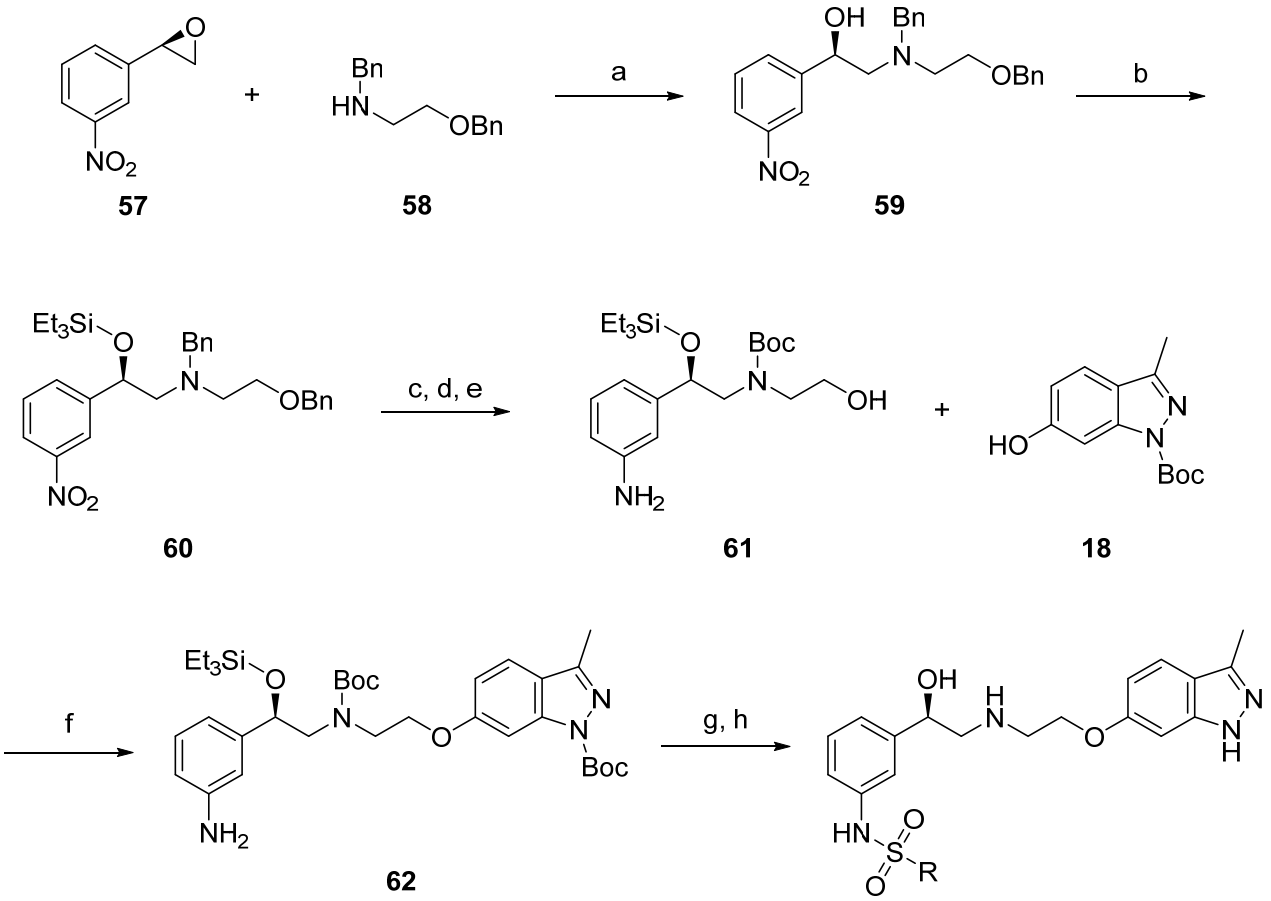
Figure 7. Relaxation of marmoset urinary bladder smooth muscle by **56d**. Each point represents the mean of two experiments. See Experimental Section for further details.

第６節　インダゾール化合物 **56a**～**56f** の合成

（１）ターゲット化合物 **56a**～**56f** の合成

化合物 **56a**～**56f** の合成ルートを Scheme 8 に示す。アミノアルコール **59** は２－プロパノール中、ベンジルアミン **58** を用いてエポキシド **57**[33]を開環させることで得た。**59** の水酸基をトリエチルシリル基で保護し、**60** を得た。**60** のニトロ基とベンジル基を水素化分解にて還元し、その後、アミノ基選択的に Boc 保護、再び水素化分解にて *O*－ベンジル基の脱保護を行うことで **61** を得た。**61** と **18** を DMEAD[34] とトリフェニルホスフィンを用いた光延反応にて結合させることで **62** を得た。アニリン **62** と種々のスルホニルクロリドを反応させ、スルホンアミド体を得た後、Boc 基と TES 基の脱保護を行うことでターゲット化合物 **56a**～**56f** を得た。

Scheme 8. Synthesis of target compounds **56a-56f**[a]



[a]Reagents and conditions: (a) 2-PrOH, reflux; (b) TESCl, imidazole, DMF, rt; (c) H_2, 10% Pd/C, EtOH, 50 °C; (d) Boc_2O, THF, rt; (e) H_2, 20% $Pd(OH)_2/C$, THF, MeOH, 50 °C; (f) DMEAD, PPh_3, toluene, rt; (g) R-SO_2Cl (R = *n*-Pr, cyclopropyl, cyclobutyl, or phenyl), pyridine, CH_2Cl_2 or R-SO_2Cl (R = *i*-Pr or cyclopentyl), DBU, CH_2Cl_2, rt; (h) for **56a-56c**, **56e**, and **56f**: 4 M HCl in dioxane, rt, for **56d**[*]: 1 M TBAF in THF, THF, rt then 4 M HCl in dioxane, rt.

[*] 固体の化合物 **56d** を取得するためには塩酸を用いた脱保護の前に一度トリエチルシリル基を除去し、カラムクロマトグラフィー精製することが効果的であった。

第７節　第２章まとめ

筆者は高活性、高選択的なβ_3-AR アゴニストである化合物 **56d** の創出に成功した。化合物 **56d** は用量依存的にマーモセットの膀胱平滑筋を弛緩させる優れた薬効を示し、臨床薬であるミラベグロンよりも心血管系の副作用が少なかった。さらに化合物 **56d** はミラベグロンよりも薬物間相互作用のリスクが低く、代謝的に安定であり、ラットにおいて十分な経口吸収が確認できた。ゆえに、化合物 **56d** は心血管系の副作用を軽減した過活動膀胱の治療薬として期待される。

第３章 β_3-AR と化合物 **56d** の相互作用解析

第１節　ドッキングスタディと分子動力学シミュレーションによるβ_3-AR と化合物 **56d** の結合様式の推定

第 1 章並びに第 2 章で述べたインダゾールシリーズβ_3-AR アゴニストとβ_3-AR とのドッキングスタディにより、結合様式を推定することはβ_3-AR アゴニスト活性の構造活性相関に対する理解とα_{1A}-AR、β_1-AR、β_2-AR に対する選択性を考察するために非常に有用である。β_3-AR の結晶構造は報告されていないため、β_3-AR のホモロジーモデルの構築を行う必要がある。これまでに、β_3-AR のサブタイプであるβ_2-AR のアンタゴニストもしくはインバースアゴニストとの複合体の結晶構造を基に、β_3-AR ホモロジーモデルを構築し、化合物の結合様式の解析並びに QSAR 解析を行った文献が報告されている。[14b, 14e, 35]一方、近年β_2-AR のアゴニストとの複合体の結晶構造が報告されたため、β_3-AR のホモロジーモデルとβ_3-AR アゴニストの推定結合様式を予測する精度向上が期待される。[36]ゆえに、私はβ_2-AR のアゴニストとの複合体の結晶構造（PDB entry 3P0G）を基にβ_3-AR ホモロジーモデルを構築することを計画した。Figure 8 にβ_3-AR ホモロジーモデルの構築からドッキングスタディまでの流れを示す。はじめに、β_1-AR、β_2-AR、β_3-AR 並びにα_{1A}-AR のアミノ酸配列は MOE 2015 のソフトウェアを用いてアライメントを実施した。さらにホモロジーモデルの鋳型となるβ_2-AR のアミノ酸配列に対しては Roy らの文献[35b]と同一のアライメントにした（Figure 9）。β_3-AR とβ_2-AR のアミノ酸配列の相同性は高く、56%であった。[35b]

続いて、インダゾールシリーズの中でβ_3-AR アゴニスト活性が最も高い化合物 **56f** とβ_3-AR ホモロジーモデルの複合体を構築した。次に、そのモデルを用いて 8 化合物のインダゾールシリーズと KUC-7322、化合物 **55** の 2 つの類縁体、並びにイソプロテレノールの合計 12 化合物を用いてドッキングスタディを実施した。同様のドッキングスタディの手順は当研究室においてヒスタミン H_3 受容体の結合様式解析で報告している。[37]ドッキングスコア（Glide XP）*と pEC_{50}†の相関解析を Figure 10 に示す。また、ドッキングスコアと pEC_{50} の値を Table 12 に示す。Figure 10 に示した通り、ドッキングスコアと pEC_{50} の間に正の相関が見られ、その相関係数（R）は $R = 0.67$ であり（Figure 10A）、8 つのインダゾール類縁体に限定すると $R = 0.90$ であった（Figure 10B）。ゆえに、ドッキングスコアと pEC_{50} の間には強い相関があることを示している。この強い相関はβ_3-AR ホモロジーモデルとリガンドのドッキングポーズによりβ_3-AR アゴニスト活性の構造活性相関を合理的に説明できる。

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* リガンドとβ_3-AR の相互作用として水素結合、静電相互作用、ファンデルワールス力、クーロン力、疎水性相互作用に関してスコアを計算し、その合計からリガンドの配座安定性とリガンドの脱水和エネルギーをペナルティとして引いた値を最終的なドッキングスコアとして算出している

† $pEC_{50} = -\log_{10}(EC_{50})$

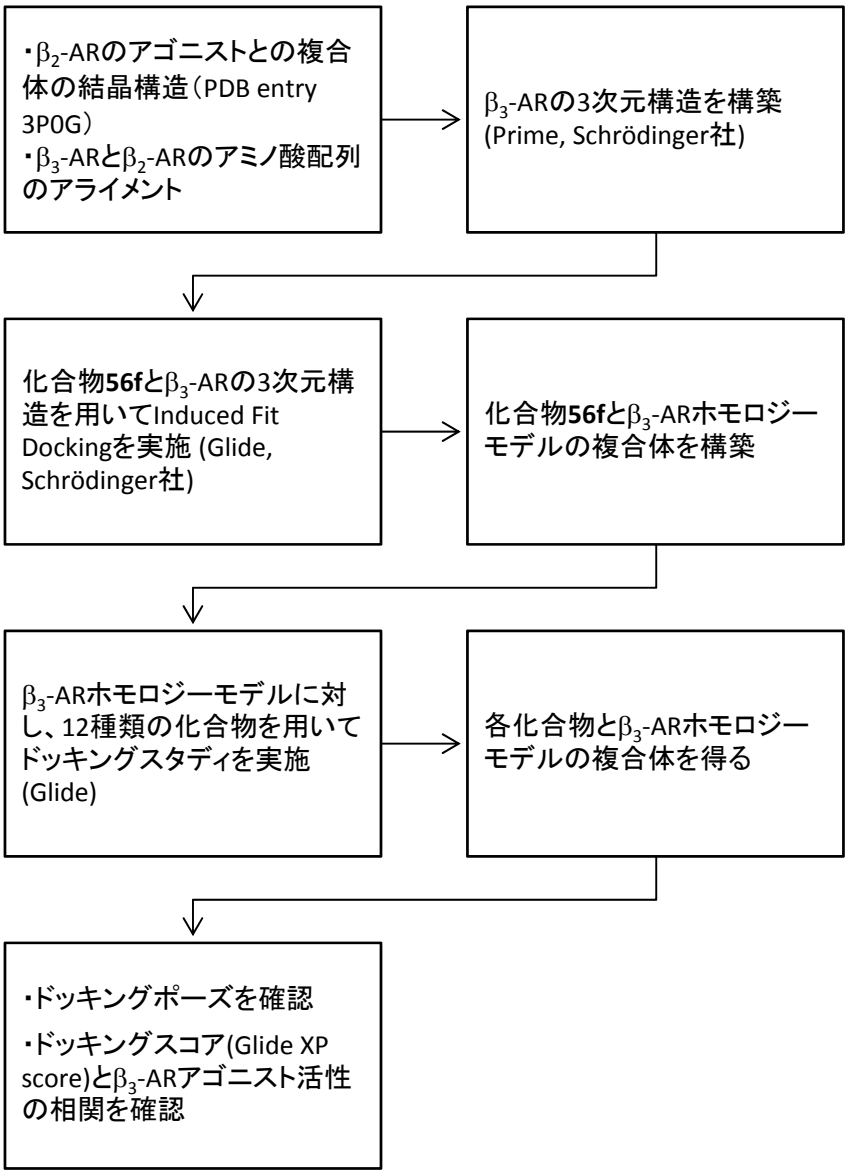

Figure 8. Flow chart of the docking procedure.

```
ADRB3_HUMAN    ---------------------MAPWPHENSSLAP-WPDLPTLAPNTANTSGLPGVPWEAA 37
ADA1A_HUMAN    --------------------------------MVFLSGNASDSSNCTQPPAPVNIS--KA 26
ADRB1_HUMAN    MGAGVLVLGASEPGNLSSAAPLPDGAATAARLLVPASPPASLLPPASESPEPLSQQ-WTA 59
ADRB2_HUMAN    ---------MGQPGN---------GSAF---LLAPNGSHA----PDHDVTQERDEV-WVV 34

ADRB3_HUMAN    LA-GALLALAVLATVGGNLLVIVAIAWTPRLQTMTNVFVTSLAAADLVMGLLVVPPAATL 97
ADA1A_HUMAN    ILLGVILGGLILFGVLGNILVILSVACHRHLHSVTHYYIVNLAVADLLLTSTVLPFSAIF 86
ADRB1_HUMAN    GM-GLLMALIVLLIVAGNVLVIVAIAKTPRLQTLTNLFIMSLASADLVMGLLVVPFGATI 118
ADRB2_HUMAN    GM-GIVMSLIVLAIVFGNVLVITAIAKFERLQTVTNYFITSLACADLVMGLAVVPFGAAH 93

ADRB3_HUMAN    ALTGHWPLGATGCELWTSVDVLCVTASIETLCALAVDRYLAVTNPLRYGALVTKRCARTA 157
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ADA1A_HUMAN    EVLGYWAFGRVFCNIWAAVDVLCCTASIMGLCIISIDRYIGVSYPLRYPTIVTQRRGLMA 146
ADRB1_HUMAN    VVWGRWEYGSFFCELWTSVDVLCVTASIETLCVIALDRYLAITSPFRYQSLLTRARARGL 178
ADRB2_HUMAN    ILMKMWTFGNFWCEFWTSIDVLCVTASIETLCVIAVDRYFAITSPFKYQSLLTKNKARVI 153

ADRB3_HUMAN    VVLVWVVSAAVSFAPIMSQWWRVGADAEAQRCHSNPRCCAFASNMPYVLLSSSVSFYLPL 217
ADA1A_HUMAN    LLCVWALSLVISIGPLF--GWRQPAP-------EDETICQINEEPGYVLFSALGSFYLPL 197
ADRB1_HUMAN    VCTVWAISALVSFLPILMHWWRAESD-EARRCYNDPKCCDFVTNRAYAIASSVVSFYVPL 237
ADRB2_HUMAN    ILMVWIVSGLTSFLPIQMHWYRA-THQEAINCYANETCCDFFTNQAYAIASSIVSFYVPL 212

ADRB3_HUMAN    LVMLFVYARVFVVATRQLRLLRGELGRF-PPEESPPAPSRSLAPAPVGTCAPPEGVP--- 273
ADA1A_HUMAN    AIILVMYCRVYVVAKRESRGLKSGL-KTDKSDSEQVTLRIHRKNAPAGGSGM-------- 248
ADRB1_HUMAN    CIMAFVYLRVFREAQKQVKKIDSCERRFLGGPARPPSPSPSPVPAPAPPPGPPRPAAAAA 297
ADRB2_HUMAN    VIMVFVYSRVFQEAKRQLQKIDKSEGRF-------HVQNLSQVEQDG------------- 252

ADRB3_HUMAN    ----ACG----RRPARLLPL-REHRALCTLGLIMGTFTLCWLPFFLANVLRALGGPSLVP 324
ADA1A_HUMAN    ----ASAKTKTHFSVRLLKFSREKKAAKTLGIVVGCFVLCWLPFFLVMPIGSFFPDFKPS 304
ADRB1_HUMAN    TAPLANGRAGKRRPSRLVAL-REQKALKTLGIIMGVFTLCWLPFFLANVVKAF-HRELVP 355
ADRB2_HUMAN    ----RTGH-GLRRSSKF-CL-KEHKALKTLGIIMGTFTLCWLPFFIVNIVHVI-QDNLIR 304

ADRB3_HUMAN    GPAFLALNWLGYANSAFNPLIY-CRSPDFRSAFRRLL-CRCGRRLPPEPCAAARPALFPS 382
ADA1A_HUMAN    ETVFKIVFWLGYLNSCINPIIYPCSSQEFKKAFQNVLRIQCLCRKQSSKHALGYTLHPPS 364
ADRB1_HUMAN    DRLFVFFNWLGYANSAFNPIIY-CRSPDFRKAFQRLL-C-CARRAARRRHATHGDRPRAS 412
ADRB2_HUMAN    KEVYILLNWIGYVNSGFNPLIY-CRSPDFRIAFQELL---CLRRSSLK---AYGNGYSSN 357

ADRB3_HUMAN    GVPAARSSPAQP-RLCQRLDGASWGVS--------------------------------- 408
ADA1A_HUMAN    QAVEGQHKDMVRIPVGSRETFYRISKTDGVCEWKFFSSMPRGSARITVSKDQSSCTTARV 424
ADRB1_HUMAN    GCLARPGPPPSPGAASDDDDDDVVGATPPARLLEPWAGCNGGAAADSDSSLDEPCRPGFA 472
ADRB2_HUMAN    G---NTG-EQSGYHVEQEKENKLLCEDLPGT--EDFVGHQGTVPSDNIDSQGRNCS---T 408

ADRB3_HUMAN    ----------------------------------------
ADA1A_HUMAN    RSKSFLQVCCCVGPSTPSLDKNHQVPTIKVHTISLSENGEEV 466
ADRB1_HUMAN    SESKV------------------------------------ 477
ADRB2_HUMAN    NDSLL------------------------------------ 413
```
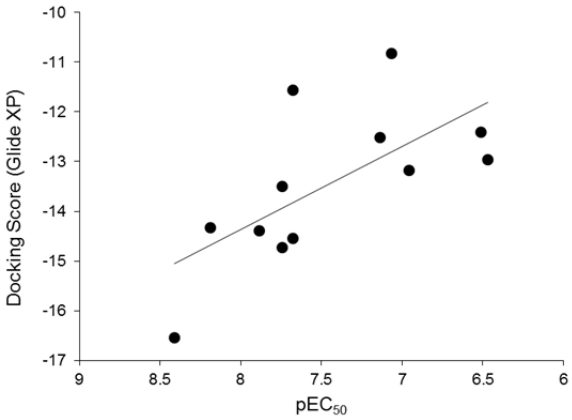

Figure 9. Multiple sequence alignment of human β_3-AR (ADRB3_HUMAN), human α_{1A}-AR (ADA1A_HUMAN), human β_1-AR (ADRB1_HUMAN), and human β_2-AR (ADRB2_HUMAN) using MOE 2015 software (Chemical Computing Group). Sequences were drawn from UniProt (β_3-AR, P13945; α_{1A}-AR, P35348; β_1-AR, P08588; β_2-AR, P07550).

(A) All 12 compounds



(B) Eight indazole analogues

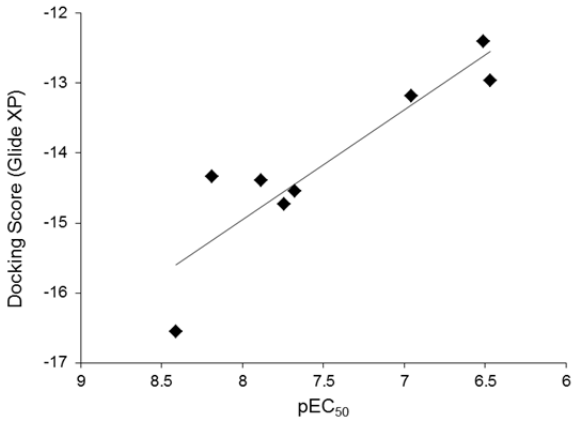
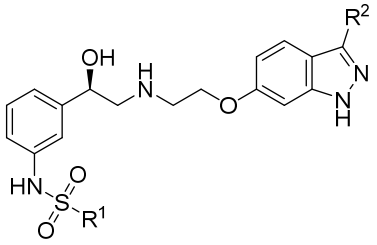
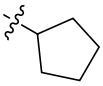
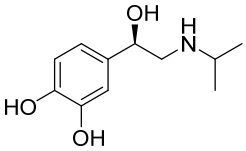
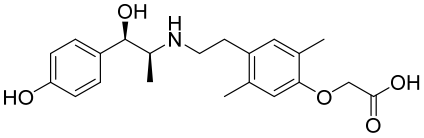
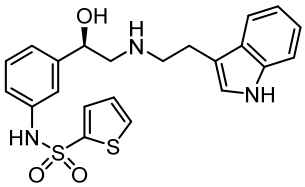
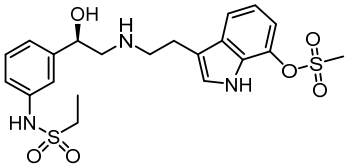


Figure 10. (A) Plot of docking score calculated by Glide extra precision (XP) based on the compound **56f** β_3-AR model versus experimental β_3-AR agonistic activity (pEC_{50}) for all 12 compounds. The coefficient of determination, R^2, was 0.45 for the docking scores and pEC_{50} values of all 12 compounds. (B) Plot of docking score calculated by Glide extra precision (XP) based on the compound **56f** β_3-AR model versus β_3-AR agonistic activity (pEC_{50}) for eight indazole analogues. The coefficient of determination, R^2, was 0.82 for the docking scores and pEC_{50} values of eight indazole analogues. See Table 12 for individual pEC_{50} values and docking scores.

Table 12. Summary of pEC_{50} values for β_3-AR and docking scores (Glide XP[a])



Compound	R^1	R^2	β_3-AR pEC_{50}	Docking Score (Glide XP)
5	Me	Me	7.7	-14.5
6	Me	CH_2OH	7.0	-13.2
11	Me	*i*-Pr	7.9	-14.4
12	Me	*t*-Bu	6.5	-13.0
56a	*n*-Pr	Me	8.2	-14.3
56d		Me	7.7	-14.7
56e		Me	6.5	-12.4
56f	Ph	Me	8.4	-16.6
isoproterenol			7.1	-10.8
KUC-7322 (active form of ritobegron)[b]			7.1	-12.5
S1[c]			7.7	-11.6
S2[c]			7.7	-13.5

[a]XP, Extra precision. [b]Reference compound[17, 38]. [c]Reference compounds[32].

次に、インダゾールシリーズの中でβ_3-AR に対するアゴニスト活性、選択性、薬物動態プロファイルが最も良い化合物 **56d** とβ_3-AR のドッキングポーズに対して分子動力学（Molecular Dynamics, MD）シミュレーションによる解析を行った。なぜならば、ドッキングスコアが良好にもかかわらず、ドッキングにて得られたポーズと MD シミュレーション後に得られたポーズに大きく乖離がある例が報告されている[39]からである。Figure 11 に 20 ns 間の MD シミュレーション中、化合物 **56d** とβ_3-AR の主鎖 Cαの二乗平均偏差（Root Mean Square Deviation, RMSD）*の推移を示す。その結果、β_3-AR の構造、並びに化合物 **56d** の位置の RMSD は 1～3 Å以内であり、さらに 5 ns～20 ns の間においてその値に変化が無く、安定な結合ポーズを定常的に保っていることが示唆された。ゆえに、20 ns の MD シミュレーション後の化合物 **56d** とβ_3-AR の結合様式に対して、詳細な相互作用解析を行った。

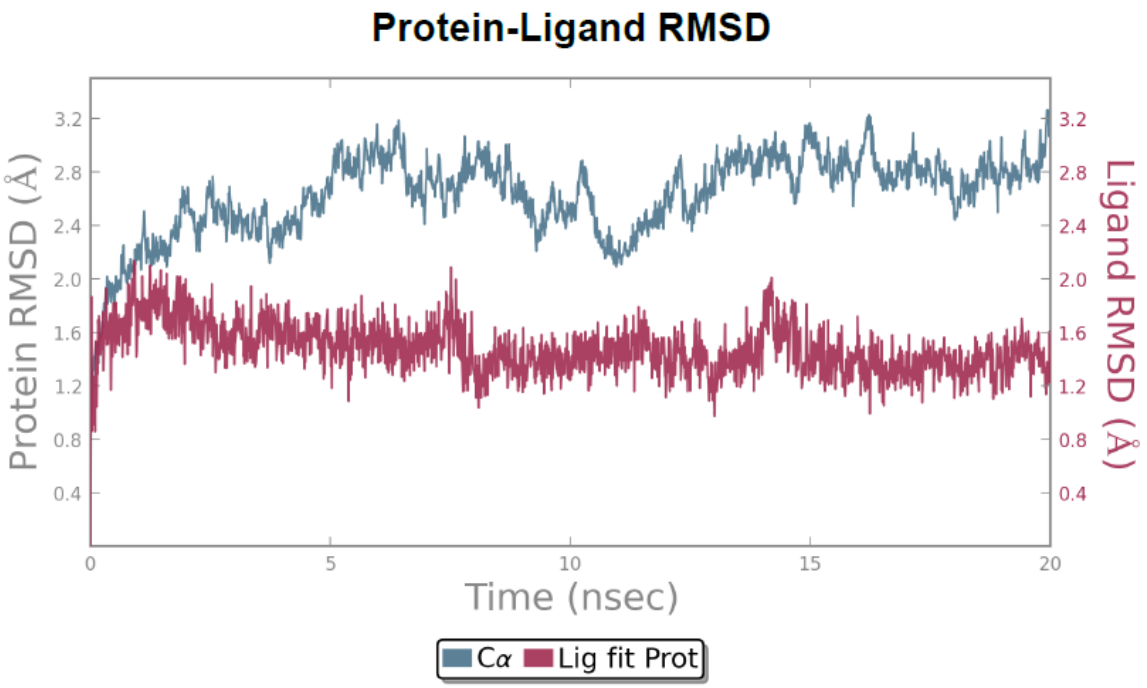


Figure 11. The plot showed the Root Mean Square Deviation (RMSD) evolution of β_3-AR (left *Y*-axis) and compound **56d** (right *Y*-axis). The RMSD was used to measure the average change in displacement of a selection of atoms for a particular frame with respect to a reference frame (the first frame was used as the reference and it is regarded as time *t*=0). 'C_α' shows the RMSD of C_α in β_3-AR backbone. 'Lig fit Prot' shows the RMSD of a ligand (**56d**) when the β_3-AR-**56d** complex is first aligned on the protein backbone of the reference and then the RMSD of the **56d** heavy atoms is measured. It was calculated for all frames in the trajectory.

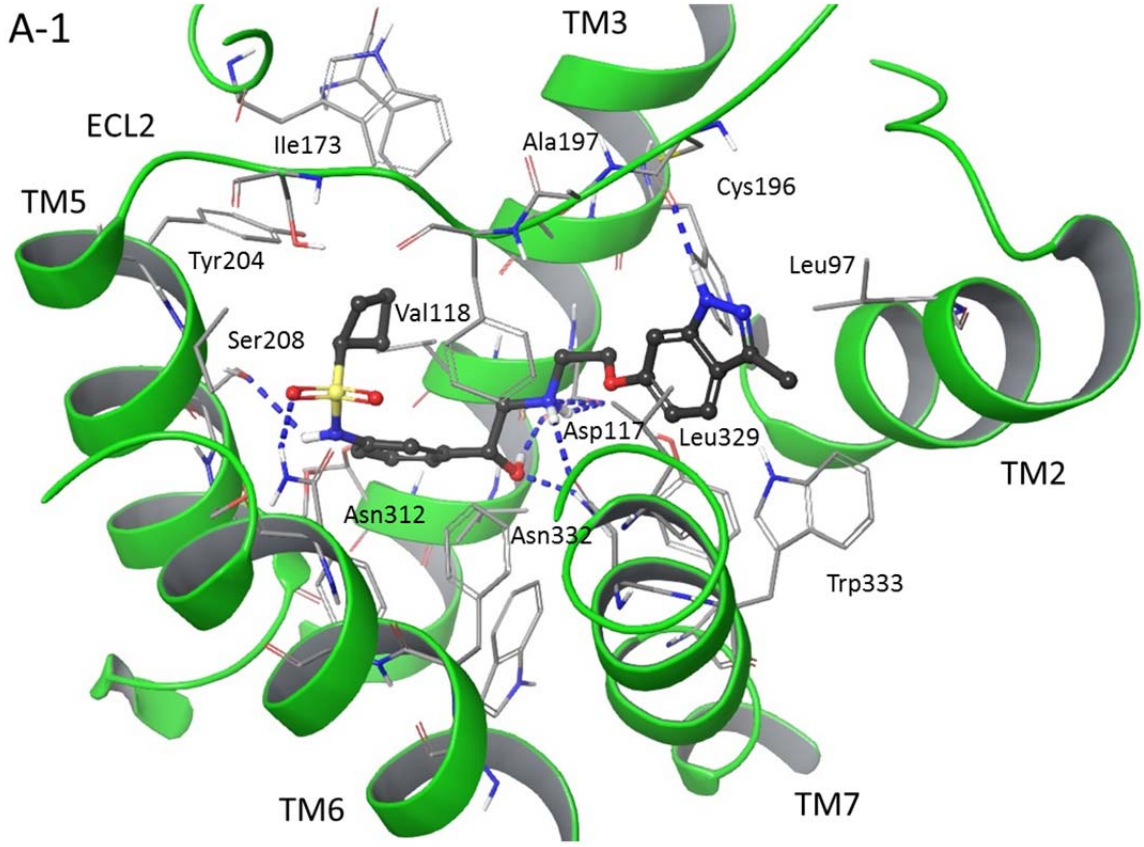
* β_3-AR と化合物 **56d** のドッキングポーズを初期構造とし、MD シミュレーション中の各原子の二乗平均偏差を取ることで、初期構造からどのくらいβ_3-AR の構造や化合物 **56d** が動いたか示すことができる。

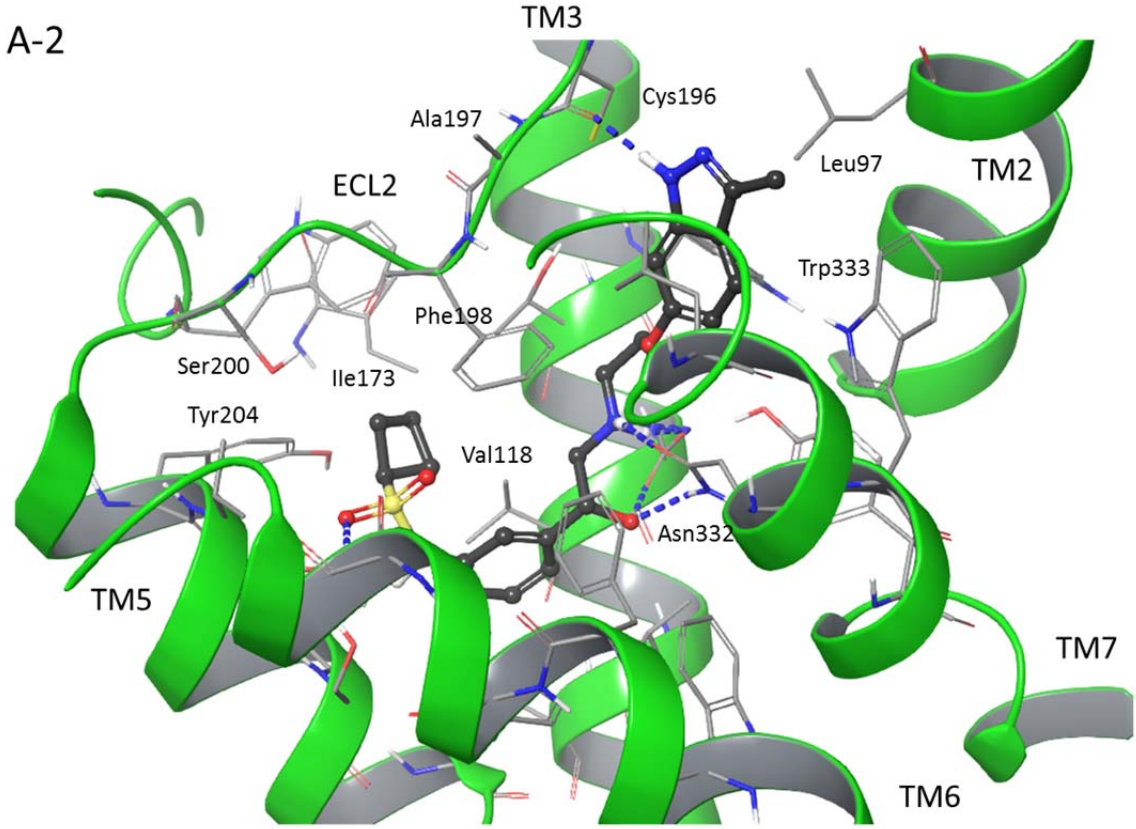
第２節　β_3-AR と化合物 **56d** の相互作用解析とβ_3-AR 選択性の考察

Figure 12 に 20ns MD シミュレーション後の化合物 **56d** とβ_3-AR の推定結合様式を示す。化合物 **56d** のアミノアルコールはβ_3-AR の Asp117、Asn332 と水素結合が確認された。また、化合物 **56d** のスルホンアミドNHはSer208と水素結合が確認された。これらの3つの水素結合はBI-167107-β_2-AR複合体（PDB entry 3P0G）の結晶構造においても確認されており、アドレナリン受容体とアゴニストリガンドの間に重要な相互作用であると考えられている。さらに、化合物 **56d** のインダゾール NH と Cys196 の主鎖カルボニルの間に水素結合が見られ、インダゾール基の周辺は Leu97、Leu329、Trp333 との疎水性相互作用が予測された。また、化合物 **56d** のスルホンアミド基の酸素と Asn312 の間に水素結合が想定され、フェニル環と Phe309 との間に T 型のπ-π相互作用が予測された。化合物 **56d** のシクロブタン基は Val118、IIe173、Tyr204 が形成する疎水性ポケットを占めていると推定された。

続いて、α_{1A}-AR/β_3-AR の選択性に関して、化合物 **56d** とβ_3-AR の推定結合様式を基にβ_3-AR とα_{1A}-AR のアミノ酸残基の違いから考察した。Figure 4 と Table 3 に示した通り、インダゾール 3 位置換基を変換した結果、α_{1A}-AR に対するβ_3-AR 選択性の順番はイソプロピル **11** > シクロブチル **14** > エチル **10** > メチル **5** > ヒドロキシメチル **6** であった。この構造活性相関は疎水性相互作用がα_{1A}-AR に対するβ_3-AR 選択性を高めることを示唆している。先に述べたように、インダゾール基はβ_3-AR の疎水性残基である Leu97、Leu329、Trp333 と相互作用している。一方、α_{1A}-AR においてβ_3-AR の Leu329 に対応する残基は極性残基である Lys309 である。したがって、α_{1A}-AR においてインダゾール環が相互作用しているポケットの環境はβ_3-AR よりもより極性が高いと推測される。ゆえに、α_{1A}-AR/β_3-AR 選択性の構造活性相関は化合物 **56d** とβ_3-AR の推定結合ポーズから得られた結果を支持している。一方、スルホンアミド基の置換基を変えた時のα_{1A}-AR に対するβ_3-AR 選択性の順番はフェニル **56f** > シクロブチル **56d** >> *n*－プロピル **56a** > メチル **5** であった（Table 8）。この構造活性相関はより嵩高い置換基がα_{1A}-AR/β_3-AR 選択性を向上させていることを示す。化合物 **56d** のシクロブチル基はβ_3-AR の Val118、 Ile173、Tyr204 側鎖と疎水性相互作用をしている。一方、α_{1A}-AR においてβ_3-AR の Ile173 に対応する残基は Leu162 である。さらに、スルホンアミド基のスルホンの近くにはβ_3-AR の細胞外第 2 ループ（Extracellular Loop 2, ECL2）に含まれる Phe198、Ala199、Ser200 があり、α_{1A}-AR における対応する残基はそれぞれ Ile178、Asn179、Glu180 である。したがって、シクロブタンスルホンアミド基の置換基周辺はα_{1A}-AR とβ_3-AR で対応する残基の種類が異なり、ポケットのサイズや水素結合に関する性質が違うため、その相互作用はα_{1A}-AR とβ_3-AR の間で大きく異なると予想される。ゆえに、これらの違いが、スルホンアミド基の置換基を嵩高くした時にα_{1A}-AR/β_3-AR 選択性が大きく向上することに関係していると推測される。

さらに、β_1-AR とβ_2-AR 対するβ_3-AR 選択性に関しても化合物 **56d** とβ_3-AR の推定結合ポーズを基に考察した。Edmondon らは化合物 **4** のアニリドのカルボニルがβ_3-AR の Ala197 の近傍にあり、その Ala197 はβ_1-AR とβ_2-AR における Asp 残基と対応し、静電的性質とサイズが異なる理由で化合物 **4** がβ_3-AR に高選択的であると報告している。[14b]化合物 **56d** のインダゾール NH はβ_3-AR の Cys196 の主鎖のカルボニルと水素結合しており、Ala197 はインダゾール基の近くに存在している。Edmondon らの報告と同様に、β_3-AR の Ala197 はβ_1-AR とβ_2-AR における Asp 残基と対応する（Asp217 in β_1-AR, Asp192 in β_2-AR）ため、この Asp 残基の側鎖は静電的性質とサイズが Ala197 と大きく異なる。ゆえに、化合物 **56d** のインダゾール基はβ_1-AR 並びにβ_2-AR と水素結合形成において不利に働くと推測される。したがって、このインダゾール基はβ_1-AR とβ_2-AR に対して高いβ_3-AR 選択性を有するために重要な置換基であると考えられる。


A-1
TM3
ECL2
Ile173
Ala197
Cys196
TM5
Tyr204
Leu97
Val118
Ser208
Asp117
Leu329
TM2
Asn312
Asn332
Trp333
TM6
TM7


A-2
TM3
Cys196
Ala197
Leu97
TM2
ECL2
Trp333
Phe198
Ser200
Ile173
Tyr204
Val118
Asn332
TM5
TM7
TM6

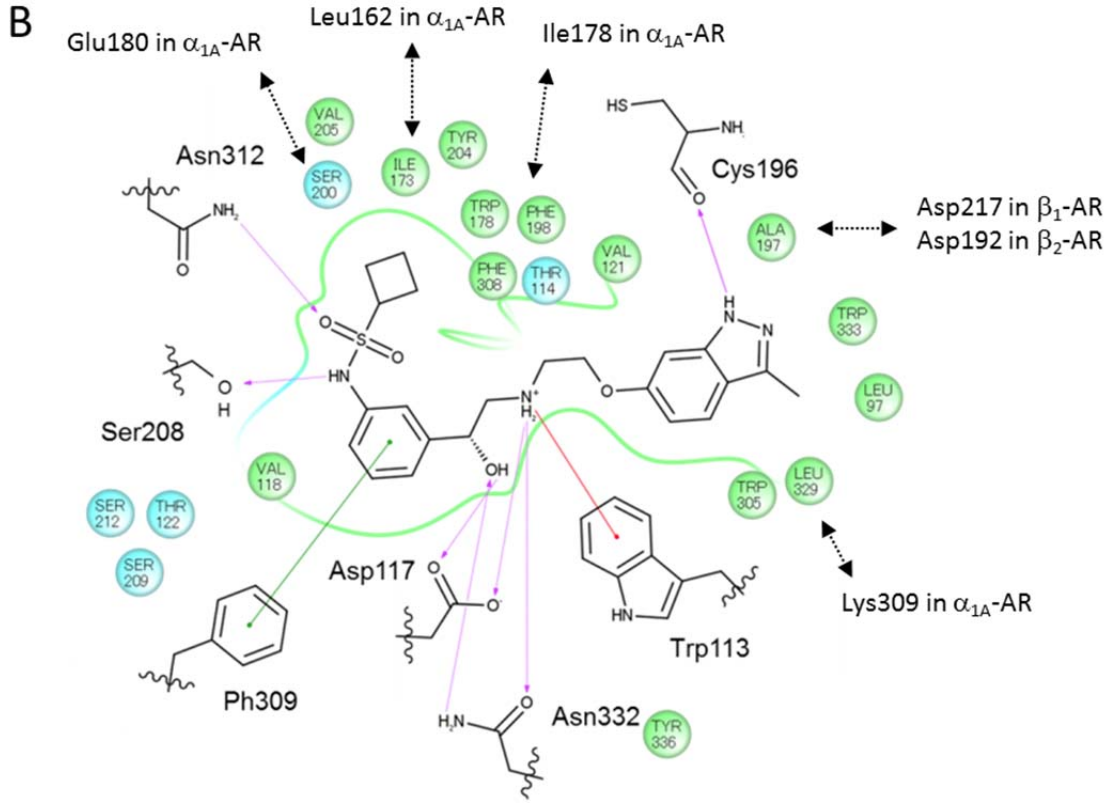


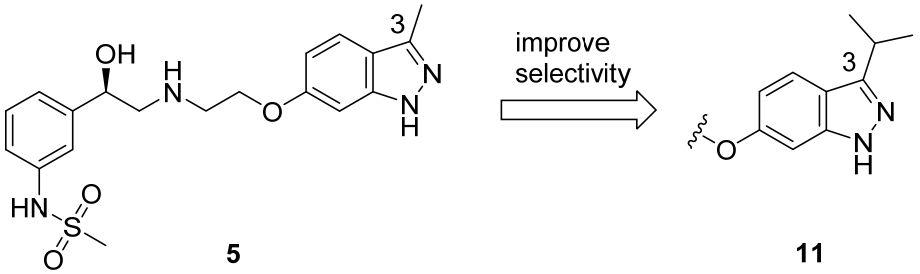
Figure 12. (A-1 and A-2) Proposed model of compound **56d** binding to the homology model of β_3-AR after MD refinement. Receptor residues within 4.0 Å of compound **56d** are represented by lines. Compound **56d** is shown as a ball and stick model. All nonpolar hydrogen atoms of the receptor residues are omitted for clarity. Hydrogen bonding and salt bridges to side chains of Asp117, Ser208, Asn312, and Asn332 are depicted by blue dots. Hydrogen bonding to the backbone of Cys196 is also depicted by blue dots. (B) Schematic representation of the interactions between compound **56d** and β_3-AR residues using the Maestro Ligand Interaction Diagram module (Schrödinger, LLC). Hydrogen bonding is indicated with dashed arrows, salt bridge with purple lines, cation-π interactions with red lines, and π-π interactions with green lines.

第３節　第３章まとめ

β_3-AR のホモロジーモデルを作成し、12 種のβ_3-AR アゴニストとドッキングスタディを実施した。その結果、pEC_{50} とドッキングスコアに高い相関が確認できた。したがって、β_3-AR ホモロジーモデルとリガンドのドッキングポーズが妥当であることが示唆された。また、化合物 **56d** の MD シミュレーションを実施し、β_3-AR と化合物 **56d** は安定に結合していることが示唆された。化合物 **56d** とβ_3-AR の相互作用解析を実施し、さらにα_{1A}-AR 、β_1-AR 、β_2-AR に対するβ_3-AR 選択性に関して考察した。β_3-AR と化合物 **56d** の相互作用に重要なアミノ酸残基を明らかにしたことにより、今後、本成果は高選択的β_3-AR アゴニストの設計に有用な情報になり得る。

結語

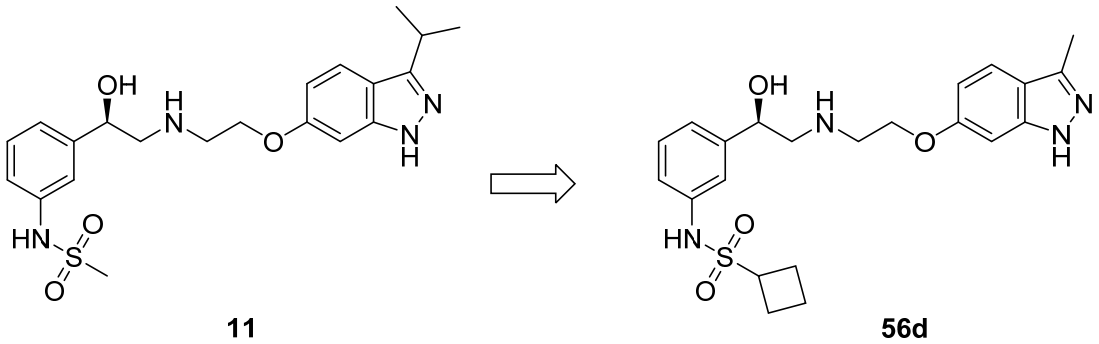
1. アリールエタノールアミンライブラリーから見出したヒット化合物（β_3-AR アゴニスト）**5** のインダゾール 3 位を変換することにより、β_3-AR に高選択的なアゴニスト **11** を見出した。化合物 **11** はマーモセットに対して用量依存的に排尿筋弛緩作用を示した。さらに、化合物 **11** は過活動膀胱治療薬として上市されているミラベグロン（**1**）と比較し、ラット静脈内投与における血圧心拍に対して影響を与えなかった。β_3-AR に対する高選択性がミラベグロン（**1**）よりも心血管系に対する副作用が少ない要因と考えられる。



β_3-AR EC_{50} = 21 nM
α_{1A}-AR EC_{50} = 219 nM

β_3-AR EC_{50} = 13 nM
α_{1A}-AR EC_{50} = >10000 nM

2. 化合物 **11** は経口剤としての薬物動態プロファイルに問題があったので、その最適化を実施した結果、化合物 **56d** の創出に成功した。化合物 **56d** はラットにおいて顕著な経口吸収が確認できた。さらに、化合物 **11** と同様に化合物 **56d** は用量依存的にマーモセットの膀胱平滑筋を弛緩させる優れた薬効を示し、ミラベグロン（**1**）よりも心血管系の副作用が少なかった。さらに化合物 **56d** はミラベグロン（**1**）よりも薬物間相互作用のリスクが低いことも示された。ゆえに、化合物 **56d** は心血管系の副作用を軽減した経口投与可能なβ_3-AR 高選択的アゴニストである。



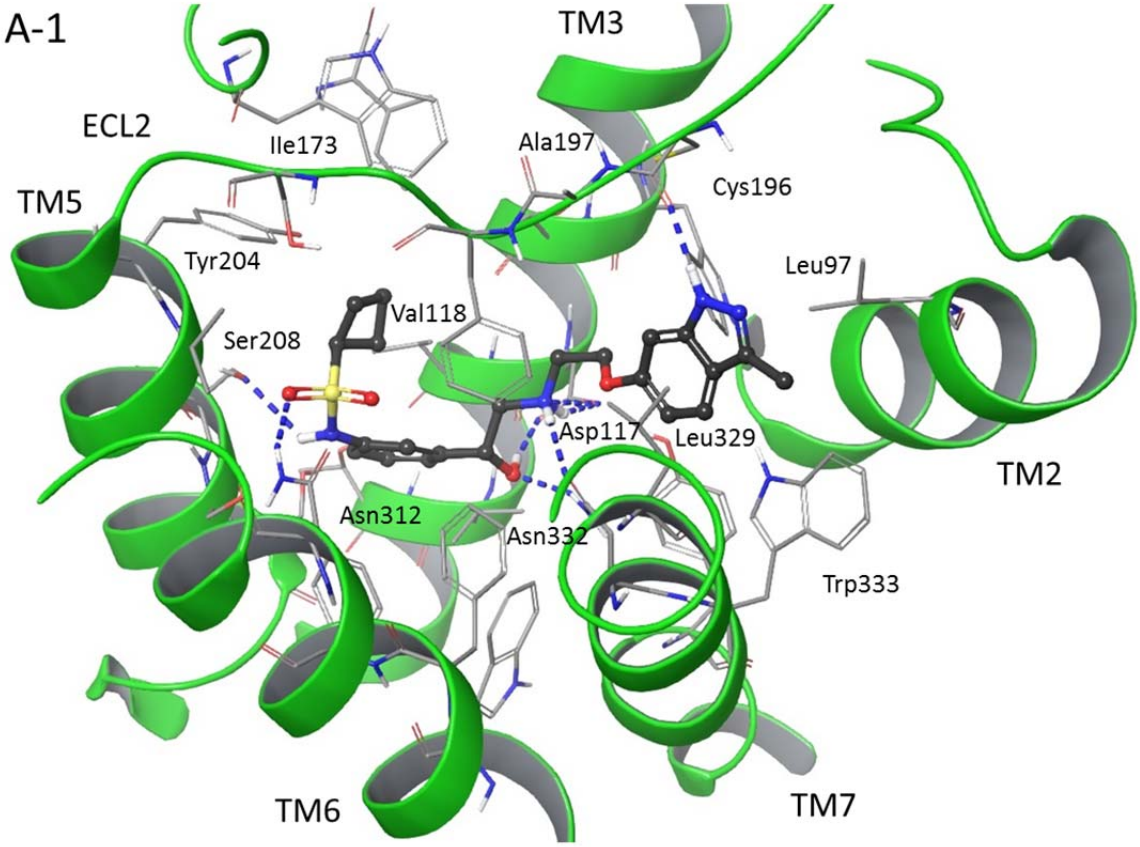
11

β_3-AR EC_{50} = 13 nM
Poor metabolic stability
Poor pharmacokinetics
(C_{max} and AUC)

56d

β_3-AR EC_{50} = 18 nM
Improved metabolic stability
Orally available
No cardiovascular side effects

3. β_3-AR のホモロジーモデルを作成し、12 種のβ_3-AR アゴニストに対するドッキングスタディを実施した。その結果、pEC_{50}とドッキングスコアに高い相関が確認できた。さらに、β_3-AR と化合物 **56d** の複合体に対して MD シミュレーションを実施した結果、β_3-AR と化合物 **56d** は安定に結合していることが示唆された。得られた化合物 **56d** とβ_3-AR の推定結合様式を基に相互作用解析を実施し、結合に重要なアミノ酸残基の推定とβ_3-AR 選択性に関して考察した。これら解析により、β_3-AR に高選択的な化合物 **56d** の相互作用に重要なアミノ酸残基を明らかにした結果は、今後、高選択的β_3-AR アゴニストの設計に有用な情報になり得る。



β_3-AR と化合物 **56d** の推定結合様式

実験の部

General Methods

All reagents and solvents were purchased from commercial sources and were used as received. Anhydrous solvents were obtained from commercial sources. Thin layer chromatography (TLC) was carried out using the Merck GmbH Precoated silica gel 60 F254. Compounds were visualized by irradiation under a 254 nm UV light. Chromatography on silica gel was carried out using pre-packed silica gel cartridges (Yamazen Hi-Flash Column Silicagel or Purifpack-Si series) and the indicated solvent system on either a Yamazen Multi Prep YFLC or a Moritex Purif-α2 (50F). ^{1}H NMR spectra were recorded on either a JEOL AL-300 (300 MHz) or a Bruker BIOPIN AVANCE3HD (400 MHz), with chemical shifts reported in δ values (ppm) relative to trimethylsilane. The following abbreviations are used to describe peak patterns where appropriate: s; singlet, d; doublet, t; triplet, q; quartet, qu; quintet, dd; doublet doublet, td; triplet doublet, qd; quartet doublet, qud; quintet doublet, m; multiplet, and brs; broad singlet. Electrospray ionization (ESI) high-resolution mass spectra were recorded using a Thermo Fisher Orbitrap Velos Pro. Liquid chromatography-mass spectrometry (LC/MS) data were recorded using either a Micromass platform-LC type mass spectrometer or a Waters single quadrupole type mass spectrometer, the UPLC/SQD system, by an electrospray ionization (ESI) method. HPLC analyses were performed following conditions: (Method A) Shiseido CAPCELL CORE ADME column (2.7 μm, 2.1 x 50 mm), 40 °C column temperature, 1.0 mL/min flow rate, photodiode array detection (254 nm), linear mobile phase gradient of 10-95% B over 2 min, holding 1.5 min at 95% B, holding 1.5 min at 10% B (mobile phase A: 10 mM ammonium acetate in water; mobile phase B: acetonitrile), (Method B) YMC Meteoric Core C18 column (2.7 μm, 3.0 x 50 mm), 40 °C column temperature, 1.0 mL/min flow rate, photodiode array detection (254 nm), linear mobile phase gradient of 10-95% B over 2 min, holding 2 min at 95% B, holding 2 min at 10% B (mobile phase A: 10 mM ammonium acetate in water; mobile phase B: acetonitrile). All animal experiments were approved by the Committee on Ethics in Animal Experiments of Asahi Kasei Pharma Corporation.

Chemistry

***N*-(3-((1*R*)-1-Hydroxy-2-(2-((3-methyl-1*H*-indazol-6-yl)oxy)ethylamino)ethyl)phenyl)methanesulfonamide (5).** To a stirred solution of **18** (128 mg, 0.52 mmol), **17** (2 mL, 1.0 mmol, 0.5 M toluene solution), and triphenylphosphine (261 mg, 1.0 mmol) in anhydrous toluene (5 mL) was added *N,N,N',N'*-tetramethylazodi-carboxamide (195 mg, 1.1 mmol) at room temperature, and the solution was stirred for 4 days. The reaction solution was then purified by flash column chromatography on silica gel (74:26 to 53:47 n-hexane/ethyl acetate) to give 371 mg (90% yield) of the coupling product. ^{1}H-NMR (400 MHz, $CDCl_3$, 1:1 rotamers): δ 0.54 (6H, q, *J* = 8.0), 0.89 (9H, t, *J* = 8.0), 1.44 (9H, s), 1.48 and 1.52 (9H, each s), 1.69 and 1.70 (9H, each s), 2.52 and 2.53 (3H, each s), 3.24-3.26 (7H, m), 4.03-4.11 (2H, m), 4.94-4.97 and 5.10-5.13 (1H, each m), 6.86 (1H, dd, *J* = 1.7, 8.6), 7.12-7.16 (1H, m), 7.21-7.47 (4H, m), 7.54 (1H, s); LC/MS (ESI, $[M+H]^+$, *m/z*) 819. To the solution of the obtained product (287 mg, 0.35 mmol) in 1,4-dioxane (0.4 mL) was added 4 M HCl in 1,4-dioxane (4 mL), and the mixture was stirred at room temperature for 6.5 h. The resultant solid was collected by suction filtration and dried. The crude solid (170 mg) was treated with water (0.5 mL) and ethanol (2.0 mL) to promote crystallization. The crystals were filtered, and washed with ethanol to afford 61 mg (37% yield) of the title compound as dihydrochloride salt.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 2.46 (3H, s), 3.00 (3H, s), 3.04-3.10 (1H, m), 3.24-3.28 (1H, m), 3.44-3.47 (2H, m), 4.34-4.41 (2H, m), 5.02 (1H, dd, J = 2.1, 10.1), 6.80 (1H, dd, J = 2.1, 8.8), 6.92 (1H, d, J = 2.1), 7.12-7.17 (2H, m), 7.31 (1H, s), 7.35 (1H, t, J = 7.8), 7.63 (1H, d, J = 8.8), 9.05 (1H, brs), 9.38 (1H, brs), 9.86 (1H, brs); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 11.3, 39.1, 45.9, 53.6, 63.4, 67.9, 92.1, 111.9, 116.9, 117.0, 118.9, 121.0, 121.1, 129.3, 138.5, 140.7, 141.6, 143.0, 157.4; HRMS calculated for $C_{19}H_{24}N_4O_4S$ + H^+ 405.1591, found (ESI, $[M+H]^+$) 405.1588; LC/MS (ESI, $[M+H]^+$, *m/z*) 405; HPLC (Method A): purity 100% R_T 1.7 min.

***N*-(3-((1*R*)-1-Hydroxy-2-(2-((3-(hydroxymethyl)-1*H*-indazol-6-yl)oxy)ethylamino)ethyl)phenyl)methane-sulfonamide (6).** To a stirred solution of **30** (139 mg, 0.16 mmol) in THF (1.2 mL) and methanol (2.0 mL) was added 2 M NaOH (1.6 mL, 3.2 mmol) at 40 °C, and the solution was stirred for 5 h. The reaction mixture was poured into water (50 mL) and washed twice with diethyl ether. The aqueous layer was added 5 M HCl (5 mL) and extracted three times with ethyl acetate. The combined organic layers were dried over magnesium sulfate and filtered. The solution was concentrated to give carboxylic acid (95 mg), which was used without further purification. This material (77 mg, 0.11 mmol) was dissolved in anhydrous THF (4.3 mL) under nitrogen and the solution was added borane tetrahydrofuran complex (0.5 mL, 0.6 mmol, 1.2 M in THF) at 0 °C. The resulting solution was allowed to warm to room temperature and was then stirred overnight. The reaction mixture was added methanol (0.5 mL) at 0 °C. The solution was poured into water and extracted twice with ethyl acetate. The combined organic layers were washed twice with water, dried over sodium sulfate, and filtered. The organic layer was concentrated to give alcohol (95 mg), which was used without further purification. This material (68 mg, 0.1 mmol) was dissolved in ethanol (0.5 mL), hydrochloric acid (0.17 mL) was then added at room temperature and the mixture was stirred for 24 h. The resultant solid was collected by filtration and dried to give 39 mg (80% yield) of the title compound as dihydrochloride salt. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.00 (3H, s), 3.04-3.11 (1H, m), 3.24-3.28 (1H, m), 3.41-3.46 (2H, m), 4.32-4.41 (2H, m), 4.73 (2H, s), 5.02 (1H, dd, J = 2.1, 10.2), 6.79 (1H, dd, J = 2.1, 8.8), 6.93 (1H, d, J = 2.1), 7.12-7.17 (2H, m), 7.31 (1H, s), 7.35 (1H, t, J = 7.8), 7.74 (1H, d, J = 8.8), 9.02 (1H, brs), 9.32 (1H, brs), 9.86 (1H, s), 12.65 (1H, brs); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 39.1, 45.9, 53.6, 56.6, 63.3, 67.9, 92.1, 111.6, 116.5, 116.9, 118.9, 121.2, 121.5, 129.3, 138.5, 141.9, 143.0, 145.4, 156.9; HRMS calculated for $C_{19}H_{24}N_4O_5S$ + H^+ 421.1540, found (ESI, $[M+H]^+$) 421.1537; LC/MS (ESI, $[M+H]^+$, *m/z*) 421; HPLC (Method A): purity 100% R_T 1.5 min.

***N*-(3-((1*R*)-1-Hydroxy-2-(2-((3-methoxy-1*H*-indazol-6-yl)oxy)ethylamino)ethyl)phenyl)methanesulfonamide (7).** To a stirred solution of **19** (215 mg, 0.8 mmol), **17** (942 mg, 1.6 mmol), and triphenylphosphine (448 mg, 1.7 mmol) in anhydrous toluene (12 mL) was added *N*,*N*,*N'*,*N'*-tetramethylazodicarboxamide (288 mg, 1.7 mmol) at room temperature, and the solution was stirred overnight. The reaction solution was then purified by flash column chromatography on silica gel (100:0 to 74:26 n-hexane/ethyl acetate) to give 587 mg (87% yield) of the coupling product. ^{1}H-NMR (300 MHz, $CDCl_3$, 1:1 rotamers): δ 0.53 (6H, q, J = 7.9), 0.87 (9H, t, J = 7.9), 1.42 (9H, s), 1.46 and 1.51 (9H, each s), 1.67 (9H, s), 3.20-3.60 (7H, m), 3.99-4.04 (m, 2H), 4.12 (3H, s), 4.92-4.96 and 5.08-5.12 (1H, each m), 6.80 (1H, dd, J = 2.1, 8.7), 7.11-7.47 (6H, m); LC/MS (ESI, $[M+H]^+$, *m/z*) 835. To the solution of the obtained product (580 mg, 0.7 mmol) in *tert*-butyl methyl ether (1 mL) was added 4 M HCl in 1,4-dioxane (7 mL),

and the mixture was stirred overnight at room temperature. The resultant solid was collected by suction filtration, washed with *tert*-butyl methyl ether, and dried to afford 321 mg (93% yield) of the title compound as dihydrochloride salt. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.00 (3H, s), 3.03-3.10 (1H, m), 3.23-3.27 (1H, m), 3.44-3.46 (2H, m), 3.96 (3H, s), 4.33-4.41 (2H, m), 5.04 (1H, dd, *J* = 2.1, 10.2), 6.70 (1H, dd, *J* = 2.0, 8.8), 6.81 (1H, d, *J* = 2.0), 7.13 (1H, d, *J* = 7.8), 7.16 (1H, dd, *J* = 1.3, 7.8), 7.30 (1H, s), 7.35 (1H, t, *J* = 7.8), 7.48 (1H, d, *J* = 8.8), 9.09 (1H, brs), 9.47 (1H, brs), 9.87 (1H, s), 11.83 (1H, brs); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 39.7, 46.4, 54.2, 56.1, 63.9, 66.8, 92.8, 106.5, 111.4, 117.5, 119.5, 120.5, 121.8, 129.8, 139.1, 143.5, 143.6, 156.7, 158.4; HRMS calculated for $C_{19}H_{24}N_4O_5S$ + H^+ 421.1540, found (ESI, $[M+H]^+$) 421.1538; LC/MS (ESI, $[M+H]^+$, *m/z*) 421; HPLC (Method A): purity 100% R_T 1.8 min.

***N*-(3-((1*R*)-2-(2-((3-Ethoxy-1H-indazol-6-yl)oxy)ethylamino)-1-hydroxy-ethyl)phenyl)methanesulfonamide (8).** To a stirred solution of **20** (51 mg, 0.18 mmol), **17** (212 mg, 0.36 mmol), and triphenylphosphine (94 mg, 0.36 mmol) in anhydrous toluene (2.7 mL) was added *N*,*N*,*N'*,*N'*-tetramethylazodicarboxamide (62 mg, 0.36 mmol) at room temperature, and the solution was stirred overnight. The reaction solution was then purified by flash column chromatography on silica gel (88:12 to 67:33 n-hexane/ethyl acetate) to give 137 mg (89% yield) of the coupling product. ^{1}H-NMR (300 MHz, $CDCl_3$, 1:1 rotamers): δ 0.54 (6H, t, *J* = 7.9), 0.89 (9H, q, *J* = 7.9), 1.44-1.47 (12H, m), 1.48 and 1.52 (9H, each s), 1.68 (9H, s), 3.22-3.61 (7H, m), 4.01-4.11 (2H, m), 4.51 (2H, q, *J* = 7.0), 4.93-4.98 and 5.09-5.13 (1H, each m), 6.80 (1H, dd, *J* = 2.1, 8.7), 7.13-7.50 (6H, m); LC/MS (ESI, $[M+H]^+$, *m/z*) 849. To the solution of the obtained product (130 mg, 0.15 mmol) in *tert*-butyl methyl ether (0.2 mL) was added 4 M HCl in 1,4-dioxane (1.5 mL), and the mixture was shaken (600 min^{-1}) for overnight at room temperature. Nitrogen gas was blown into the reaction solution to evaporate the solvent. Subsequently, water was added to dissolve the residue and the solution was freeze-dried to give 76 mg (98 % yield) of the title compound as dihydrochloride salt. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.39 (3H, t, *J* = 7.0), 3.00 (3H, s), 3.03-3.10 (1H, m), 3.23-3.27 (1H, m), 3.43-3.49 (2H, m), 4.33 (2H, q, *J* = 7.0), 4.34-4.41 (2H, m), 5.03 (1H, dd, *J* = 2.1, 10.2), 6.70 (1H, dd, *J* = 2.0, 8.8), 6.81 (1H, d, *J* = 2.0), 7.13 (1H, d, *J* = 7.7), 7.15-7.18 (1H, m), 7.30 (1H, s), 7.35 (1H, t, *J* = 7.8), 7.47 (1H, d, *J* = 8.8), 9.07 (1H, brs), 9.42 (1H, brs), 9.87 (1H, s), 11.78 (1H, s); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 14.7, 39.1, 45.8, 53.6, 63.3, 63.8, 67.9, 92.2, 106.1, 110.8, 117.0, 119.0, 120.0, 121.2, 129.2, 138.5, 142.7, 143.0, 155.5, 157.7; HRMS calculated for $C_{20}H_{26}N_4O_5S$ + H^+ 435.1967, found (ESI, $[M+H]^+$) 435.1696; LC/MS (ESI, $[M+H]^+$, *m/z*) 435; HPLC (Method A): purity 100% R_T 1.9 min.

***N*-(3-((1*R*)-2-(2-((3-Chloro-1*H*-indazol-6-yl)oxy)ethylamino)-1-hydroxy-ethyl)phenyl)methanesulfonamide (9).** To a stirred solution of **21** (26 mg, 0.1 mmol), **17** (118 mg, 0.2 mmol), and triphenylphosphine (55 mg, 0.2 mmol) in anhydrous toluene (1.5 mL) was added *N*,*N*,*N'*,*N'*-tetramethylazodicarboxamide (35 mg, 0.2 mmol) at room temperature, and the solution was stirred overnight. Then the reaction solution was then purified by flash column chromatography on silica gel (81:19 to 60:40 n-hexane/ethyl acetate) to give 71 mg (85% yield) of the coupling product. ^{1}H-NMR (300 MHz, $CDCl_3$, 1:1 rotamers): δ 0.54 (6H, q, *J* = 7.9), 0.89 (9H, t, *J* = 7.9), 1.44 (9H, s), 1.49 and 1.52 (9H, each s), 1.69 (9H, s), 3.25-3.63 (7H, m), 4.03-4.11 (2H, m), 4.94-4.98 and 5.10-5.14 (1H, each m), 6.93 (1H, dd, *J* = 2.0, 8.8), 7.12-7.44 (4H, m), 7.50 (1H, dd, *J* = 3.6, 8.8), 7.57 (1H, d, *J* = 1.5); LC/MS

(ESI, $[M+H]^+$, *m/z*) 839. To the solution of the obtained product (71 mg, 0.085 mmol) in *tert*-butyl methyl ether (0.2 mL) was added 4 M HCl in 1,4-dioxane (1.2 mL), and the mixture was shaken (600 min^{-1}) overnight at room temperature. The resultant solid was collected by suction filtration, washed with *tert*-butyl methyl ether, and dried to afford 41 mg (96% yield) of the title compound as dihydrochloride salt. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.00 (3H, s), 3.04-3.10 (1H, m), 3.24-3.28 (1H, m), 3.46-3.48 (2H, m), 4.38-4.43 (2H, m), 5.03 (1H, dd, *J* = 2.1, 10.2), 6.91 (1H, dd, *J* = 2.0, 8.9), 7.01 (1H, d, *J* = 2.0), 7.12-7.17 (2H, m), 7.31 (1H, s), 7.35 (1H, t, *J* = 7.8), 7.56 (1H, d, *J* = 8.9), 9.08 (1H, brs), 9.43 (1H, brs), 9.86 (1H, s), 13.21 (1H, brs); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 39.1, 45.8, 53.6, 63.5, 67.9, 92.6, 113.8, 114.4, 116.9, 118.9, 119.5, 121.2, 129.2, 132.0, 138.5, 142.1, 143.0, 157.9; HRMS calculated for $C_{18}H_{21}ClN_4O_4S$ + H^+ 425.1045, found (ESI, $[M+H]^+$) 425.1045; LC/MS (ESI, $[M+H]^+$, *m/z*) 425; HPLC (Method A): purity 100% R_T 1.9 min.

***N*-(3-((1*R*)-2-(2-((3-Ethyl-1*H*-indazol-6-yl)oxy)ethylamino)-1-hydroxy-ethyl)phenyl)methanesulfonamide (10).** To a stirred solution of **22** (1.326 g, 5 mmol), **17** (10 mL, 10 mmol, 1 M toluene solution), and triphenylphosphine (2.905 g, 11 mmol) in anhydrous toluene (15 mL) was added *N,N,N',N'*-tetramethylazodicarboxamide (1.953 g, 11 mmol) at room temperature, and the solution was stirred overnight. Triphenylphosphine (1.344 g) and *N,N,N',N'*-tetramethylazodicarboxamide (0.973 g) were further added to the reaction solution, and the mixture was stirred for 2 h at room temperature. Triphenylphosphine (1.24 g) and *N,N,N',N'*-tetramethylazodicarboxamide (0.923 g) were further added to the reaction solution, and the mixture was stirred for 0.5 h at room temperature. The reaction solution was then purified by flash column chromatography on silica gel (88:12 to 67:33 n-hexane/ethyl acetate) to give 3.71 g (88% yield) of the coupling product. ^{1}H-NMR (300 MHz, $CDCl_3$, 1:1 rotamers): δ 0.54 (6H, q, *J* = 7.9), 0.89 (9H, t, *J* = 7.9), 1.38 (3H, t, *J* = 7.6), 1.43 (9H, s), 1.48 and 1.52 (9H, each s), 1.70 (9H, s), 2.95 (2H, q, *J* = 7.6), 3.22-3.62 (7H, m), 4.02-4.11 (2H, m), 4.93-4.97 and 5.09-5.13 (1H, each m), 6.77 (1H, dd, *J* = 2.0, 8.7), 7.13-7.60 (6H, m); LC/MS (ESI, $[M+H]^+$, *m/z*) 833. The obtained product (3.56 g, 4.3 mmol) and 4 M HCl in ethyl acetate (70 mL) were stirred overnight at room temperature. The resultant solid was collected by suction filtration, washed with diethyl ether, and dried to afford 2.056 g (97% yield) of the title compound as dihydrochloride salt. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.31 (3H, t, *J* = 7.6), 2.93 (2H, q, *J* = 7.6), 3.00 (3H, s), 3.04-3.11 (1H, m), 3.23-3.27 (1H, m), 3.43-3.48 (2H, m), 4.37-4.43 (2H, m), 5.05 (1H, dd, *J* = 2.1, 10.2), 6.83 (1H, dd, *J* = 2.1, 8.8), 6.95 (1H, d, *J* = 2.1), 7.13 (1H, d, *J* = 7.8), 7.16-7.18 (1H, m), 7.31 (1H, s), 7.35 (1H, t, *J* = 7.8), 7.70 (1H, d, *J* = 8.8), 9.13 (1H, brs), 9.54 (1H, brs), 9.88 (1H, s); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 13.3, 19.2, 39.1, 45.8, 53.7, 63.4, 67.9, 92.1, 112.4, 115.8, 117.0, 119.0, 121.2, 121.3, 129.2, 138.5, 141.6, 143.0, 145.9, 157.8; HRMS calculated for $C_{20}H_{26}N_4O_4S$ + H^+ 419.1748, found (ESI, $[M+H]^+$) 419.1747; LC/MS (ESI, $[M+H]^+$, *m/z*) 419; HPLC (Method A): purity 100% R_T 1.8 min.

***N*-(3-((1*R*)-1-Hydroxy-2-(2-((3-isopropyl-1*H*-indazol-6-yl)oxy)ethylamino)ethyl)phenyl)methanesulfonamide (11).** To a stirred solution of **23** (146 mg, 0.5 mmol), **17** (1.19 g, 2.0 mmol), and triphenylphosphine (574 mg, 2.2 mmol) in anhydrous toluene (5 mL) was added *N,N,N',N'*-tetramethylazodicarboxamide (371 mg, 2.1 mmol) at room temperature and the solution was stirred overnight. The reaction solution was then purified by flash column chromatography on silica gel (100:0 to 67:33 n-hexane/ethyl acetate) to give 400 mg (95% yield) of the coupling

product. ^{1}H-NMR (300 MHz, $CHCl_3$, 1:1 rotamers): δ 0.54 (6H, q, *J* = 7.9), 0.89 (9H, t, *J* = 7.9), 1.43-1.45 (15H, m), 1.47 and 1.52 (9H, each s), 1.70 (9H, s), 3.22-3.62 (8H, m), 4.02-4.11 (2H, m), 4.94-4.98 and 5.10-5.14 (1H, each m), 6.85 (1H, dd, *J* = 1.6, 8.8), 7.12-7.44 (4H, m), 7.51 (1H, s), 7.57 (1H, dd, *J* = 2.4, 8.8); LC/MS (ESI, $[M+H]^+$, *m/z*) 847. To the solution of the obtained product (400 mg, 0.47 mmol) in THF (2.4 mL) was added 4 M HCl in 1,4-dioxane (1.5 mL) and the mixture was stirred overnight at room temperature. The resultant solid was collected by suction filtration, washed with diethyl ether, and dried under reduced pressure. The solid was dissolved with water, and the solution was freeze-dried to give 191 mg (93 % yield) of the title compound as dihydrochloride salt. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.38 (6H, d, *J* = 7.0), 3.00 (3H, s), 3.05-3.12 (1H, m), 3.24-3.28 (1H, m), 3.40 (1H, septet, *J* = 7.0), 3.45-3.49 (2H, m), 4.37-4.47 (2H, m), 5.07 (1H, dd, *J* = 2.0, 10.1), 6.86 (1H, dd, *J* = 2.0, 8.9), 6.97 (1H, d, *J* = 2.0), 7.15 (1H, d, *J* = 7.8), 7.17-7.19 (1H, m), 7.32 (1H, s), 7.35 (1H, t, *J* = 7.8), 7.78 (1H, d, *J* = 8.9), 9.20 (1H, brs), 9.62 (1H, brs), 9.90 (1H, s); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 22.1, 26.6, 39.3, 45.9, 53.9, 63.6, 68.1, 92.4, 113.0, 115.0, 117.2, 119.2, 121.4, 121.9, 129.4, 138.7, 141.9, 143.2, 149.8, 158.1; HRMS calculated for $C_{21}H_{28}N_4O_4S$ + H^+ 433.1904, found (ESI, $[M+H]^+$) 433.1903; LC/MS (ESI, $[M+H]^+$, *m/z*) 433; HPLC (Method A): purity 100% R_T 1.9 min.

***N*-(3-((1*R*)-2-(2-((3-*tert*-Butyl-1*H*-indazol-6-yl)oxy)ethylamino)-1-hydroxy-ethyl)phenyl)methanesulfonamide (12).** To a stirred solution of **24** (141 mg, 0.5 mmol), **17** (0.92 mL, 0.9 mmol, 1 M toluene solution), and triphenylphosphine (401 mg, 1.5 mmol) in anhydrous toluene (5 mL) was added *N,N,N',N'*-tetramethylazodi-carboxamide (284 mg, 1.6 mmol) at room temperature, and the solution was stirred overnight. The reaction solution was then purified by flash column chromatography on silica gel (95:5 to 74:26 n-hexane/ethyl acetate) to give 354 mg (82% yield) of the coupling product. ^{1}H-NMR (300 MHz, $CDCl_3$, 1:1 rotamers): δ 0.54 (6H, t, *J* = 7.9), 0.89 (9H, t, *J* = 7.9), 1.43 (9H, s), 1.47 and 1.51 (9H, each s), 1.50 (9H, s), 1.69 (9H, s), 3.22-3.62 (7H, m), 4.01-4.10 (2H, m), 4.93-4.97 and 5.1-5.13 (1H, each m), 6.83 (1H, d, *J* = 8.8), 7.13-7.44 (4H, m), 7.51 (1H, d, *J* = 1.7), 7.67 (1H, dd, *J* = 2.4, 8.8); LC/MS (ESI, $[M+H]^+$, *m/z*) 861. To the solution of the obtained product (232 mg, 0.27 mmol) in 1,4-dioxane (0.45 mL) was added 4 M HCl in 1,4-dioxane (1.5 mL), and the mixture was shaken (600 min^{-1}) overnight at room temperature. The reaction mixture was added ethanol and was shaken (600 min^{-1}) for 4 h at room temperature. Nitrogen gas was blown into the reaction solution to evaporate the solvent. Subsequently, water was added to dissolve the residue and the solution was freeze-dried to give 140 mg (quantitative yield) of the title compound as dihydrochloride salt. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.44 (9H, s), 3.00 (3H, s), 3.06-3.08 (1H, m), 3.24-3.28 (1H, m), 3.45-3.49 (2H, m), 4.36-4.39 (2H, m), 5.03 (1H, dd, *J* = 2.0, 10.1), 6.77 (1H, dd, *J* = 2.0, 8.9), 6.93 (1H, d, *J* = 2.0), 7.13 (1H, d, *J* = 7.8), 7.15-7.17 (1H, m), 7.31 (1H, s), 7.35 (1H, t, *J* = 7.8), 7.80 (1H, d, *J* = 8.9), 9.08 (1H, brs), 9.45 (1H, brs), 9.87 (1H, s); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 29.9, 33.1, 39.1, 45.8, 53.6, 63.4, 67.9, 92.4, 111.3, 114.7, 117.0, 119.0, 121.2, 122.4, 129.2, 138.5, 142.5, 143.0, 152.1, 156.6; HRMS calculated for $C_{22}H_{30}N_4O_4S$ + H^+ 447.2061, found (ESI, $[M+H]^+$) 447.2061; LC/MS (ESI, $[M+H]^+$, *m/z*) 447; HPLC (Method A): purity 100% R_T 2.0 min.

***N*-(3-((1*R*)-2-(2-((3-Cyclopropyl-1*H*-indazol-6-yl)oxy)ethylamino)-1-hydroxy-ethyl)phenyl)methanesulfon-amide (13).** To a stirred solution of **25** (1.415 g, 5 mmol), **17** (10 mL, 10 mmol, 1 M toluene solution), and

triphenylphosphine (2.910 g, 11 mmol) in anhydrous toluene (50 mL) was added *N*,*N*,*N'*,*N'*-tetramethylazodicarboxamide (1.915 g, 11 mmol) at room temperature, and the solution was stirred overnight. The reaction solution was purified by flash column chromatography on silica gel (95:5 to 74:26 n-hexane/ethyl acetate) to give 3.779 g (89% yield) of the coupling product. ^{1}H-NMR (300 MHz, $CDCl_3$, 1:1 rotamers): δ 0.54 (6H, q, *J* = 7.9), 0.89 (9H, t, *J* = 7.9), 1.03-1.05 (2H, m), 1.15-1.20 (2H, m), 1.44 (9H, s), 1.48 and 1.52 (9H, each s), 1.68 (9H, s), 2.13-2.21 (1H, m), 3.22-3.63 (7H, m), 4.02-4.13 (2H, m), 4.94-4.98 and 5.10-5.14 (1H, each m), 6.84 (1H, dd, *J* = 2.0, 8.7), 7.13-7.53 (6H, m); LC/MS (ESI, $[M+H]^+$, *m/z*) 845. To the solution of the obtained product (3.770 g, 4.5 mmol) in 1,4-dioxane (9 mL) was added 4 M HCl in 1,4-dioxane (20 mL), and the mixture was stirred overnight at room temperature. Then, 4 M HCl in 1,4-dioxane (14 mL) was further added to the reaction solution, and the mixture was stirred for 2 h at room temperature. The resultant solid was collected by suction filtration, washed with diethyl ether, and dried under reduced pressure. The solid was dissolved with water, and the solution was freeze-dried to give 2.033 g (90 % yield) of the title compound as dihydrochloride salt. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 0.96-1.02 (4H, m), 2.23-2.30 (1H, m), 3.00 (3H, s), 3.04-3.10 (1H, m), 3.24-3.28 (1H, m), 3.44-3.47 (2H, m), 4.34-4.42 (2H, m), 5.04 (1H, dd, *J* = 2.1, 10.2), 6.80 (1H, dd, *J* = 2.1, 8.8), 6.91 (1H, d, *J* = 2.1), 7.13 (1H, d, *J* = 8.0), 7.17 (1H, dd, *J* = 1.3, 8.0), 7.31 (1H, s), 7.34 (1H, t, *J* = 8.0), 7.70 (1H, d, *J* = 8.8), 9.11 (1H, brs), 9.50 (1H, brs), 9.87 (1H, s); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 7.6, 7.7, 39.1, 45.8, 53.7, 63.4, 67.9, 92.2, 112.1, 116.2, 117.0, 119.0, 121.0, 121.2, 129.2, 138.5, 141.7, 143.0, 146.1, 157.6; HRMS calculated for $C_{21}H_{26}N_4O_4S + H^+$ 431.1748, found (ESI, $[M+H]^+$) 431.1743; LC/MS (ESI, $[M+H]^+$, *m/z*) 431; HPLC (Method B): purity 96% R_T 1.9 min.

***N*-(3-((1*R*)-2-(2-((3-Cyclobutyl-1*H*-indazol-6-yl)oxy)ethylamino)-1-hydroxy-ethyl)phenyl)methanesulfonamide (14).** To a stirred solution of **26** (1.426 g, 5 mmol), **17** (10 mL, 10 mmol, 1 M toluene solution), and triphenylphosphine (2.914 g, 11 mmol) in anhydrous toluene (25 mL) was added *N*,*N*,*N'*,*N'*-tetramethylazodicarboxamide (1.911 g, 11 mmol) at room temperature, and the solution was stirred overnight. The reaction solution was purified by flash column chromatography on silica gel (88:12 to 67:33 n-hexane/ethyl acetate) to give 3.848 g (90% yield) of the coupling product. ^{1}H-NMR (300 MHz, $CDCl_3$, 1:1 rotamers): δ 0.54 (6H, q, *J* = 7.9), 0.89 (9H, t, *J* = 7.9), 1.44 (9H, s), 1.48 and 1.52 (9H, each s), 1.70 (9H, s), 1.95-2.02 (1H, m), 2.11-2.20 (1H, m), 2.39-2.59 (4H, m), 3.22-3.63 (7H, m), 3.87 (1H, qu, *J* = 8.8), 4.02-4.08 (2H, m), 4.94-4.98 and 5.10-5.14 (1H, each m), 6.84 (1H, dd, *J* = 1.7, 8.6), 7.13-7.54 (6H, m); LC/MS (ESI, $[M+H]^+$, *m/z*) 859. The obtained product (3.840 g, 4.3 mmol) and 4 M HCl in ethyl acetate (80 mL) were stirred overnight at room temperature. The resultant solid was collected by suction filtration, washed with diethyl ether, and dried to afford 2.166 g (93% yield) of the title compound as dihydrochloride salt. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.90-1.98 (1H, m), 2.02-2.13 (1H, m), 2.35-2.43 (4H, m), 3.00 (3H, s), 3.04-3.11 (1H, m), 3.23-3.28 (1H, m), 3.45-3.48 (2H, m), 3.90 (1H, qu, *J* = 8.7), 4.37-4.44 (2H, m), 5.05 (1H, dd, *J* = 2.0, 10.2), 6.82 (1H, dd, *J* = 2.1, 8.8), 6.95 (1H, d, *J* = 2.1), 7.14 (1H, d, *J* = 7.8), 7.16-7.18 (1H, m), 7.31 (1H, s), 7.35 (1H, t, *J* = 7.8), 7.69 (1H, d, *J* = 8.8), 9.15 (1H, brs), 9.56 (1H, brs), 9.89 (1H, s); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 18.4, 27.9, 32.0, 39.1, 45.8, 53.7, 63.4, 67.9, 92.2, 112.4, 115.3, 117.0, 119.0, 121.2, 121.3, 129.2, 138.5, 141.8, 143.0, 147.6, 157.7; HRMS calculated for $C_{22}H_{28}N_4O_4S + H^+$ 445.1904, found (ESI, $[M+H]^+$) 445.1897; LC/MS (ESI, $[M+H]^+$, *m/z*) 445; HPLC (Method A): purity 96% R_T 2.0 min.

***N*-(3-((1*R*)-1-Hydroxy-2-(2-((3-(trifluoromethyl)-1*H*-indazol-6-yl)oxy)ethylamino)ethyl)phenyl)methane-sulfonamide (15).** To a stirred solution of **27** (1.511 g, 5 mmol), **17** (10 mL, 10 mmol, 1 M toluene solution), and triphenylphosphine (2.600 g, 9.9 mmol) in anhydrous toluene (15 mL) was added *N*,*N*,*N'*,*N'*-tetramethylazodi-carboxamide (1.733 g, 10 mmol) at room temperature, and the solution was stirred overnight. The reaction solution was then purified by flash column chromatography on silica gel (82:18 to 61:39 n-hexane/ethyl acetate) to give 3.544 g (81% yield) of the coupling product. ^{1}H-NMR (300 MHz, $CDCl_3$, 1:1 rotamers): δ 0.54 (6H, q, *J* = 8.1), 0.89 (9H, t, *J* = 8.1), 1.44 (9H, s), 1.49 and 1.53 (9H,each s), 1.71 (9H, s), 3.22-3.64 (7H, m), 4.04-4.13 (2H, m), 4.94-4.99 and 5.10-5.14 (1H, each m), 6.98 (1H, dd, *J* = 2.2, 8.8), 7.14-7.45 (4H, m) 7.59 (1H, d, *J* = 1.8), 7.64(1H, dd, *J* = 4.0, 8.8); LC/MS (ESI, $[M+H]^+$, *m/z*) 873. The obtained product (3.514 g, 4 mmol) and 4 M HCl in ethyl acetate (80 mL) were stirred overnight at room temperature. The resultant solid was collected by suction filtration, washed with *tert*-butyl methyl ether, and dried to afford 1.930 g (93% yield) of the title compound as dihydrochloride salt. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.00 (3H, s), 3.05-3.12 (1H, m), 3.24-3.28 (1H, m), 3.49 (2H, brs), 4.40-4.47 (2H, m), 5.02 (1H, d, *J* = 9.8), 6.28 (1H, brs), 7.03 (1H, dd, *J* = 2.1, 8.8), 7.13-7.18 (3H, m) 7.31 (1H, s), 7.35 (1H, t, *J* = 7.8), 7.71 (1H, d, *J* = 8.8), 9.11 (1H, brs), 9.46 (1H, brs), 9.87 (1H, s), 13.96 (1H, s); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 39.2, 45.8, 53.6, 63.6, 67.9, 92.8, 113.7, 115.4, 117.0, 119.0, 119.6, 121.2, 122.2 (q, *J* = 268), 129.2, 133.0 (q, *J* = 36), 138.5, 141.9, 143.0, 157.7; HRMS calculated for $C_{19}H_{21}F_3N_4O_4S + H^+$ 459.1308, found (ESI, $[M+H]^+$) 459.1307; LC/MS (ESI, $[M+H]^+$, *m/z*) 459; HPLC (Method A): purity 99% R_T 2.0 min.

***N*-(3-((1*R*)-1-Hydroxy-2-(2-((3-phenyl-1*H*-indazol-6-yl)oxy)ethylamino)ethyl)phenyl)methanesulfonamide (16).** To a stirred solution of **28** (34 mg, 0.13 mmol), **17** (0.5 mL, 0.20 mmol, 0.4 M toluene solution), and triphenylphosphine (56 mg, 0.21 mmol) in anhydrous toluene (1 mL) was added *N*,*N*,*N'*,*N'*-tetramethylazodi-carboxamide (40 mg, 0.23 mmol) at room temperature, and the solution was stirred overnight. The reaction solution was then purified by flash column chromatography on silica gel (88:12 to 67:33 n-hexane/ethyl acetate) to give 77 mg (87% yield) of the coupling product. ^{1}H-NMR (300 MHz, $CDCl_3$, 1:1 rotamers): δ 0.55 (6H, q, *J* = 7.9), 0.89 (9H, t, *J* = 7.9), 1.44 (9H, s), 1.46 and 1.49 (9H, each s), 1.73 (9H, s), 3.24-3.64 (7H, m), 4.06-4.15 (2H, m), 4.95-4.99 and 5.10-5.15 (1H, each m), 6.93 (1H, dd, *J* = 1.9, 8.8), 7.13-7.52 (7H, m), 7.64 (1H, d, *J* = 1.9), 7.79 (1H, dd, *J* = 3.0, 8.8), 7.96 (2H, dd, *J* = 1.5, 8.0); LC/MS (ESI, $[M+H]^+$, *m/z*) 881. To the solution of the obtained product (75 mg, 0.09 mmol) in *tert*-butyl methyl ether (0.2 mL) was added 4 M HCl in 1,4-dioxane (1 mL) and the mixture was stirred overnight at room temperature. The resultant solid was collected by suction filtration, washed with *tert*-butyl methyl ether, and dried to afford 48 mg (quantitative yield) of the title compound as dihydrochloride salt. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.00 (s, 3H), 3.08-3.12 (1H, m), 3.25-3.30 (1H, m), 3.47-3.50 (2H, m), 4.42-4.44 (2H, m), 5.04 (1H, dd, *J* = 2.0, 10.2), 6.91 (1H, dd, *J* = 2.0, 8.9), 7.05 (1H, d, *J* = 2.0), 7.13-7.18 (2H, m), 7.32 (1H, s), 7.35 (1H, t, *J* = 7.9), 7.38-7.42 (1H, m), 7.49-7.53 (2H, m), 7.96-7.99 (3H, m), 9.08 (1H, brs), 9.41 (1H, brs), 9.87 (1H, s); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 39.1, 45.9, 53.6, 63.4, 67.9, 92.5, 113.0, 115.1, 116.9, 118.9, 121.2, 121.5, 126.5, 127.6, 128.7, 129.2, 133.6, 138.5, 142.6, 142.9, 143.0, 156.9; HRMS calculated for $C_{24}H_{26}N_4O_4S + H^+$ 467.1748, found (ESI, $[M+H]^+$) 467.1746; LC/MS (ESI, $[M+H]^+$, *m/z*) 467; HPLC (Method A): purity 99% R_T 2.0 min.

***tert*-Butyl *N*-(3-((1*R*)-2-(*tert*-butoxycarbonyl(2-hydroxyethyl)amino)-1-triethylsilyloxy-ethyl)phenyl)-*N*-methylsulfonyl-carbamate (17).** A stirred mixture of **53** (494 mg, 0.88 mmol) and 20% palladium hydroxide on carbon (103 mg) in THF (1.8 mL) and methanol (1.8 mL) was evacuated, placed under a hydrogen atmosphere, and stirred overnight at 50 °C. The reaction mixture was passed through a membrane filter, and the solvent was concentrated under reduced pressure to give *N*-(3-((1*R*)-2-(2-hydroxyethylamino)-1-triethylsilyloxy-ethyl)phenyl)-methanesulfonamide (364 mg), which was used without further purification. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.50-0.58 (6H, m), 0.81-0.91 (9H, t, *J* = 7.9), 2.71-2.86 (4H, m), 2.99 (3H, s), 3.59 (2H, t, *J* = 5.2), 4.79 (1H, dd, *J* = 4.5, 7.2), 7.11-7.23 (3H, m), 7.31 (1H, t, *J* = 7.8); LC/MS (ESI, $[M+H]^+$, *m/z*) 291. The *N*-(3-((1*R*)-2-(2-hydroxyethylamino)-1-triethylsilyloxy-ethyl)phenyl)methanesulfonamide (337 mg, 0.86 mmol) was dissolved in anhydrous THF (4 mL). The solution was added triethylamine (0.12 mL, 0.86 mmol), Boc_2O (0.44 mL, 1.9 mmol) and 4-*N*,*N*-dimethylaminopyridine (21 mg, 0.17 mmol) at room temperature under a nitrogen atmosphere, and was stirred overnight. The reaction mixture was added ethyl acetate, was washed twice with water, washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue (524 mg) was purified by flash column chromatography (71:29 to 50:50 n-hexane/ethyl acetate) to give the title compound (254 mg, 50% yield). ^{1}H-NMR (300 MHz, $CDCl_3$, 1:1 rotamers): δ 0.49-0.58 (6H, m), 0.85-0.91 (9H, m), 1.44 (9H, s), 1.49 and 1.53 (9H, each s), 3.03-3.72 (9H, m), 5.00-5.04 and 5.27-5.29 (1H, each m), 7.12-7.16 (1H, m), 7.20-7.42 (3H, m); LC/MS (ESI, $[M+H]^+$, *m/z*) 589.

***tert*-Butyl 6-hydroxy-3-methyl-1*H*-indazole-1-carboxylate (18).** To a stirred mixture of **34a** (16.74 g, 70 mmol) and 10% palladium on activated charcoal (5.12 g, PE-type, N.E.Chemcat.) in ethanol (166 mL) was added concentrated hydrochloric acid (5.83 mL, 70 mmol). The reaction was evacuated, placed under a hydrogen atmosphere and stirred for 10 h at 60 °C. The reaction mixture was added 10% palladium on activated charcoal (1.51 g, PE-type, N.E.Chemcat.). The mixture was evacuated, placed under a hydrogen atmosphere and stirred for 5 h at 60 °C. The reaction mixture was cooled to room temperature, passed through a membrane filter, and the solvent was concentrated under reduced pressure. The residue was partitioned between ethyl acetate and saturated aq $NaHCO_3$. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to give 3-methyl-1*H*-indazol-6-ol (10.816 g). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.38 (3H, s), 6.58 (1H, dd, *J* = 2.0, 8.6), 6.67 (1H, d, *J* = 2.0), 7.44 (1H, d, *J* = 8.6), 9.47 (1H, brs), 12.10 (1H, brs). LC/MS (ESI, $[M+H]^+$, *m/z*) 149. To a stirred solution of 3-methyl-1*H*-indazol-6-ol (10.72 g, 70 mmol) and imidazole (9.55 g, 140 mmol) in anhydrous DMF (140 mL) was added TBDPSCl (38.53 g, 140 mmol) at room temperature, and the mixture was stirred overnight. The reaction mixture was poured into water and the aqueous layer was extracted twice with ethyl acetate. The combined organic layers were washed twice with water, washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue (41.36 g) was dissolved in dichloromethane (350 mL). The solution was added triethylamine (8.55 g, 84 mmol), Boc_2O (18.36 g, 84 mmol) and 4-*N*,*N*-dimethylaminopyridine (0.85 g, 7 mmol) at room temperature, and the mixture was stirred overnight. The reaction solution was washed twice with 1 M HCl and washed with brine. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue (52.57 g) was dissolved in anhydrous THF (350 mL). The solution was added 1 mol/L TBAF-THF solution

(140 mL, 140 mmol) at room temperature, and the mixture was stirred for 1 h. The reaction mixture was added ethyl acetate, and the organic layer was washed with brine and water. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (74:26 to 47:53 n-hexane/ethyl acetate) to afford the product (10.934 g, 63% yield). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.66 (9H, s), 2.52 (3H, s), 6.42 (1H, brs), 6.88 (1H, dd, *J* = 2.2, 8.6), 7.48 (1H, d, *J* = 8.6), 7.57 (1H, d, *J* = 2.2); LC/MS (ESI, $[M+H]^+$, *m/z*) 249.

***tert*-Butyl 6-hydroxy-3-methoxy-1*H*-indazole-1-carboxylate (19).** A stirred mixture of **42a** (206 mg, 0.58 mmol) and 5% palladium on activated charcoal (113 mg, STD-type, N.E.Chemcat.) in THF (5.8 mL) was evacuated, placed under a hydrogen atmosphere, and stirred overnight at room temperature. The reaction mixture was passed through a membrane filter, and the solvent was concentrated under reduced pressure to give the title compound (164 mg, quantitative yield). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.65 (9H, s), 4.11 (3H, s), 6.48 (1H, brs), 6.83 (1H, dd, *J* = 2.1, 8.6), 7.43 (1H, brs), 7.47 (1H, d, *J* = 8.6); LC/MS (ESI, $[M+H]^+$, *m/z*) 265.

***tert*-Butyl 3-ethoxy-6-hydroxy-1*H*-indazole-1-carboxylate (20).** A stirred mixture of **42b** (67 mg, 0.18 mmol) and 5% palladium on activated charcoal (35 mg, STD-type, N.E.Chemcat.) in THF (2 mL) was evacuated, placed under a hydrogen atmosphere, and stirred overnight at room temperature. The reaction mixture was passed through a membrane filter, and the solvent was concentrated under reduced pressure to give the title compound (54 mg, quantitative yield). ^{1}H-NMR (300 MHz, $CDCl_3$, 3:2 rotamers): δ 1.42 and 1.42 (3H, each t, *J* = 7.0), 1.62 and 1.63 (9H, each s), 4.47 and 4.47 (2H, each q, *J* = 7.1), 6.84 (1H, dd, *J* = 1.5, 8.6), 7.43 (1H, brs), 7.48 (1H, d, *J* = 8.6); LC/MS (ESI, $[M+H]^+$, *m/z*) 279.

***tert*-Butyl 3-chloro-6-hydroxy-1*H*-indazole-1-carboxylate (21). 38a** (17.42 g, 34 mmol) was dissolved in anhydrous THF (150 mL). The solution was added 1 mol/L TBAF-THF solution (42 mL, 42 mmol) at room temperature, and the mixture was stirred overnight. The reaction mixture was added ethyl acetate, washed with brine, washed with water, washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was added *n*-hexane (150 mL), and the precipitates were collected by suction filtration to give the product (6.38 g, 70% yield). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.69 (9H, s), 6.03 (1H, s), 6.95 (1H, dd, *J* = 2.0, 8.7), 7.53 (1H, d, *J* = 8.7), 7.60 (1H, d, *J* = 2.0). LC/MS (ESI, $[M+H]^+$, *m/z*) 269.

***tert*-Butyl 3-ethyl-6-hydroxy-1*H*-indazole-1-carboxylate (22).** To a stirred mixture of **34b** (4.78 g, 19 mmol) and 10% palladium on activated charcoal (1.953 g, PE-type, N.E.Chemcat.) in ethanol (189 mL) was added concentrated hydrochloric acid (1.58 mL, 19 mmol). The mixture was evacuated, placed under a hydrogen atmosphere and stirred for 1.2 h at 60 °C. The reaction mixture was cooled to room temperature, passed through a membrane filter, and the solvent was concentrated under reduced pressure to give 3-ethyl-1*H*-indazol-6-ol hydrochloride (3.918 g). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.31 (3H, t, *J* = 7.6), 2.94 (2H, q, *J* = 7.6), 6.73 (1H, dd, *J* = 1.7, 8.7), 6.78 (1H, d, *J* = 1.7), 7.65 (1H, d, *J* = 8.7); LC/MS (ESI, $[M+H]^+$, *m/z*) 163. To a stirred solution of 3-ethyl-1*H*-indazol-6-ol hydrochloride (3.76 g, 19 mmol) and imidazole (4.51 g, 66 mmol) in anhydrous DMF (100

mL) was added TBDPSCl (17.01 mL, 66 mmol) at 0 °C. The mixture was allowed to warm to room temperature and was then stirred overnight. The reaction mixture was added imidazole (1.29 g, 19 mmol) and TBDPSCl (4.86 mL, 19 mmol) at 30 °C, and the mixture was stirred for 2.5 h at 30 °C. The reaction mixture was poured into water, and the aqueous layer was extracted twice with ethyl acetate. The combined organic layers were washed twice with water, washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue (28.72 g) was purified by flash column chromatography (88:12 to 67:33 n-hexane/ethyl acetate) to afford 6-((*tert*-butyldiphenyl-silyl)oxy)-3-ethyl-1*H*-indazole (5.98 g, 74% yield). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.11 (9H, s), 1.35 (3H, t, *J* = 7.6), 2.90 (2H, q, *J* = 7.6), 6.61 (1H, d, *J* = 2.0), 6.74 (1H, dd, *J* = 2.0, 8.7), 7.33-7.45 (7H, m), 7.72-7.76 (4H, m); LC/MS (ESI, $[M+H]^+$, *m/z*) 401. The 6-((*tert*-butyldiphenylsilyl)oxy)-3-ethyl-1*H*-indazole (5.98 g, 15 mmol) was dissolved in THF (150 mL). The solution was added triethylamine (2.5 mL, 18 mmol), Boc_2O (4.1 mL, 18 mmol), and 4-*N*,*N*-dimethylaminopyridine (1.01 g, 8 mmol) at room temperature, and the mixture was stirred overnight. The reaction mixture was concentrated under reduced pressure, and remaining oil was partitioned between ethyl acetate and water. The aqueous layer was extracted twice with ethyl acetate. The combined organic layers were washed twice with water, washed with brine, and dried over Na_2SO_4. The organic solution was concentrated under reduced pressure to give *tert*-butyl 6-((*tert*-butyldiphenylsilyl)oxy)-3-ethyl-1*H*-indazole-1-carboxylate (8.02 g, 89%). LC/MS (ESI, $[M+H]^+$, *m/z*) 501. The residue (8.02 g, 16 mmol) was dissolved in anhydrous THF (53 mL). The solution was added 1 mol/L TBAF-THF solution (31.5 mL, 32 mmol) at room temperature, and the mixture was stirred for 0.5 h. The reaction mixture was concentrated under reduced pressure, and remaining oil was partitioned between ethyl acetate and water. The aqueous layer was extracted twice with ethyl acetate. The combined organic layers were washed twice with water, washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue (10.0 g) was purified by flash column chromatography (95:5 to 74:26 n-hexane/ethyl acetate) to afford the product (3.27 g, 78%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.37 (3H, t, *J* = 7.6), 1.63 (9H, s), 2.94 (2H, q, *J* = 7.6), 6.89 (1H, dd, *J* = 2.1, 8.6), 6.93 (1H, brs) 7.52 (1H, d, *J* = 8.6), 7.57 (1H, s); LC/MS (ESI, $[M+H]^+$, *m/z*) 263.

***tert*-Butyl 6-hydroxy-3-isopropyl-1*H*-indazole-1-carboxylate (23).** To a stirred mixture of **34c** (6.51 g, 24 mmol) and 10% palladium on activated charcoal (2.69 g, PE-type, N.E.Chemcat.) in ethanol (244 mL) was added concentrated hydrochloric acid (2.0 mL, 24 mmol). The reaction was evacuated and placed under a hydrogen atmosphere, and stirred for 1.5 h at 60 °C. The reaction mixture was cooled to room temperature, passed through a membrane filter, and the solvent was concentrated under reduced pressure to give 3-isopropyl-1*H*-indazol-6-ol hydrochloride (6.17 g). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.35 (6H, d, *J* = 7.0), 3.33 (1H, septet, *J* = 7.0), 6.67 (1H, dd, *J* = 2.0, 8.8), 6.75 (1H, d, *J* = 1.8), 7.64 (1H, d, *J* = 8.8); LC/MS (ESI, $[M+H]^+$, *m/z*) 177. To a stirred solution of 3-isopropyl-1*H*-indazol-6-ol hydrochloride (6.17 g) and imidazole (4.15 g, 61 mmol) in anhydrous DMF (122 mL) was added TBDPSCl (16.77 g, 61 mmol) at 0 °C. The mixture was allowed to warm to room temperature and was then stirred overnight. The reaction mixture was added imidazole (2.47 g, 36 mmol) and TBDPSCl (9.4 mL, 36 mmol) at 20 °C, and the mixture was stirred for 3 h at 30 °C. The reaction mixture was poured into water, and the aqueous layer was extracted twice with ethyl acetate. The combined organic layers were washed twice with water, washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue (33.17 g)

was purified by flash column chromatography (95:5 to 74:26 n-hexane/ethyl acetate) to afford 6-((*tert*-butyldiphenylsilyl)oxy)-3-isopropyl-1*H*-indazole (6.65 g, 67% yield). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.07 (9H, s), 1.39 (6H, d, *J* = 7.0), 3.30 (1H, septet, *J* = 7.0), 6.61 (1H, d, *J* = 1.8), 6.73 (1H, dd, *J* = 2.0, 8.7), 7.33-7.50 (7H, m), 7.72-7.76 (4H, m); LC/MS (ESI, $[M+H]^+$, *m/z*) 415. The 6-((*tert*-butyldiphenyl-silyl)oxy)-3-isopropyl-1*H*-indazole (6.65 g, 16 mmol) was dissolved in CH_3CN (160 mL). The solution was added triethylamine (2.68 mL, 19 mmol), Boc_2O (4.4 mL, 19 mmol) and 4-*N*,*N*-dimethylaminopyridine (0.98 g, 8 mmol) at room temperature, and the mixture was stirred overnight. The reaction mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography (100:0 to 90:10 n-hexane/ethyl acetate) to afford *tert*-butyl 6-((*tert*-butyldiphenylsilyl)oxy)-3-isopropyl-1*H*-indazole-1-carboxylate (8.12 g, 98%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.11 (9H, s), 1.40 (9H, s), 1.41 (6H, d, *J* = 7.0), 3.30 (1H, septet, *J* = 7.0), 6.79 (1H, dd, *J* = 2.1, 8.6), 7.33-7.46 (8H, m), 7.71-7.74 (4H, m); LC/MS (ESI, $[M+H]^+$, *m/z*) 515. The *tert*-butyl 6-((*tert*-butyl-diphenylsilyl)oxy)-3-isopropyl-1*H*-indazole-1-carboxylate (8.12 g, 16 mmol) was dissolved in anhydrous THF (56 mL). The solution was added 1 mol/L TBAF-THF solution (31.5 mL, 32 mmol) at 0 °C, and the mixture was allowed to warm to room temperature. After the reaction mixture was stirred for 1 h, the mixture was added water and brine. The aqueous layer was extracted three times with ethyl acetate. The combined organic layers were washed with water, washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column chromatography (95:5 to 74:26 n-hexane/ethyl acetate) to afford the product (3.39 g, 78%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.43 (6H, d, *J* = 7.0), 1.64 (9H, s), 3.35 (1H, septet, *J* = 7.0), 6.22 (1H, brs), 6.85 (1H, dd, *J* = 2.2, 8.6), 7.53 (1H, s), 7.59 (1H, d, *J* = 8.6); LC/MS (ESI, $[M+H]^+$, *m/z*) 277.

***tert*-Butyl 3-(*tert*-butyl)-6-hydroxy-1*H*-indazole-1-carboxylate (24).** To a stirred mixture of **34d** (1.51 g, 5.4 mmol) and 10% palladium on activated charcoal (0.30 g, PE-type, N.E.Chemcat.) in ethanol (54 mL) was added concentrated hydrochloric acid (0.45 mL, 5.4 mmol). The reaction was evacuated, placed under a hydrogen atmosphere, and stirred for 1.5 h at 60 °C. The reaction mixture was cooled to room temperature, passed through a membrane filter, and the solvent was concentrated under reduced pressure to give 3-(*tert*-butyl)-1*H*-indazol-6-ol hydrochloride (1.27 g). LC/MS (ESI, $[M+H]^+$, *m/z*) 191. To a stirred solution of 3-(*tert*-butyl)-1*H*-indazol-6-ol hydrochloride (1.22 g) and imidazole (1.29 g, 19 mmol) in anhydrous DMF (27 mL) was added TBDPSCl (4.85 mL, 19 mmol) at room temperature, and the mixture was stirred overnight. The reaction mixture was poured into water, and the aqueous layer was extracted twice with ethyl acetate. The combined organic layers were washed twice with water, washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue (7.8 g) was purified by flash column chromatography (100:0 to 87:13 n-hexane/ethyl acetate) to afford 3-(*tert*-butyl)-6-((*tert*-butyldiphenylsilyl)oxy)-1*H*-indazole (1.35 g, 58% yield). LC/MS (ESI, $[M+H]^+$, *m/z*) 429. The 3-(*tert*-butyl)-6-((*tert*-butyldiphenylsilyl)oxy)-1*H*-indazole (1.35 g, 3.1 mmol) was dissolved in CH_3CN (31 mL). The solution was added triethylamine (0.53 mL, 3.8 mmol), Boc_2O (0.87 mL, 3.8 mmol) and 4-*N*,*N*-dimethylaminopyridine (0.22 g, 0.18 mmol) at room temperature, and the mixture was stirred for 4 h. The reaction mixture was added water, concentrated under reduced pressure, and the remaining oil was partitioned between ethyl acetate and water. The aqueous layer was extracted twice with ethyl acetate. The combined organic layers were washed twice with water, washed with brine. The organic layer was dried over Na_2SO_4, and

concentrated under reduced pressure to give *tert*-butyl 3-(*tert*-butyl)-6-((*tert*-butyldiphenylsilyl)oxy)-1*H*-indazole-1-carboxylate (1.71 g). LC/MS (ESI, $[M+H]^+$, *m/z*) 529. The residue (1.66 g, 3.1 mmol) was dissolved in anhydrous THF (11.2 mL). The solution was added 1 mol/L TBAF-THF solution (6.3 mL, 6.3 mmol) at room temperature, and the mixture was stirred for 2 h. The reaction mixture was added water and brine. The aqueous layer was extracted three times with ethyl acetate. The combined organic layers were washed with water, washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column chromatography (95:5 to 74:26 n-hexane/ethyl acetate) to afford the product (0.73 g, 79%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.50 (9H, s), 1.67 (9H, s), 5.79 (1H, brs), 6.82 (1H, dd, *J* = 2.0, 8.7), 7.51 (1H, d, *J* = 2.0), 7.70 (1H, d, *J* = 8.7); LC/MS (ESI, $[M-H]^+$, *m/z*) 289.

***tert*-Butyl 3-cyclopropyl-6-hydroxy-1*H*-indazole-1-carboxylate (25).** To a stirred mixture of **34e** (6.68 g, 25 mmol) and 10% palladium on activated charcoal (2.68 g, PE-type, N.E.Chemcat.) in ethanol (246 mL) was added concentrated hydrochloric acid (2.0 mL, 24 mmol). The reaction was evacuated, placed under a hydrogen atmosphere, and stirred for 2.5 h at 60 °C. The reaction mixture was cooled to room temperature, passed through a membrane filter, and the solvent was concentrated under reduced pressure to give 3-cyclopropyl-1*H*-indazol-6-ol hydrochloride (5.82 g). LC/MS (ESI, $[M+H]^+$, *m/z*) 175. To a stirred solution of 3-cyclopropyl-1*H*-indazol-6-ol hydrochloride (5.18 g) and imidazole (4.21 g, 61 mmol) in anhydrous DMF (122 mL) was added TBDPSCl (15.67 mL, 61 mmol) at room temperature, and the mixture was stirred overnight. The reaction mixture was added imidazole (1.8 g, 26 mmol) and TBDPSCl (6.3 mL, 24 mmol) at room temperature, and the mixture was stirred for 1.5 h. The reaction mixture was then poured into water, and the aqueous layer was extracted twice with ethyl acetate. The combined organic layers were washed twice with water, washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue (31.38 g) was purified by flash column chromatography (95:5 to 74:26 n-hexane/ethyl acetate) to afford 6-((*tert*-butyldiphenylsilyl)oxy)-3-cyclopropyl-1*H*-indazole (4.71 g, 47% yield). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.95-1.00 (4H, m), 1.11 (9H, s), 2.04-2.14 (1H, m), 6.59 (1H, d, *J* = 2.0), 6.73 (1H, dd, *J* = 2.0, 8.7), 7.33-7.49 (7H, m), 7.72-7.75 (4H, m); LC/MS (ESI, $[M+H]^+$, *m/z*) 413. The 6-((*tert*-butyldiphenylsilyl)oxy)-3- cyclopropyl-1*H*-indazole (4.70 g, 11 mmol) was dissolved in THF (120 mL). The solution was added triethylamine (1.9 mL, 14 mmol), Boc_2O (3.1 mL, 14 mmol) and 4-*N*,*N*-dimethylamino-pyridine (0.69 g, 5.7 mmol) at room temperature, and the mixture was stirred overnight. The reaction mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography (100:0 to 90:10 n-hexane/ethyl acetate) to afford *tert*-butyl 6-((*tert*-butyldiphenylsilyl)oxy)-3-cyclopropyl-1*H*-indazole-1-carboxylate (5.08 g, 87%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.97-1.28 (13H, m), 1.41 (9H, s), 2.07-2.15 (1H, m), 6.78 (1H, dd, *J* = 2.0, 8.7), 7.33-7.45 (8H, m), 7.66-7.74 (4H, m); LC/MS (ESI, $[M+H]^+$, *m/z*) 513. The *tert*-butyl 6-((*tert*-butyldiphenylsilyl)oxy)-3-cyclopropyl-1*H*-indazole-1-carboxylate (5.08 g, 9.9 mmol) was dissolved in anhydrous THF (35 mL). The solution was added 1 mol/L TBAF-THF solution (19.8 mL, 20 mmol) at room temperature, and the mixture was stirred for 0.5 h. The reaction mixture was added water and brine. The aqueous layer was extracted three times with ethyl acetate. The combined organic layers were washed with water, washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column chromatography (95:5 to 74:26 n-hexane/ethyl acetate) to afford the product (2.54 g, 93% yield). ^{1}H-NMR (300

MHz, $CDCl_3$): δ 0.99-1.06 (2H, m), 1.16-1.21 (2H, m), 1.64 (9H, s), 2.12-2.21 (1H, m), 6.25 (1H, brs), 6.86 (1H, dd, *J* = 2.0, 8.7), 7.52 (1H, brs), 7.53 (1H, d, *J* = 8.7); LC/MS (ESI, $[M+H]^+$, *m/z*) 275.

***tert*-Butyl 3-cyclobutyl-6-hydroxy-1*H*-indazole-1-carboxylate (26).** To a stirred mixture of **34f** (8.00 g, 29 mmol) and 10% palladium on activated charcoal (3.22 g, PE-type, N.E.Chemcat.) in ethanol (287 mL) was added concentrated hydrochloric acid (2.4 mL, 29 mmol). The reaction was evacuated, placed under a hydrogen atmosphere, and stirred for 1.5 h at 60 °C. The reaction mixture was cooled to room temperature, passed through a membrane filter, and the solvent was concentrated under reduced pressure to give 3-cyclobutyl-1*H*-indazol-6-ol hydrochloride (6.50 g). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.84-2.14 (2H, m), 2.25-2.42 (4H, m), 3.89 (1H, q, *J* = 8.7), 6.66 (1H, dd, *J* = 1.9, 8.7), 6.73 (1H, d, *J* = 1.9), 7.56 (1H, d, *J* = 8.7); LC/MS (ESI, $[M+H]^+$, *m/z*) 189. To a stirred solution of 3-cyclobutyl-1*H*-indazol-6-ol hydrochloride (6.45 g) and imidazole (5.04 g, 74 mmol) in anhydrous DMF (100 mL) was added TBDPSCl (18.4 mL, 72 mmol) at room temperature, and the mixture was stirred overnight. The reaction mixture was poured into water, and the aqueous layer was extracted twice with ethyl acetate. The combined organic layers were washed twice with water, washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue (27.90 g) was purified by flash column chromatography (95:5 to 74:26 n-hexane/ethyl acetate) to afford 6-((*tert*-butyldiphenylsilyl)oxy)-3-cyclobutyl-1*H*-indazole (9.93 g, 83% yield). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.10 (9H, s), 1.94-2.18 (2H, m), 2.39-2.49 (4H, m), 3.82 (1H, q, *J* = 8.8), 6.61 (1H, d, *J* = 2.0), 6.72 (1H, dd, *J* = 2.0, 8.8), 7.33-7.47 (7H, m), 7.72-7.75 (4H, m); LC/MS (ESI, $[M+H]^+$, *m/z*) 427. The 6-((*tert*-butyldiphenylsilyl)oxy)-3-cyclobutyl-1*H*-indazole (9.93 g, 23 mmol) was dissolved in anhydrous THF (233 mL). The solution was added triethylamine (3.9 mL, 28 mmol), Boc_2O (6.4 mL, 28 mmol) and 4-*N*,*N*-dimethylaminopyridine (1.51 g, 12 mmol) at room temperature, and the mixture was stirred for 4 h. The reaction mixture was concentrated under reduced pressure, and the residue was partitioned between ethyl acetate and 1 M HCl. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with water, washed with brine, and dried over Na_2SO_4. The organic layer was concentrated in vacuo to give *tert*-butyl 6-((*tert*-butyldiphenylsilyl)oxy)-3-cyclobutyl-1*H*-indazole-1-carboxylate (12.92 g). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.11 (9H, s), 1.42 (9H, s), 1.94-2.20 (2H, m), 2.34-2.60 (4H, m), 3.82 (1H, q, *J* = 8.8), 6.77 (1H, dd, *J* = 2.0, 8.8), 7.32-7.44 (8H, m), 7.70-7.74 (4H, m); LC/MS (ESI, $[M+H]^+$, *m/z*) 527. The *tert*-butyl 6-((*tert*-butyldiphenylsilyl)oxy)-3-cyclobutyl-1*H*-indazole-1-carboxylate (12.26 g, 23 mmol) was dissolved in THF (80 mL). The solution was added 1 mol/L TBAF-THF solution (46 mL, 46 mmol) at room temperature, and the mixture was stirred for 1 h. The reaction mixture was added water and brine. The aqueous layer was extracted twice with ethyl acetate. The combined organic layers were washed with water, washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue (15.1 g) was purified by flash column chromatography (95:5 to 74:26 n-hexane/ethyl acetate) to afford the product (6.45 g, 96% yield). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.63 (9H, s), 1.91-2.02 (1H, m), 2.06-2.21 (1H, m), 2.38-2.63 (4H, m), 3.87 (1H, q, *J* = 8.8), 6.86 (1H, dd, *J* = 2.1, 8.6), 7.54 (1H, d, *J* = 8.6), 7.55 (1H, brs); LC/MS (ESI, $[M+H]^+$, *m/z*) 289.

***tert*-Butyl 6-hydroxy-3-(trifluoromethyl)-1*H*-indazole-1-carboxylate (27).** To a stirred mixture of **47** (1.82 g, 9.1 mmol) and imidazole (1.36 g, 20 mmol) in anhydrous DMF (22 mL) was added TBDPSCl (5.14 mL, 20 mmol) at

room temperature, and the mixture was stirred overnight. The reaction mixture was added ethyl acetate. The organic layer was washed twice with water, washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue (22.86 g) was purified by flash column chromatography (97:3 to 76:24 n-hexane/ethyl acetate) to afford 6-((*tert*-butyldiphenylsilyl)oxy)-3-(trifluoromethyl)-1*H*-indazole (3.37 g, 83% yield). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.06 (9H, s), 6.76 (1H, d, *J* = 2.0), 6.93 (1H, dd, *J* = 2.0, 8.9), 7.37-7.54 (6H, m), 7.62 (1H, d, *J* = 8.9), 7.69-7.73 (4H, m), 13.49 (1H, brs). To a solution of 6-((*tert*-butyldiphenylsilyl)oxy)-3-(trifluoromethyl)-1*H*-indazole (3.35 g, 7.6 mmol), triethylamine (1.3 mL, 9.1 mmol) and Boc_2O (2.1 mL, 9.1 mmol) in anhydrous THF (35 mL) was added 4-*N*,*N*-dimethylaminopyridine (93 mg, 0.76 mmol) at room temperature, and the mixture was stirred overnight. The reaction mixture was added ethyl acetate, washed twice with 1 M HCl, washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue (4.48 g) was dissolved in THF (40 mL). The solution was added 1 mol/L TBAF-THF solution (12 mL, 12 mmol) at room temperature, and the mixture was stirred for 20 min. The reaction mixture was poured into brine and extracted with ethyl acetate. The organic layer was washed twice with water, washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue (4.30 g) was purified by flash column chromatography (86:24 to 65:35 n-hexane/ethyl acetate) to afford the product (1.65 g, 72%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.72 (9H, s), 5.39 (1H, s), 6.98 (1H, dd, *J* = 2.2, 8.8), 7.63 (1H, d, *J* = 2.2), 7.69 (1H, d, *J* = 8.8); LC/MS (ESI, $[M-H]^+$, *m/z*) 301.

***tert*-Butyl 6-hydroxy-3-phenyl-1*H*-indazole-1-carboxylate (28). 38b** (183 mg, 0.3 mmol), phenylboronic acid (78 mg, 0.6 mmol), $Pd_2(dba)_3$ (28 mg, 0.03 mmol), P(o-Tol)$_3$ (48 mg, 0.15 mmol) and K_3PO_4 (128 mg) were dissolved in DMF (1.4 mL) and water (0.14 mL) under a nitrogen atmosphere. The mixture was stirred for 2 h at 80 °C, and was then cooled at room temperature. The mixture was added ethyl acetate. The organic layer was washed with water, washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography (88:12 to 67:33 n-hexane/ethyl acetate) to give the product (79 mg, 83% yield). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.69 (9H, s), 6.92 (1H, dd, *J* = 2.2, 8.7), 7.40-7.50 (3H, m), 7.63 (1H, d, *J* = 2.0), 7.77 (1H, d, *J* = 8.7), 7.90-7.94 (2H, m). LC/MS (ESI, $[M+H]^+$, *m/z*) 311.

Ethyl 6-hydroxy-1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-indazole-3-carboxylate (29). To a solution of ethyl **50** (1.30 g, 2.5 mmol) in THF (12 mL) was added 1 mol/L TBAF-THF solution (3.7 mL, 3.7 mmol) at room temperature under a nitrogen atmosphere, and the mixture was stirred for 2 h. The reaction mixture was added ethyl acetate, washed three times with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue (2.95 g) was purified by flash column chromatography (100:0 to 81:19 n-hexane/ethyl acetate) to give the title compound (0.66 g, 92% yield). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.46 (3H, t, *J* = 7.1), 1.63-1.76 (3H, m), 2.03-2.11 (2H, m), 2.42-2.53 (1H, m), 3.67-3.75 (1H, m), 4.01-4.05 (1H, m), 4.49 (2H, q, *J* = 7.1), 5.40 (1H, brs), 5.69-5.74 (1H, m), 6.88 (1H, dd, *J* = 2.1, 8.8), 7.07 (1H, d, *J* = 2.1), 8.04 (1H, d, *J* = 8.8); LC/MS (ESI, $[M+H]^+$, *m/z*) 291.

Ethyl 6-(2-(*tert*-butoxycarbonyl-((2*R*)-2-(3-(*tert*-butoxycarbonyl(methylsulfonyl)amino)phenyl)-2-triethylsilyloxy-ethyl)amino)ethoxy)-1-tetrahydropyran-2-yl-indazole-3-carboxylate (30). To a stirred solution of **17** (506 mg, 0.86 mmol), **29** (124 mg, 0.43 mmol), and triphenylphosphine (229 mg, 0.87 mmol) in anhydrous THF

(4.3 mL) under nitrogen was added diethyl azodicarboxylate (0.39 mL, 0.86 mmol, 2.2 M toluene solution) at 0 °C. The resulting solution was allowed to warm to room temperature and was then stirred overnight. The solution was concentrated and purified by flash column chromatography on silica gel (85:15 to 64:36 n-hexane/ethyl acetate) to afford 238 mg (64% yield) of the title compound. ^{1}H-NMR (300 MHz, $CDCl_3$, 2:3 rotamers): δ 0.48-0.61 (6H, m), 0.87-0.97 (12H, m), 1.22-1.33 (2H, m), 1.43-1.52 (18H, m), 1.64-1.78 (3H, m), 2.04-2.14 (2H, m), 2.47-2.59 (1H, m), 3.22-3.62 (6H, m), 3.71-3.78 (1H, m), 4.01-4.07 (2H, m), 4.48 (2H, q, J = 7.1), 4.95-4.99 and 5.10-5.15 (1H, each m), 5.72-5.77 (1H, m), 6.89-7.00 (2H, m), 7.12-7.44 (4H, m), 7.98-8.04 (1H, m); LC/MS (ESI, $[M+H]^+$, *m/z*) 861.

4-(*tert*-Butyldimethylsilyloxy)-2-fluorobenzonitrile (32). To a stirred mixture of 2-fluoro-4-hydroxybenzonitrile **31** (30.13 g, 220 mmol) and imidazole (18.37 g, 270 mmol) in anhydrous DMF (436 mL) was added TBSCl (48.32 g, 320 mmol) at 0 °C. The resulting solution was allowed to warm to room temperature, and the mixture was stirred for 1 h. The mixture was concentrated under reduced pressure and remaining oil was partitioned between ethyl acetate and water. The aqueous layer was extracted twice with ethyl acetate. The combined organic layers were washed twice with water, washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (100:0 to 94:6 n-hexane/ethyl acetate) to give the product (40.36 g, 73% yield). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.25 (6H, s), 0.98 (9H, s), 6.64 (1H, dd, J = 2.2, 10.5), 6.69 (1H, dd, J = 2.2, 8.4), 7.47 (1H, t, J = 8.4).

1-(2-Fluoro-4-hydroxyphenyl)propan-1-one (33b). 32 (10.06 g, 40 mmol) was dissolved in anhydrous diethyl ether (100 mL) under an argon atmosphere, and the solution was added 3 mol/L ethylmagnesium bromide-diethyl ether solution (35 mL, 105 mmol) at room temperature. The reaction mixture was stirred for 20 min at room temperature, and the mixture was stirred for 1.5 h at reflux. The mixture was added water (36 mL) and 5 M HCl (36 mL) at 0 °C. The mixture was stirred overnight at reflux. The aqueous layer was extracted three times with ethyl acetate. The combined organic layers were washed with water, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was dissolved in anhydrous THF (100 mL), and the solution was added 1 mol/L TBAF-THF solution (31.5 mL, 31.5 mmol) at room temperature. The reaction mixture was stirred for 20 min, and the mixture was added brine. The aqueous layer was extracted three times with ethyl acetate. The combined organic layers were washed with water, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was dissolved in diethyl ether, and the solution was extracted with 2 M NaOH. The aqueous layer was washed three times with diethyl ether and added 2 M HCl. The aqueous layer was extracted twice with ethyl acetate. The combined organic layers were washed with water, washed with brine, and dried over Na_2SO_4. The solvent was evaporated under reduced pressure to give the title compound (5.63 g, 84 %). ^{1}H-NMR (300 MHz, $CDCl_3$+DMSO-d_6): δ 1.14 (3H, t, J = 7.2), 2.90 (2H, qd, J = 3.3, 7.2), 6.55 (1H, dd, J = 2.2, 13.3), 6.67 (1H, dd, J = 2.2, 8.8), 7.74 (1H, d, J = 8.8), 10.22 (1H, brs); LC/MS (ESI, $[M-H]^+$, *m/z*) 167.

1-(2-Fluoro-4-hydroxyphenyl)-2-methylpropan-1-one (33c). 32 (14.02 g, 56 mmol) was dissolved in anhydrous THF (5 mL) under an argon atmosphere, and the solution was added 0.78 mol/L isopropylmagnesium

bromide-THF solution (89 mL, 70 mmol) at room temperature. The reaction mixture was stirred for 20 min at room temperature. The mixture was added CuBr (140 mg), and stirred for 1.5 h at 60 °C. The reaction mixture was cooled to 0 °C, and added water (21 mL) and 5 M HCl (21 mL). The mixture was stirred for 6 h at 60 °C. The reaction mixture was then added 5 M HCl (21 mL), and stirred for 13 h at 60 °C. The reaction mixture was cooled at room temperature. The aqueous layer was extracted three times with ethyl acetate. The combined organic layers were washed with water, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was added *n*-hexane, and the precipitates were collected by suction filtration to afford the product (7.91 g, 79% yield). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.07 (6H, d, J = 6.8), 3.31 (1H, septetdoublet, J = 0.8, 6.8), 6.62 (1H, dd, J = 2.3, 13.6), 6.71 (1H, dd, J = 2.3, 8.7), 7.70 (1H, d, J = 8.7); LC/MS (ESI, $[M-H]^+$, *m/z*) 181.

1-(2-Fluoro-4-hydroxyphenyl)-2,2-dimethylpropan-1-one (33d). 32 (1.60 g, 6.2 mmol) was added 1.01 mol/L *tert*-butylmagnesium chloride-THF solution (15.6 mL, 15 mmol) at room temperature under an argon atmosphere. The reaction mixture was stirred for 15 min at room temperature. The mixture was added CuBr (16 mg), and stirred for 1.5 h at 60 °C. The reaction mixture was cooled to room temperature, and added water (6 mL) and 5 M HCl (9 mL). The mixture was stirred overnight at 60 °C. The reaction mixture was then cooled at room temperature. The aqueous layer was extracted twice with ethyl acetate. The combined organic layers were washed with water, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was dissolved in anhydrous THF (22 mL), and the solution was added 1 mol/L TBAF-THF solution (6.2 mL, 6.2 mmol) at room temperature. The mixture was stirred for 1 h, and then added brine. The aqueous layer was extracted twice with ethyl acetate. The combined organic layers were washed with water, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography (15:1 to 5:1 n-hexane/ethyl acetate) to afford the product (1.13 g, 92% yield). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.25 (9H, d, J = 0.7), 6.55 (1H, dd, J = 2.2, 11.1), 6.59 (1H, dd, J = 2.2, 8.1), 7.11 (1H, t, J = 8.1); LC/MS (ESI, $[M-H]^+$, *m/z*) 195.

Cyclopropyl(2-fluoro-4-hydroxyphenyl)methanone (33e). 32 (10.00 g, 40 mmol) was dissolved in anhydrous THF (5 mL) under an argon atmosphere, and the solution was added 1 mol/L cyclopropylmagnesium bromide-THF solution (80 mL, 80 mmol) at 0 °C. The reaction mixture was stirred for 15 min at room temperature. The mixture was then stirred for 1.5 h at 60 °C. The reaction mixture was cooled to 0 °C, and added water (50 mL) and 5 M HCl (50 mL). After the reaction mixture was stirred overnight at 60 °C, the mixture was cooled at room temperature. The aqueous layer was extracted three times with ethyl acetate. The combined organic layers were washed with water, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was added *n*-hexane, and the precipitates were collected by suction filtration to afford the product (6.52 g, 90% yield). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 0.97-1.04 (4H, m), 2.56-2.65 (1H, m), 6.63 (1H, dd, J = 2.2, 13.5), 6.70 (1H, dd, J = 2.2, 8.6), 7.67 (1H, t, J = 8.6); LC/MS (ESI, $[M-H]^+$, *m/z*) 179.

Cyclobutyl(2-fluoro-4-hydroxyphenyl)methanone (33f). Preparation of a cyclobutylmagnesium bromide-diethyl ether solution: To a stirred mixture of magnesium (9.18 g, 378 mmol) in anhydrous diethyl ether (20 mL) was added a catalytic amount of iodine and anhydrous diethyl ether (10 mL). The mixture was stirred for 15 min at

room temperature. The mixture was then added a catalytic amount of dibromoethane and anhydrous diethyl ether (20 mL). The mixture was stirred for 15 min at room temperature. The reaction mixture was added bromocyclobutane (7.0 mL, 74 mmol) dissolved in anhydrous diethyl ether (30 mL). The mixture was stirred for 15 min and was then used directly in the next step. **32** (9.49 g, 38 mmol) was dissolved in anhydrous THF (30 mL) under an argon atmosphere, and the solution was added cyclobutylmagnesium bromide-diethyl ether solution (60 mL) at room temperature. The reaction mixture was stirred for 15 min at room temperature. The mixture was added CuBr (140 mg), and was stirred for 0.5 h at 60 °C. The reaction mixture was cooled to 0 °C, and added water (30 mL) and 5 M HCl (30 mL). After the reaction mixture was stirred for 1 h at 60 °C, the mixture was cooled at room temperature. The aqueous layer was extracted three times with ethyl acetate. The combined organic layers were washed with water, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was dissolved in anhydrous THF (76 mL), and the solution was added 1 mol/L TBAF-THF solution (38 mL, 38 mmol) at room temperature. The mixture was stirred for 5 min, and was added water and brine. The aqueous layer was extracted twice with ethyl acetate. The combined organic layers were washed with water, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was dissolved in diethyl ether, and the solution was extracted with 2 M NaOH. The aqueous layer was washed six times with diethyl ether, and was added 2 M HCl. The aqueous layer was extracted twice with ethyl acetate. The combined organic layers were washed with water, washed with brine, and dried over Na_2SO_4. The solvent was evaporated under reduced pressure to give the title compound (6.97 g, 95% yield). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.71-1.82 (1H, m), 1.89-2.04 (1H, m), 2.15-2.23 (4H, m), 3.76-3.88 (1H, m), 6.61 (1H, dd, J = 2.2, 13.5), 6.72 (1H, dd, J = 2.2, 8.9), 7.73 (1H, t, J = 8.9), 10.80 (1H, brs); LC/MS (ESI, $[M+H]^+$, *m/z*) 195.

1-Benzyl-3-methyl-1*H*-indazol-6-ol (34a). To a stirred mixture of 1-(2-Fluoro-4-hydroxyphenyl)ethanone **33a** (771 mg, 5 mmol) and sodium acetate (1.23 g, 15 mmol) in anhydrous xylene (35 mL) was added benzylhydrazine dihydrochloride (1.19 g, 7.5 mmol). The mixture was stirred overnight at 160 °C. The reaction mixture was cooled to room temperature and added water (15 mL) and n-hexane (15 mL). The resultant solid was collected by suction filtration and washed with toluene and n-hexane to afford 1.125 g (94% yield) of the title compound. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.52 (3H, s), 5.39 (2H, s), 5.77 (1H, brs), 6.59 (1H, d, J = 2.0), 6.68 (1H, dd, J = 2.0, 8.6), 7.13-7.15 (2H, m), 7.19-7.28 (m, 3H), 7.49 (1H, d, J = 8.6); LC/MS (ESI, $[M+H]^+$, *m/z*) 239.

1-Benzyl-3-ethyl-1*H*-indazol-6-ol (34b). To a stirred mixture of **33b** (5.37 g, 32 mmol) and sodium acetate (2.75 g, 155 mmol) in anhydrous xylene (76 mL) was added benzylhydrazine dihydrochloride (9.45 g, 48 mmol). The mixture was stirred overnight at reflux using a Dean-Stark apparatus. The reaction mixture was cooled to room temperature, and added water (50 mL). The aqueous layer was extracted twice with ethyl acetate. The combined organic layers were washed twice with water, washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. Before completely evaporating the solvent, the precipitates were filtered, and the solids were washed with n-hexane to give the title compound (4.76 g, 59% yield). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.28 (3H, t, J = 7.6), 2.84 (2H, q, J = 7.6), 5.42 (2H, s), 6.62 (1H, dd, J = 1.9, 8.7), 6.72 (1H, d, J = 1.9) 7.14-7.17 (2H, m), 7.21-7.40 (3H, m), 7.51 (1H, d, J = 8.7), 9.58 (1H, brs); LC/MS (ESI, $[M+H]^+$, *m/z*) 253.

1-Benzyl-3-isopropyl-1*H*-indazol-6-ol (34c). To a stirred mixture of **33c** (0.76 g, 4.3 mmol) and sodium acetate (1.72 g, 21 mmol) in anhydrous xylene (43 mL) was added benzylhydrazine dihydrochloride (1.25 g, 48 mmol). The mixture was stirred overnight at reflux. The reaction mixture was cooled to room temperature and added water. The aqueous layer was extracted twice with ethyl acetate. The combined organic layers were washed twice with water, washed with brine, and dried over anhydrous Na_2SO_4. The organic layer was concentrated under reduced pressure. The residue was added *n*-hexane, and the precipitates were collected by suction filtration to afford the product (0.91 g, 80% yield). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.35 (6H, d, *J* = 6.9), 3.26 (1H, septet, *J* = 6.9), 5.42 (2H, s), 6.61 (1H, dd, *J* = 1.9, 8.7), 6.70 (1H, d, *J* = 1.7), 7.12-7.14 (2H, m), 7.20-7.32 (3H, m), 7.56 (1H, d, *J* = 8.7), 9.57 (1H, brs); LC/MS (ESI, $[M+H]^+$, *m/z*) 267.

1-Benzyl-3-(*tert*-butyl)-1*H*-indazol-6-ol (34d). To a stirred mixture of **33d** (1.13 g, 5.9 mmol) and sodium acetate (2.31 g, 28 mmol) in anhydrous xylene (29 mL) was added benzylhydrazine dihydrochloride (1.72 g, 8.8 mmol). The mixture was stirred overnight at reflux using a Dean-Stark apparatus. The reaction mixture was cooled to room temperature, and added water. The aqueous layer was extracted twice with ethyl acetate. The combined organic layers were washed twice with water, washed with brine, and dried over anhydrous Na_2SO_4. The organic layer was concentrated under reduced pressure. The residue was added *n*-hexane, and the precipitates were collected by suction filtration to afford the product (1.51 g, 91% yield). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 0.69 (9H, s), 4.62 (2H, s), 5.79-5.80 (1H, m), 5.84 (1H, dd, *J* = 2.0, 8.8), 6.27-6.29 (2H, m), 6.33-6.55 (5H, m), 6.90 (1H, d, *J* = 8.8); LC/MS (ESI, $[M-H]^+$, *m/z*) 279.

1-Benzyl-3-cyclopropyl-1*H*-indazol-6-ol (34e). To a stirred mixture of **33e** (1.20 g, 6.7 mmol) and sodium acetate (2.62 g, 32 mmol) in anhydrous xylene (61 mL) was added benzylhydrazine dihydrochloride (1.95 g, 10 mmol). The mixture was stirred overnight at reflux using a Dean-Stark apparatus. The reaction mixture was cooled to room temperature, and added water. The aqueous layer was extracted twice with ethyl acetate. The combined organic layers were washed with water, washed with brine, and dried over anhydrous Na_2SO_4. The organic layer was concentrated under reduced pressure. The residue was added *n*-hexane/ethyl acetate=10/1, and the precipitates were collected by suction filtration to afford the product (0.89 g, 51% yield). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 0.87-0.99 (4H, m), 2.14-2.26 (1H, m), 5.38 (2H, s), 6.61 (1H, dd, *J* = 1.8, 8.7), 6.69 (1H, d, *J* = 1.8), 7.11-7.13 (2H, m), 7.20-7.31 (3H, m), 7.53 (1H, d, *J* = 8.7), 9.57 (1H, brs); LC/MS (ESI, $[M-H]^+$, *m/z*) 263.

1-Benzyl-3-cyclobutyl-1*H*-indazol-6-ol (34f). To a stirred mixture of **33f** (6.97 g, 36 mmol) and sodium acetate (14.16 g, 172 mmol) in anhydrous xylene (85 mL) was added benzylhydrazine dihydrochloride (10.55 g, 54 mmol). The mixture was stirred overnight at reflux using a Dean-Stark apparatus. The reaction mixture was cooled to room temperature, and the precipitates were collected by suction filtration. The solid was dissolved in ethyl acetate. The organic layer was washed with water, washed with brine, and was dried over Na_2SO_4. The solvent was evaporated under reduced pressure to give the title compound to afford the product (8.00 g, 80% yield). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.89-2.12 (2H, m), 2.32-2.41 (4H, m), 3.81 (1H, q, *J* = 8.7), 5.44 (2H, s), 6.62 (1H, dd, *J* = 1.9, 8.7),

6.72 (1H, d, J = 1.9), 7.14-7.17 (2H, m), 7.21-7.32 (3H, m), 7.51 (1H, d, J = 8.7), 9.59 (1H, brs); LC/MS (ESI, $[M+H]^+$, *m/z*) 279.

6-((*tert*-Butyldiphenylsilyl)oxy)-1*H*-indazole (36). 1*H*-indazol-6-amine **35** (24.33 g, 181 mmol) and 48% HBF_4 solution (242 mL) were dissolved in water (100 mL) and cooled to 0 °C. A solution of sodium nitrite (13.87 g, 201 mmol) in water (20 mL) was added dropwise to the reaction mixture at 0 °C. The mixture was stirred for 30 min at 0 °C. The precipitates were collected by suction filtration, and washed with chloroform. The solid was dissolved in acetic acid (250 mL) and was stirred for 10 min at 50 °C, 10 min at 110 °C, and then 10 min at 130 °C. The solution was cooled at room temperature, and added saturated Na_2CO_3 solution. The aqueous layer was extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was dissolved in ethanol (240 mL). The solution was added 2 M NaOH (365 mL) at room temperature, and the mixture was stirred for 1 h. The mixture was concentrated under reduced pressure. After neutralization with 2 M HCl (200 mL) and NH_4Cl solution until pH 7, the mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was added chloroform, and the precipitates were collected by suction filtration to give 1*H*-indazol-6-ol (13.54 g, 56% yield). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 6.64 (1H, dd, J = 2.0, 8.6), 6.77 (1H, s), 7.52 (1H, d, J = 8.6), 7.85 (1H, d, J = 0.5), 9.67 (1H, s), 12.56 (1H, s); LC/MS (ESI, $[M+H]^+$, *m/z*) 135. To a stirred mixture of 1*H*-indazol-6-ol (4.03 g, 30 mmol) and imidazole (4.49 g, 66 mmol) in anhydrous DMF (60 mL) was added TBDPSCl (17.1 mL, 66 mmol) at room temperature, and the mixture was stirred overnight. The reaction mixture was poured into water, and the aqueous layer was extracted twice with ethyl acetate. The combined organic layers were washed three times with water, dried over $MgSO_4$, and concentrated in vacuo. The residue (22.86 g) was purified by flash column chromatography (92:8 to 71:29 n-hexane/ethyl acetate) to afford the title compound (9.21 g, 82% yield). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.11 (9H, s), 6.66-6.67 (1H, m), 6.78 (1H, dd, J = 2.0, 8.7), 7.33-7.45 (6H, m), 7.49 (1H, dd, J = 0.4, 8.7), 7.71-7.74 (4H, m), 7.88 (1H, s); LC/MS (ESI, $[M+H]^+$, *m/z*) 373.

6-((*tert*-Butyldiphenylsilyl)oxy)-3-chloro-1*H*-indazole (37a). 36 (29.25 g, 79 mmol) was dissolved in anhydrous THF (200 mL) under a nitrogen atmosphere, and was cooled to 0 °C. The solution was added potassium *tert*-butoxide (18.22 g, 162 mmol) and *N*-chlorosuccinimide (17.05 g, 128 mmol) at 0 °C. The mixture was allowed to warm to room temperature, and was stirred for 4 h. The reaction mixture was added a saturated NH_4Cl solution, and the aqueous layer was extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue (22.86 g) was purified by flash column chromatography (88:12 to 67:33 n-hexane/ethyl acetate) to afford the product (18.59 g, 57% yield). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.11 (9H, s), 6.60 (1H, d, J = 2.0), 6.83 (1H, dd, J = 2.0, 8.8), 7.33-7.47 (7H, m), 7.70-7.74 (4H, m), 9.54 (1H, brs); LC/MS (ESI, $[M+H]^+$, *m/z*) 407.

6-((*tert*-Butyldiphenylsilyl)oxy)-3-iodo-1*H*-indazole (37b). 36 (9.21 g, 25 mmol) was dissolved in anhydrous THF (247 mL) under a nitrogen atmosphere and was cooled to 0 °C. The solution was added potassium *tert*-butoxide (5.56 g, 50 mmol) and iodine (12.62 g, 50 mmol) at 0 °C. The mixture was allowed to warm to room

temperature, and was stirred for 40 min. The reaction mixture was added a solution of sodium thiosulfate, and the aqueous layer was extracted twice with ethyl acetate. The combined organic layers were washed twice with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography (88:12 to 67:33 n-hexane/ethyl acetate) to afford the product (11.05 g, 90% yield). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.10 (9H, s), 6.71-6.72 (1H, m), 6.84 (1H, dd, *J* = 1.9, 8.8), 7.24 (1H, d, *J* = 9.3), 7.32-7.45 (6H, m), 7.70-7.73 (4H, m), 10.69 (1H, brs); LC/MS (ESI, $[M+H]^+$, *m/z*) 499.

***tert*-Butyl 6-((*tert*-butyldiphenylsilyl)oxy)-3-chloro-1*H*-indazole-1-carboxylate (38a).** To a solution of **37a** (18.46 g, 46 mmol), triethylamine (7.7 mL, 55 mmol) and Boc_2O (12.56 g, 58 mmol) in anhydrous THF (200 mL) was added 4-*N*,*N*-dimethylaminopyridine (0.55 g, 4.6 mmol) at room temperature, and the mixture was stirred overnight. The reaction mixture was added ethyl acetate. The organic layer was washed twice with 1 M HCl, washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column chromatography (97:3 to 80:20 n-hexane/ethyl acetate) to give the product (17.51 g, 75% yield). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.12 (9H, s), 1.48 (9H, s), 6.83 (1H, dd, *J* = 1.9, 8.6), 7.34-7.43 (7H, m), 7.70-7.73 (5H, m).

***tert*-Butyl 6-((*tert*-butyldiphenylsilyl)oxy)-3-iodo-1*H*-indazole-1-carboxylate (38b).** To a solution of **37b** (2.43 g, 5 mmol), triethylamine (0.77 mL, 6 mmol) and Boc_2O (1.37 mL, 6 mmol) in anhydrous acetonitrile (25 mL) was added 4-*N*,*N*-dimethylaminopyridine (0.61 g, 5 mmol) at room temperature, and the mixture was stirred overnight. The reaction mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography (100:0 to 87:13 n-hexane/ethyl acetate) to give the product (2.30 g, 76% yield). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.11 (9H, s), 1.47 (9H, s), 6.83 (1H, dd, *J* = 2.1, 8.7), 7.17 (1H, d, *J* = 8.7), 7.32-7.45 (6H, m), 7.69-7.72 (5H, m).

6-(Benzyloxy)-1*H*-indazol-3-ol (40). To a stirred mixture of methyl 2-fluoro-4-hydroxybenzoate **39** (1.47 g, 8.6 mmol) and K_2CO_3 powder (3.69 g, 27 mmol) in anhydrous DMF (21 mL) was added benzyl bromide (1.2 mL 10 mmol) at room temperature under a nitrogen atmosphere, and the mixture was stirred overnight at 50 °C. The reaction mixture was poured into water, and the aqueous layer was extracted twice with ethyl acetate. The combined organic layers were washed twice with water, washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue (2.42 g) was purified by flash column chromatography (97:3 to 77:23 n-hexane/ethyl acetate) to afford methyl 4-(benzyloxy)-2-fluorobenzoate (2.20 g, 98% yield). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 3.89 (3H, s), 5.09 (2H, s), 6.70 (1H, dd, *J* = 2.4, 12.6), 6.79 (1H, dd, *J* = 2.4, 8.8), 7.33-7.41 (5H, m), 7.89 (1H, t, *J* = 8.8). The methyl 4-(benzyloxy)-2-fluorobenzoate (1.72 g, 6.6 mmol) was dissolved in *n*-butanol (32.5 mL). The solution was added hydrazine monohydrate (3.14 mL, 65 mmol) and was irradiated in a microwave for 1 h at 160 °C. The reaction mixture was cooled and the precipitates were collected by suction filtration to give the product (1.25 g, 79% yield). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 5.13 (2H, s), 6.67 (1H, dd, *J* = 2.0, 8.7), 6.75 (1H, d, *J* = 2.0), 7.30-7.49 (6H, m); LC/MS (ESI, $[M+H]^+$, *m/z*) 241.

***tert*-Butyl 6-(benzyloxy)-3-hydroxy-1H-indazole-1-carboxylate (41).** To a solution of **40** (1.92 g, 8 mmol),

triethylamine (2.8 mL, 20 mmol) and Boc_2O (4.6 mL, 20 mmol) in dichloromethane (80 mL) was added 4-*N*,*N*-dimethylaminopyridine (0.49 g, 4 mmol) at room temperature under a nitrogen atmosphere, and the mixture was stirred overnight. The reaction mixture was washed twice with 1 M HCl, washed with water, dried over $MgSO_4$, and concentrated in vacuo. The residue (3.65 g) was dissolved in methanol (64 mL). The solution was added 7 M NH_3-MeOH (16 mL) at room temperature, and was stirred for 4 h. The reaction mixture was concentrated under reduced pressure. The residue was added ethanol, and the precipitates were collected by suction filtration to give the product (1.58 g, 58% yield). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.70 (9H, s), 5.15 (2H, s), 6.96 (1H, dd, *J* = 2.1, 8.7), 7.33-7.47 (6H, m), 7.69 (1H, d, *J* = 8.7); LC/MS (ESI, $[M+H]^+$, *m/z*) 341.

***tert*-Butyl 6-(benzyloxy)-3-methoxy-1*H*-indazole-1-carboxylate (42a).** A mixture of **41** (207 mg, 0.6 mmol), Ag_2CO_3 (509 mg, 1.8 mmol) and methyl iodide (0.37 mL, 6 mmol) in anhydrous toluene (6 mL) was irradiated in a microwave for 2 h at 60 °C. The reaction mixture was filtered and concentrated in vacuo. The residue (226 mg) was purified by flash column chromatography (99:1 to 78:22 n-hexane/ethyl acetate) to afford the title compound (153 mg, 72% yield). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.68 (9H, s), 4.14 (3H, s), 5.13 (2H, s), 6.94 (1H, dd, *J* = 2.2, 8.7), 7.31-7.47 (5H, m), 7.51 (1H, d, *J* = 8.7), 7.57 (1H, brs); LC/MS (ESI, $[M+H]^+$, *m/z*) 355.

***tert*-Butyl 6-(benzyloxy)-3-ethoxy-1*H*-indazole-1-carboxylate (42b).** A mixture of **41** (69 mg, 0.2 mmol), Ag_2CO_3 (168 mg, 0.6 mmol) and ethyl iodide (0.16 mL, 2 mmol) in anhydrous toluene (2 mL) was irradiated in a microwave for 2 h at 90 °C. The reaction mixture was purified by flash column chromatography (99:1 to 78:22 n-hexane/ethyl acetate) to afford the title compound (68 mg, 90% yield). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.47 (3H, t, *J* = 7.1), 1.70 (9H, s), 4.52 (2H, q, *J* = 7.1), 5.13 (2H, s), 6.94 (1H, dd, *J* = 2.2, 8.7), 7.31-7.46 (5H, m), 7.53 (1H, d, *J* = 8.7), 7.56 (1H, brs); LC/MS (ESI, $[M+H]^+$, *m/z*) 369.

1-(Bromomethyl)-4-methoxy-2-nitrobenzene (44). To a stirred mixture of 4-methoxy-1-methyl-2-nitrobenzene **43** (2.7 mL, 20 mmol) and *N*-bromosuccinimide (4.05 g, 23 mmol) in CCl_4 (20 mL) was added 70% benzoyl peroxide (348 mg, 1 mmol) at room temperature under a nitrogen atmosphere, and the mixture was stirred for 3 h at reflux. The reaction mixture was filtered and the reaction solution was washed with saturated aq $NaHCO_3$, washed with water, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column chromatography (94:6 to 74:27 n-hexane/ethyl acetate) to give the title compound (3.25 g, 66% yield). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 3.88 (3H, s), 4.80 (2H, s), 7.13 (1H, dd, *J* = 2.7, 8.6), 7.46 (1H, d, *J* = 8.6), 7.56 (1H, d, *J* = 2.7).

4-Methoxy-2-nitro-1-(2,2,2-trifluoroethyl)benzene (45). To a stirred mixture of **44** (2.71 g, 11 mmol) and 2,2-difluoro-2-(fluorosulfonyl)acetate (3.1 mL, 24 mmol) in DMF (22 mL) was added CuI (0.52 g, 2.8 mmol) at room temperature under a nitrogen atmosphere, and the mixture was stirred for 4 h at 100 °C. The reaction mixture was added ethyl acetate, and the organic layer was washed with 28% NH_3 solution, brine and water. The organic layer was dried over $MgSO_4$, and concentrated in vacuo. The residue (2.95 g) was purified by flash column chromatography (97:3 to 77:23 n-hexane/ethyl acetate) to give the title compound (1.58 g, 61% yield). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 3.83 (2H, q, *J* = 10.4), 3.89 (3H, s), 7.14 (1H, dd, *J* = 2.7, 8.6), 7.35 (1H, d, *J* = 8.6), 7.52

(1H, d, J = 2.7).

1-(6-Methoxy-3-(trifluoromethyl)-*1H*-indazol-1-yl)ethan-1-one (46). A stirred mixture of **45** (1.81 g, 7.7 mmol) and 5% palladium on activated charcoal (0.93 g, STD-type, N.E.Chemcat.) in methanol (30 mL) was evacuated, placed under a hydrogen atmosphere, and stirred overnight at room temperature. The reaction mixture was added 5% palladium on activated charcoal (2.30 g, STD-type, N.E.Chemcat.), and was then evacuated and placed under a hydrogen atmosphere, and stirred for 9 h at room temperature. The reaction mixture was passed through a membrane filter, and the solvent was concentrated under reduced pressure to give 5-methoxy-2-(2,2,2-trifluoroethyl)benzenamine (1.41 g, 90% yield). LC/MS (ESI, $[M+H]^+$, *m/z*) 206. To a stirred mixture of 5-methoxy-2-(2,2,2-trifluoroethyl)benzenamine (1.40 g, 6.9 mmol) and potassium acetate (1.70 g, 17 mmol) in chlorobenzene (23 mL) was added acetic anhydride (3.26 mL, 35 mmol) at room temperature, and the mixture was stirred for 20 min at 80 °C. The reaction mixture was added isoamyl nitrite (2.8 mL, 21 mmol), and was stirred for 15 h at 80 °C. This mixture was added isoamyl nitrite (1 mL, 7.5 mmol), and was stirred for 4 h at 80 °C. The reaction mixture was partitioned between saturated aq $NaHCO_3$ and ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue (1.96 g) was purified by flash column chromatography (100:0 to 80:20 n-hexane/ethyl acetate) to give the title compound (1.61 g, 90% yield). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.81 (3H, s), 3.93 (3H, s), 7.06 (1H, dd, J = 2.2, 8.9), 7.67 (1H, d, J = 8.9), 7.91 (1H, d, J = 2.2); LC/MS (ESI, $[M+H]^+$, *m/z*) 259.

3-(Trifluoromethyl)-1*H*-indazol-6-ol (47). A mixture of **46** (2.58 g, 10 mmol) in 48% hydrobromic acid (100 mL) was stirred overnight at 110 °C. After neutralization with 2 M NaOH until pH 7 the mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo to give the product (1.86 g, 92% yield). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 6.85 (1H, dd, J = 2.0, 8.8), 6.88 (1H, d, J = 2.0), 7.57 (1H, d, J = 8.8), 9.98 (1H, brs), 13.46 (1H, brs); LC/MS (ESI, $[M+H]^+$, *m/z*) 203.

Ethyl 6-((*tert*-butyldiphenylsilyl)oxy)-1*H*-indazole-3-carboxylate (49). A mixture of 6-methoxy-1*H*-indazole-3-carboxylic acid **48** (1.01 g, 5.2 mmol) in 48% hydrobromic acid (52 mL) was stirred overnight at reflux. The reaction mixture was concentrated in vacuo to give 6-methoxy-1*H*-indazole-3-carboxylic acid (1.50 g) which was used without further purification; LC/MS (ESI, $[M+H]^+$, *m/z*) 179. The 6-methoxy-1*H*-indazole-3-carboxylic acid (1.50 g) was dissolved in ethanol (52 mL). The solution was added dropwise thionyl chloride (7.6 mL, 104 mmol) at 0 °C, and the mixture was stirred overnight at 60 °C. The reaction mixture was concentrated under reduced pressure to give ethyl 6-hydroxy-1*H*-indazole-3-carboxylate (1.46 g) which was used without further purification; LC/MS (ESI, $[M+H]^+$, *m/z*) 207. The ethyl 6-hydroxy-1*H*-indazole-3-carboxylate (1.46 g) was dissolved in anhydrous DMF (16 mL). The solution was added imidazole (1.43 g, 21 mmol) and TBDPSCl (4.06 mL, 16 mmol) at room temperature, and was stirred overnight. The reaction mixture was poured into saturated aq $NaHCO_3$ and extracted twice with ethyl acetate. The combined organic layers were washed with brine, washed twice with water, dried over $MgSO_4$, and concentrated in vacuo. The residue (5.51 g) was purified by flash column chromatography (81:19 to 60:40 n-hexane/ethyl acetate) to afford the title compound (1.47 g, 63% yield). ^{1}H-NMR (300 MHz,

$CDCl_3$): δ 1.12 (9H, s), 1.32 (3H, t, *J* = 7.1), 4.30 (2H, q, *J* = 7.1), 6.88-6.94 (2H, m), 7.29-7.38 (6H, m), 7.70-7.75 (4H, m), 7.91 (1H, d, *J* = 8.8), 11.99 (1H, brs); LC/MS (ESI, $[M+H]^+$, *m/z*) 445.

Ethyl 6-((*tert*-butyldiphenylsilyl)oxy)-1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-indazole-3-carboxylate (50). To a stirred mixture of **49** (1.46 g, 3.3 mmol) and 3,4-dihydro-2H-pyrane (0.6 mL, 6.6 mmol) in toluene (17 mL) was added *p*-toluenesulfonic acid monohydrate (0.13 g, 0.7 mmol) at room temperature under a nitrogen atmosphere, and the mixture was stirred overnight at 60 °C. The reaction mixture was poured into saturated aq $NaHCO_3$ and extracted with ethyl acetate. The organic layer was washed with water, washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography (96:4 to 75:25 n-hexane/ethyl acetate) to afford the title compound (1.33 g, 76%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.14 (9H, s), 1.42 (3H, t, *J* = 7.1), 1.47-1.57 (2H, m), 1.82-2.18 (3H, m), 3.46-3.54 (1H, m), 3.84-3.87 (1H, m), 4.45 (2H, q, *J* = 7.1), 5.48 (1H, dd, *J* = 2.7, 9.8), 6.86-6.91 (2H, m), 7.33-7.46 (6H, m), 7.72-7.77 (4H, m), 7.91 (1H, d, *J* = 8.7); LC/MS (ESI, $[M+H]^+$, *m/z*) 529.

***N*-Benzyl-*N*-(3-((1*R*)-2-(benzyl(2-hydroxyethyl)amino)-1-triethylsilyloxy-ethyl)phenyl)methanesulfonamide (53).** A mixture of *N*-benzyl-*N*-(3-((1*R*)-2-iodo-1-triethylsilyloxy-ethyl)phenyl)methanesulfonamide[30] **51** (77.99 g, 143 mmol) and 2-(benzylamino)ethanol **52** (119 mL, 795 mmol) was stirred overnight at 100 °C under a nitrogen atmosphere. The reaction mixture was added toluene (100 mL) and diethyl ether (500 mL), was washed three times with water, dried over $MgSO_4$, and concentrated in vacuo. The residue (2.95 g) was purified by column chromatography (2:1 to 81:19 n-hexane/ethyl acetate) to give the title compound (80.40 g, 99% yield). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.36-0.44 (6H, m), 0.79 (9H, t, *J* = 7.9), 2.49-2.80 (4H, m), 2.93 (3H, s), 3.37 (2H, brs), 3.58 (1H, d, *J* = 13.6), 3.66 (1H, d, *J* = 13.6), 3.82 (1H, d, *J* = 2.3), 4.49 (1H, d, *J* = 6.2), 4.80 (1H, d, *J* = 14.5), 4.89 (1H, d, *J* = 14.5), 7.11-7.34 (14H, m); LC/MS (ESI, $[M+H]^+$, *m/z*) 569.

(*R*)-N-(3-(1-Hydroxy-2-((2-((3-methyl-1H-indazol-6-yl)oxy)ethyl)amino)ethyl)phenyl)propane-1-sulfonamide (56a). To a solution of **62** (95 mg, 0.15 mmol) and pyridine (75 μL, 0.9 mmol) in anhydrous CH_2Cl_2 (1 mL) was added propane-1-sulfonyl chloride (86 mg, 0.6 mmol) at room temperature, and the solution was shaken (600 min^{-1}) overnight. The mixture was purified by flash column chromatography (4:3 n-hexane/ethyl acetate). The sulfonamide product was diluted with MTBE (100 μL) and 4 M HCl in 1,4-dioxane (1.5 mL, 6.0 mmol) was added at room temperature. The mixture was shaken (600 min^{-1}) overnight and nitrogen gas was blown into the reaction solution to evaporate the solvent. The residue was dried to give compound **56a** (40.9 mg, 0.095 mmol, 63% yield) as dihydrochloride salt. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 0.93 (3H, t, *J* = 7.6), 1.68 (2H, quin, *J* = 7.6), 2.44 (3H, s), 3.03-3.09 (1H, m), 3.07 (2H, t, *J* = 7.6), 3.23-3.27 (1H, m), 3.46 (2H, t, *J* = 5.1), 4.33-4.37 (2H, m), 4.99 (1H, dd, *J* = 2.2, 10.2), 6.21 (1H, brs), 6.77 (1H, dd, *J* = 1.8, 8.7), 6.90 (1H, d, *J* = 1.8), 7.11 (1H, d, *J* = 7.8), 7.15 (1H, dd, *J* = 1.3, 7.8), 7.30 (1H, s), 7.33 (1H, t, *J* = 7.8), 7.60 (1H, d, *J* = 8.7), 8.97 (1H, brs), 9.22 (1H, brs), 9.88 (1H, s); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 11.4, 12.5, 16.7, 45.9, 52.2, 53.6, 63.4, 67.9, 92.1, 111.6, 116.6, 117.1, 118.6, 120.9, 121.0, 129.3, 138.5, 140.7, 141.7, 143.0, 157.2; HRMS calculated for $C_{21}H_{28}N_4O_4S + H^+$, 433.1904, found ESI: $[M+H]^+$, 443.1897; LC/MS-ESI (*m/z*): $[M+H]^+$, 433; HPLC: purity 100%, R_T 2.0 min.

(*R*)-*N*-(3-(1-Hydroxy-2-((2-((3-methyl-1H-indazol-6-yl)oxy)ethyl)amino)ethyl)phenyl)propane-2-sulfonamide (56b). *Step 1.* To a solution of **62** (96 mg, 0.15 mmol) and pyridine (18 μL, 0.23 mmol) in anhydrous CH_2Cl_2 (2 mL) was added propane-2-sulfonyl chloride (26 mg, 0.18 mmol) at room temperature, and the solution was stirred overnight. To the mixture was added DBU (134 μL, 0.9 mmol) and propane-2-sulfonyl chloride (20 μL, 0.18 mmol) at room temperature. The mixture was stirred overnight and DBU (33.5 μL, 0.23 mmol) and propane-2-sulfonyl chloride (20 μL, 0.18 mmol) were added at room temperature. The mixture was stirred for 4 days and DBU (33.5 μL, 0.23 mmol) and propane-2-sulfonyl chloride (30 μL, 0.26 mmol) were added at room temperature. The mixture was stirred overnight and purified by flash column chromatography (81:19 to 60:40 n-hexane/ethyl acetate) to give a mixture of **62** and sulfonamide product (41.5 mg). The mixture was diluted with CH_2Cl_2 (1.5 mL) and 1.7 mmol/g MP-Isocyanate (118 mg, 0.2 mmol) was added at room temperature. The mixture was stirred overnight, filtered, and concentrated to give *tert*-butyl (*R*)-6-(2-((tert-butoxycarbonyl)(2-(3-((1-methylethyl)sulfonamido)phenyl)-2-((triethylsilyl)oxy)ethyl)amino)ethoxy)-3-methyl-1*H*-indazole-1-carboxylate (40 mg). LC/MS-ESI (*m/z*): $[M+H]^+$, 747.

Step 2. To *tert*-butyl (*R*)-6-(2-((tert-butoxycarbonyl)(2-(3-((1-methylethyl)sulfonamido)phenyl)-2-((triethylsilyl)-oxy)ethyl)amino)ethoxy)-3-methyl-1*H*-indazole-1-carboxylate (40 mg) was added 4 M HCl in 1,4-dioxane (1.5 mL, 6.0 mmol) at room temperature. The mixture was shaken (600 min^{-1}) overnight and nitrogen gas was blown into the reaction solution to evaporate the solvent. The residue was dried to give compound **56b** (28 mg, 0.055 mmol, 36% yield) as dihydrochloride salt. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.24 (6H, d, *J* = 6.8), 2.44 (3H, s), 3.02-3.09 (1H, m), 3.23 (1H, septet, *J* = 6.8), 3.24-3.26 (1H, m), 3.44-3.47 (2H, m), 4.31-4.40 (2H, m), 4.98 (1H, dd, *J* = 2.2, 10.3), 6.22 (1H, brs), 6.77 (1H, dd, *J* = 2.1, 8.7), 6.90 (1H, d, *J* = 2.1), 7.09 (1H, d, *J* = 7.7), 7.17 (1H, dd, *J* = 1.3, 8.1), 7.30-7.34 (2H, m), 7.60 (1H, d, *J* = 8.7), 8.95 (1H, brs), 9.20 (1H, brs), 9.85 (1H, s); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 11.4, 16.0, 45.9, 51.2, 53.6, 63.4, 68.0, 92.1, 111.6, 116.5, 117.1, 118.5, 120.8, 120.9, 129.3, 138.8, 140.8, 141.6, 143.0, 157.0; HRMS calculated for $C_{21}H_{28}N_4O_4S + H^+$, 433.1904, found ESI: $[M+H]^+$, 433.1895; LC/MS-ESI (*m/z*): $[M+H]^+$, 433; HPLC: purity 100%, R_T 1.9 min.

(*R*)-*N*-(3-(1-Hydroxy-2-((2-((3-methyl-1*H*-indazol-6-yl)oxy)ethyl)amino)ethyl)phenyl)cyclopropanesulfon-amide (56c). To a solution of **62** (96 mg, 0.15 mmol) and pyridine (18 μL, 0.23 mmol) in anhydrous CH_2Cl_2 (2 mL) was added cyclopropanesulfonyl chloride (25 mg, 0.18 mmol) at room temperature, and the solution was stirred overnight. To the mixture was added pyridine (24 μL, 0.3 mmol) and cyclopropanesulfonyl chloride (42 mg, 0.3 mmol) at room temperature. The mixture was stirred overnight and purified by flash column chromatography (4/3 n-hexane/ethyl acetate). The sulfonamide product was diluted with 1,4-dioxane (0.2 mL) to which was added 4 M HCl in 1,4-dioxane (1.6 mL, 6.4 mmol) at room temperature. The mixture was shaken (600 min^{-1}) overnight and nitrogen gas was blown into the reaction solution to evaporate the solvent. The residue was dried to give compound **56c** (63 mg, 0.12 mmol, 83% yield) as dihydrochloride salt. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 0.90-0.97 (4H, m), 2.44 (3H, s), 2.58-2.64 (1H, m), 3.03-3.10 (1H, m), 3.23-3.28 (1H, m), 3.46 (2H, t, *J* = 5.0), 4.32-4.44 (2H, m), 5.01 (1H, dd, *J* = 2.2, 10.3), 6.25 (1H, brs), 6.77 (1H, dd, *J* = 2.1, 8.8), 6.91 (1H, d, *J* = 2.1), 7.12 (1H, d, *J* = 7.8), 7.18 (1H, dd, *J* = 1.3, 7.8), 7.32-7.36 (2H, m), 7.60 (1H, d, *J* = 8.8), 8.99 (1H, brs), 9.26 (1H, brs), 9.82 (1H, s);

^{13}C-NMR (100 MHz, DMSO-d_6) δ 4.9, 11.5, 29.5, 45.9, 53.6, 63.4, 67.9, 92.1, 111.5, 117.2, 117.4, 119.4, 120.8, 121.1, 129.1, 138.5, 140.8, 141.7, 142.9, 157.1; HRMS calculated for $C_{21}H_{26}N_4O_4S$ + H^+, 431.1748, found ESI: $[M+H]^+$, 431.1754; LC/MS-ESI (*m/z*): $[M+H]^+$, 431; HPLC: purity 100%, R_T 1.9 min.

(*R*)-*N*-(3-(1-Hydroxy-2-((2-((3-methyl-1*H*-indazol-6-yl)oxy)ethyl)amino)ethyl)phenyl)cyclobutanesulfonamide (56d). *Step 1.* To a solution of **62** (14.95 g, 23.4 mmol) and pyridine (13.2 mL, 164 mmol) in anhydrous CH_2Cl_2 (100 mL) was added cyclobutanesulfonyl chloride (12.73 g, 81.9 mmol) at room temperature, and the solution was stirred overnight. The mixture was diluted with ethyl acetate, washed twice with 0.5 M HCl, washed with brine, dried over Mg_2SO_4, and concentrated in vacuo. The residue (28.72 g) was purified by flash column chromatography (73:27 to 52:48 n-hexane/ethyl acetate) to afford the sulfonamide product (11.14 g, 14.7 mmol, 63% yield). ^{1}H-NMR (300 MHz, $CDCl_3$, 1:1 rotamers) δ 0.54 (6H, q, *J* = 7.8), 0.88 (9H, t, *J* = 7.8), 1.23-1.31 (2H, m), 1.47 (9H, s), 1.71 (9H, s), 1.88-2.02 (2H, m), 2.19-2.23 (2H, m), 2.47-2.60 (5H, m), 3.21-4.16 (5H, m), 4.88-4.93 and 5.02-5.06 (1H, each m), 6.03 and 6.44 (1H, each s), 6.86 (1H, d, *J* = 8.7), 7.09-7.12 (1H, m), 7.15-7.30 (2H, m), 7.47 (1H, dd, *J* = 1.6, 8.7), 7.54 (1H, s); LC/MS-ESI (*m/z*): $[M+H]^+$, 759.

Step 2. To a solution of sulfonamide (11.14 g, 14.7 mmol) in anhydrous THF (100 mL) was added 1 M TBAF in THF (30 mL, 30 mmol) at room temperature, and the mixture was stirred for 2 h. The mixture was diluted with ethyl acetate and the organic layer was washed with brine, water, and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash column chromatography (39:61 to 18:82 n-hexane/ethyl acetate) to give the alcohol product (8.99 g, 13.9 mmol, 95% yield). LC/MS-ESI (*m/z*): $[M+H]^+$, 645.

Step 3. To a solution of the alcohol (8.99 g, 13.9 mmol) in anhydrous 1,4-dioxane (13.9 mL) was added 4 M HCl in 1,4-dioxane (139 mL, 556 mmol) at room temperature, and the mixture was stirred for 2 h. The sticky precipitate was allowed to settle to the bottom of the flask, and the supernatant solution was decanted. The residue was diluted with ethanol (150 mL) and water (15 mL). The solution was concentrated under reduced pressure. To the residue was added ethanol, and the precipitate was collected by suction filtration to give compound **56d** (5.15 g, 10 mmol, 72% yield) as dihydrochloride salt. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.81-1.93 (2H, m), 2.11-2.19 (2H, m), 2.27-2.36 (2H, m), 2.48 (3H, s), 3.02-3.09 (1H, m), 3.22-3.25 (1H, m), 3.46 (2H, t, *J* = 5.0), 3.86-3.94 (1H, quintet, *J* = 8.2), 4.34-4.43 (2H, m), 5.81 (1H, brs), 5.02 (1H, dd, *J* = 1.9, 10.2), 6.82 (1H, dd, *J* = 2.0, 8.8), 6.93 (1H, dd, *J* = 2.0), 7.11 (1H, d, *J* = 7.8), 7.14 (1H, d, *J* = 7.8), 7.29 (1H, s), 7.31 (1H, t, *J* = 7.8), 7.65 (1H, d, *J* = 8.8), 9.09 (1H, brs), 9.47 (1H, brs), 9.80 (1H, s); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 11.2, 16.2, 23.2, 45.8, 53.1, 53.7, 63.4, 67.9, 92.1, 112.2, 116.8, 117.2, 119.1, 121.1, 121.2, 129.1, 138.5, 140.7, 141.5, 142.9, 157.6; HRMS calculated for $C_{22}H_{28}N_4O_4S$ + H^+ 445.1904, found ESI: $[M+H]^+$, 445.1895; LC/MS-ESI (*m/z*): $[M+H]^+$, 445; HPLC: purity 100%, R_T 2.0 min.

(*R*)-*N*-(3-(1-Hydroxy-2-((2-((3-methyl-1*H*-indazol-6-yl)oxy)ethyl)amino)ethyl)phenyl)cyclopentanesulfonamide (56e). *Step 1.* To a solution of **62** (95 mg, 0.15 mmol) and DBU (134 μL, 0.9 mmol) in anhydrous CH_2Cl_2 (1 mL) was added cyclopentanesulfonyl chloride (111 mg, 0.6 mmol) in CH_2Cl_2 (1 mL) at room temperature, and the mixture was stirred for 2 days. Nitrogen gas was blown into the reaction solution to evaporate the solvent. The

residue was diluted with methanol (2 mL) and 5 M NaOH (200 µL, 1 mmol) was added at room temperature. The mixture was stirred for 3 days and poured into saturated aq NH_4Cl. The aqueous layer was extracted twice with ethyl acetate. The organic layers were washed twice with 2 M HCl, washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column chromatography (47:53 to 20:80 n-hexane/ethyl acetate) and preparative TLC (10:1 CH_3Cl/MeOH) to give *tert*-butyl (*R*)-(2-(3-(cyclopentanesulfonamido)phenyl)-2-hydroxyethyl)(2-((3-methyl-1*H*-indazol-6-yl)oxy)ethyl)carbamate (35 mg, 0.063 mmol, 42% yield). LC/MS-ESI (*m/z*): $[M+H]^+$, 559.

Step 2. To the *tert*-butyl (*R*)-(2-(3-(cyclopentanesulfonamido)phenyl)-2-hydroxyethyl)(2-((3-methyl-1*H*-indazol-6-yl)oxy)ethyl)carbamate (35 mg, 0.063 mmol) was added 4 M HCl in 1,4-dioxane (1.5 mL) at room temperature. The mixture was shaken (600 min^{-1}) overnight and nitrogen gas was blown into the reaction solution to evaporate the solvent. The residue was dried to provide the product (34 mg, 0.064 mmol, quant.) as dihydrochloride salt. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.48-1.57 (2H, m), 1.61-1.70 (2H, m), 1.79-1.95 (4H, m), 2.43 (3H, s), 3.02-3.09 (1H, m), 3.22-3.28 (1H, m), 3.46 (2H, t, *J* = 4.7), 3.50-3.56 (1H, m), 4.32-4.40 (2H, m), 4.98 (1H, dd, *J* = 2.0, 10.1), 6.23 (1H, brs), 6.76 (1H, dd, *J* = 2.1, 8.7), 6.90 (1H, d, *J* = 2.1), 7.10 (1H, d, *J* = 7.8), 7.17 (1H, dd, *J* = 1.4, 7.8), 7.31 (1H, s), 7.33 (1H, t, *J* = 7.8), 7.59 (1H, d, *J* = 8.7), 8.94 (1H, brs), 9.16 (1H, brs), 9.82 (1H, s); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 11.5, 25.3, 27.2, 45.9, 53.6, 59.6, 63.4, 67.9, 92.1, 111.5, 116.9, 117.2, 118.9, 120.9, 121.0, 129.2, 138.7, 140.1, 141.6, 143.0, 157.1; HRMS calculated for $C_{23}H_{30}N_4O_4S + H^+$, 459.2061, found ESI: $[M+H]^+$, 459.2068; LC/MS-ESI (*m/z*): $[M+H]^+$, 459; HPLC: purity 100%, R_T 2.0 min.

(*R*)-*N*-(3-(1-Hydroxy-2-((2-((3-methyl-1*H*-indazol-6-yl)oxy)ethyl)amino)ethyl)phenyl)benzenesulfonamide (56f). To a solution of **62** (96 mg, 0.15 mmol) and pyridine (18 µL, 0.23 mmol) in anhydrous CH_2Cl_2 (2 mL) was added benzenesulfonyl chloride (32 mg, 0.18 mmol) at room temperature, and the solution was stirred overnight. The mixture was purified by flash column chromatography (4:3 n-hexane/ethyl acetate). The sulfonamide product was diluted with 1,4-dioxane (0.2 mL) and 4 M HCl in 1,4-dioxane (1.6 mL, 6.4 mmol) was added at room temperature. The mixture was shaken (600 min^{-1}) overnight and nitrogen gas was blown into the reaction solution to evaporate the solvent. The residue was dried to provide the product (67 mg, 0.12 mmol, 83% yield) as dihydrochloride salt. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 2.44 (3H, s), 2.88-2.99 (1H, m), 3.14-3.17 (1H, m), 3.43 (2H, t, *J* = 4.9), 4.29-4.38 (2H, m), 4.92 (1H, dd, *J* = 2.0, 10.4), 6.20 (1H, brs), 6.76 (1H, dd, *J* = 2.1, 8.8), 6.90 (1H, d, *J* = 2.1), 7.02 (1H, d, *J* = 8.0), 7.05 (1H, d, *J* = 8.0), 7.22 (1H, s), 7.23 (1H, t, *J* = 8.0), 7.52-7.56 (2H, m), 7.58-7.62 (2H, m), 7.77-7.79 (2H, m), 8.93 (1H, brs), 9.17 (1H, brs), 10.41 (1H, s); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 11.4, 45.9, 53.5, 63.4, 67.8, 92.1, 111.6, 117.1, 117.2, 119.0, 120.9, 121.4, 126.6, 129.1, 132.8, 137.8, 139.4, 140.8, 141.6, 142.9, 157.2; HRMS calculated for $C_{24}H_{26}N_4O_4S + H^+$, 467.1748, found ESI: $[M+H]^+$, 467.1735; LC/MS-ESI (*m/z*): $[M+H]^+$, 467; HPLC: purity 95% R_T 2.0 min.

(*R*)-2-(Benzyl(2-(benzyloxy)ethyl)amino)-1-(3-nitrophenyl)ethan-1-ol (59). *Step 1.* A mixture of 2-(benzyloxy)ethan-1-amine (12.31 g, 81 mmol), benzaldehyde (8.72 g, 82 mmol), and Na_2SO_4 (67.79 g, 477 mmol) in CH_2Cl_2 (150 mL) was stirred overnight at ambient temperature. The mixture was filtered and concentrated under reduced pressure. The residue was diluted with methanol (150 mL) and sodium borohydride

(3.41 g, 90 mmol) was added at 0 °C. The mixture was allowed to warm to ambient temperature and was stirred for 2 h. The mixture was concentrated under reduced pressure, quenched with water, and diluted with ethyl acetate. The layers were separated, and the aqueous layer was extracted twice with ethyl acetate. The combined organic layers were washed twice with water, washed with brine, dried over Na_2SO_4, filtered, and concentrated. The crude *N*-benzyl-2-(benzyloxy)ethan-1-amine **58** (20.19 g, >100% yield) was carried forward without further purification. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.72 (1H, brs), 2.84 (2H, t, *J* = 5.2), 3.62 (2H, t, *J* = 5.2), 3.80 (2H, s), 4.52 (2H, s), 7.20-7.37 (10H, m).

Step 2. A mixture of (*R*)-2-(3-nitrophenyl)oxirane[33] **57** (13.65 g, 83 mmol) and **58** (20.21 g, 84 mmol) in 2-propanol (205 mL) was stirred for 36 h at reflux. The mixture was concentrated under reduced pressure. The residue was diluted with toluene (100 mL) and concentrated under reduced pressure. The residue was purified by flash column chromatography (85:15 to 80:20 n-hexane/ethyl acetate) to give compound **59** (30.76 g, 76 mmol, 92% yield). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 2.62 (1H, dd, *J* = 10.2, 13.1), 2.75-2.87 (2H, m), 2.92-3.01 (1H, m), 3.51-3.64 (2H, m), 3.78 (2H, dd, *J* = 13.6, 69.1), 4.54 (2H, s), 4.70 (1H, dd, *J* = 3.3, 10.2), 7.27-7.39 (10H, m), 7.45 (1H, t, *J* = 7.9), 7.59 (1H, d, *J* = 7.9), 8.09 (1H, dd, *J* = 1.1, 2.3, 7.9), 8.16 (1H, d, *J* = 2.3); LC/MS-ESI (*m/z*):$[M+H]^+$, 407.

(*R*)-*N*-Benzyl-*N*-(2-(benzyloxy)ethyl)-2-(3-nitrophenyl)-2-((triethylsilyl)oxy)ethan-1-amine (60). To a solution of **59** (30.37 g, 75 mmol) and imidazole (6.13 g, 90 mmol) in anhydrous DMF (150 mL) was added chlorotriethylsilane (15.1 mL, 90 mmol) at room temperature, and the solution was stirred overnight. The mixture was quenched with water and extracted twice with ethyl acetate. The combined organic layers were washed twice with water, washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was purified by flash column chromatography (100:0 to 87:13 n-hexane/ethyl acetate) to give compound **60** (36.66 g, 70 mmol, 93% yield). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.42-0.56 (6H, m), 0.85 (9H, t, *J* = 7.9), 2.65-2.85 (4H, m), 3.37-3.47 (2H, m), 3.62 (2H, dd, *J* = 13.6, 42.9), 4.43 (2H, s), 4.68 (1H, dd, *J* = 5.4, 7.4), 7.06-7.11 (2H, m), 7.16-7.20 (3H, m), 7.26-7.37 (6H, m), 7.56 (1H, d, *J* = 7.7), 8.05 (1H, dd, *J* = 1.1, 2.3, 7.7), 8.12 (1H, dd, *J* = 2.3, 2.3); LC/MS-ESI (*m/z*): $[M+H]^+$, 521.

***tert*-Butyl (*R*)-(2-(3-aminophenyl)-2-((triethylsilyl)oxy)ethyl)(2-hydroxyethyl)carbamate (61).** *Step 1.* A mixture of **60** (36.46 g, 70 mmol) and 10% palladium on activated charcoal (15.12 g, PE-type, NE Chemcat) in ethanol (175 mL) was evacuated, placed under a hydrogen atmosphere, and stirred for 9 h at 50 °C. The mixture was evacuated, placed under a hydrogen atmosphere, and stirred for 4 h at 50 °C. The reaction mixture was cooled to room temperature, passed through a membrane filter, and the solvent was concentrated under reduced pressure to give (*R*)-3-(2-(2-benzyloxyethylamino)-1-triethylsilyloxy-ethyl)aniline (25.08 g). LC/MS-ESI (*m/z*): $[M+H]^+$, 401.

Step 2. To a solution of (*R*)-3-(2-(2-benzyloxyethylamino)-1-triethylsilyloxy-ethyl)aniline (25.08 g, 70 mmol) in THF (175 mL) was added Boc_2O (14.60 g, 67 mmol) at room temperature, and the solution was stirred for 1.5 h. The mixture was concentrated under reduced pressure. The residue was added to $Pd(OH)_2$ on activated charcoal (15.02 g, NE Chemcat), THF (80 mL), and MeOH (80 mL). The mixture was evacuated, placed under a hydrogen atmosphere, and stirred for 8 h at 50 °C. The reaction mixture was cooled to room temperature, passed through a

membrane filter, and the solvent was concentrated under reduced pressure. The residue was purified by flash column chromatography (75:25 to 54:46 n-hexane/ethyl acetate) to give compound **61** (17.94 g, 44 mmol, 62% yield). ^{1}H-NMR (300 MHz, $CDCl_3$, 3:2 rotamers) δ 0.44-0.63 (6H, m), 0.88 (9H, t, *J* = 7.9), 1.49 and 1.51 (9H, each s), 2.95-3.87 (8H, m), 4.90-4.95 and 5.17-5.21 (1H, each m), 6.57-6.78 (3H, m), 7.09 (1H, t, *J* = 7.7); LC/MS-ESI (*m/z*): $[M+H]^+$, 411.

***tert*-Butyl (*R*)-6-(2-((2-(3-Aminophenyl)-2-((triethylsilyl)oxy)ethyl)(tert-butoxycarbonyl)amino)ethoxy)-3-methyl-1*H*-indazole-1-carboxylate (62).** To a solution of **61** (2.51 g, 6.1 mmol), **18** (1.99 g, 8.0 mmol), and triphenylphosphine (1.61 g, 6.1 mmol) in anhydrous toluene (25 mL) was added DMEAD[29] (1.43 g, 6.1 mmol) at room temperature, and the solution was stirred overnight at 50 °C under a nitrogen atmosphere. The reaction mixture was cooled to room temperature and washed three times with water. The organic layer was dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography (74:26 to 53:47 n-hexane/ethyl acetate) to give a mixture of **18** and **62** (2.29 g). The mixture was diluted with CH_2Cl_2 (25 mL), to which 2.91 mmol/g MP-Carbonate (3.4 g, 9.9 mmol) was added at room temperature. The mixture was stirred overnight and 2.91 mmol/g MP-Carbonate (2.0 g, 5.8 mmol) was added at room temperature. The mixture was stirred for 5 h and filtered to give compound **62** (1.52 g, 2.4 mmol, 47% yield). ^{1}H-NMR (300 MHz, $CDCl_3$, 3:2 rotamers) δ 0.53 (6H, t, *J* = 7.9), 0.89 (9H, q, *J* = 7.9), 1.48 (9H, s), 1.70 (9H, s), 2.53 (3H, s), 3.16-3.78 (6H, m), 4.02-4.14 (2H, m), 4.80-4.84 and 4.96-5.00 (1H, each m), 6.57-6.60 (1H, m), 6.66 (1H, d, *J* = 2.3), 6.70 and 6.78 (1H, each d, *J* = 8.7 and 7.6), 6.87 (1H, dt, *J* = 2.3, 8.7), 7.09 (1H, t, *J* = 7.6), 7.46 (1H, dd, *J* = 1.8, 8.7), 7.55 (1H, s); LC/MS-ESI (*m/z*): $[M+H]^+$, 641.

Results of ion chromatography.

Regarding compounds **5** to **16**, ion chromatographic analyses were performed using the following conditions; (Anion) ICS-1000 (Dionex), Dionex IonPac AS14 (Dionex or Thermo Scientific), 30 °C column temperature, 1.2 mL/min flow rate, electrical conductivity detection, mobile phase of 1.0 mmol/L sodium hydrogen carbonate and 3.5 mmol/L sodium carbonate in water and standard solution of Mixed Anion Standard Solution IV (Kanto Chemical Industry Co., Ltd.); (Cation) DX500 (Dionex), Dionex IonPac CS14 (Dionex), 30 °C column temperature, 1.0 mL/min flow rate, electrical conductivity detection, mobile phase of 10 mmol/L methanesulfonic acid in water, standard solution of Mixed Cation Standard Solution II (Kanto Chemical Industry Co., Ltd.).

Compound	Cl^- content rate (%)	Salt
5	15.52	2.1 HCl
6	13.77	1.9 HCl
7	14.46	2.0 HCl
8	11.04	1.5 HCl
9	14.01	2.0 HCl
10	14.26	2.0 HCl
11	14.37	2.1 HCl

12	11.70	1.7 HCl
13	14.56	2.1 HCl
14	13.12	1.9 HCl
15	9.77	1.4 HCl
16	11.25	1.7 HCl

Regarding compounds **56a** to **56f**, ion chromatographic analyses were performed using the following conditions: (Anion) ICS-5000+ (Thermo Scientific, Waltham, MA, USA), IonPac AS18-4μm (Thermo Scientific), 30 °C; 1.0 mL/min flow rate; electrical conductivity detection; mobile phase of 23 to 40 mM KOH in water; standard solution of Mixed Anion Standard Solution IV (Kanto Chemical Industry Co., Ltd., Tokyo, Japan); (Cation) ICS-1100 (Thermo Scientific), IonPac CS12A (Thermo Scientific), 30 °C; 1.0 mL/min flow rate; electrical conductivity detection; mobile phase of 20 mmol/L methanesulfonic acid in water; standard solution of Mixed Cation Standard Solution II (Kanto Chemical Industry Co., Ltd.).

Compound	Cl^- Content Rate (%)	Salt
56a	12.44	1.7 HCl
56b	11.28	1.6 HCl
56c	11.27	1.5 HCl
56d	12.86	1.9 HCl
56e	11.31	1.7 HCl
56f	10.53	1.6 HCl

Biology

Human β_1-, β_2-, β_3 or marmoset β_3-AR Agonist Assay. The measurement of human β_1-, β_2-, β_3-, or marmoset β_3-AR agonist activity was carried out using stably transfected Chinese Hamster Ovary (CHO) cells expressing recombinant human β_1-, β_2-, or β_3-AR. The cells were cultured in Ham's F-12 medium containing 10% fetal bovine serum, 400 μg/mL geneticin (Invitrogen, Waltham, MA, USA), 100 U/mL penicillin, and 100 μg/mL streptomycin. Cells were seeded on a 96-well plate at a density of 2×10^4 cells/well and cultured for approximately 20 h. The medium was then aspirated from each well and replaced with 80 μL of serum-free Ham's F-12 medium, in which the cells were incubated for a further 15 min. The compound to be tested was initially dissolved in DMSO and then diluted with Ham's F-12 medium containing 100 mmol/L HEPES and 1 mmol/L isobutylmethylxanthine. The diluted compound (20 μL) was added to the cells and the cells were incubated with it for 30 min. The medium was removed and 0.1 mL of the Assay/Lysis Buffer from the cAMP-Screen kit (Applied Biosystems, Waltham, MA, USA) was added, and the cells were then incubated at 37 °C for 30 min. The cAMP level in the resulting cell lysate was quantified using the cAMP-Screen kit. The maximum response to the positive control, isoproterenol, was defined as 100%. The maximum response of each test compound was calculated as a percentage of this maximum response; this was defined as each compound's Intrinsic Activity [IA (%)]. The concentration of the compound that induced a response that was 50% of the maximum (EC_{50}) was also determined.

Rat β_3-AR Agonist Assay. The measurement of rat β_3 adrenergic receptor agonist activity was carried out using stably transfected Chinese Hamster Ovary (CHO) cells expressing recombinant rat β_3 adrenergic receptor. The cells were cultured in Ham's F-12 medium containing 10% fetal bovine serum, 400 μg/ml geneticin (Invitrogen), 100 U/ml penicillin and 100 μg/ml streptomycin. These cells were transfected with a cAMP-response element / luciferase reporter gene plasmid using the FuGENE 6 Tranfection Reagent (Roche). Transfected cells were seeded on a 96-well white plate at a density of 3×10^4 cells/well and were cultured for 20 hours. Following test compound addition to the cells and culture for a further 6 hours, the medium was removed. The PicaGene LT2.0 Luciferase Assay System solution (30 μ L, Wako) was added to the cells, and chemiluminescence was analyzed using ARVOsx plate reader (PerkinElmer). The maximum response to the positive control isoproterenol was taken as 100%. The maximum response to each test compound was calculated as a percentage of the response of isoproterenol, and is termed the Intrinsic Activity [IA (%)]. The concentration of the compound solution that results in a response that was 50% of the maximum for that compound (EC_{50}) was determined.

Human α_{1A}-AR Agonist Assay. Human α_{1A}-AR agonist activity was measured using stably transfected HEK293 cells expressing recombinant human α_{1A}-AR. The cells were cultured in DMEM containing 10% fetal bovine serum, 400 μg/mL hygromycin B (Gibco BRL, Waltham, MA, USA), 100 U/mL penicillin, and 100 μg/mL streptomycin. Subsequently, the cells were prepared at a density of 5×10^6 cells/mL in a solution of Assay Buffer [20 mmol/L HEPES-KOH (pH 7.4), 115 mmol/L NaCl, 5.4 mmol/L KCl, 0.8 mmol/L $MgCl_2$, 1.8 mmol/L $CaCl_2$, 13.8 mmol/L D-glucose, and 0.1% bovine serum albumin] containing 0.2% Pluronic F-127 (Invitrogen) and 20 μmol/L Fura-2AM (Dojindo Laboratories, Kumamoto, Japan). The cells were incubated in a CO_2 incubator for 30 min, washed twice with the Assay Buffer to remove excess Fura-2AM, and resuspended at a density of 5×10^6 cells/mL with the Assay Buffer. Subsequently, the cells were dispensed into a 96-well plate at a volume of 80 μL/well. In addition to the cell plate, a sample plate was provided in which wells contained a test compound that had been diluted 10 times with the Assay Buffer to concentrations ranging from 10^{-5} to 10^{-12} M. The plates were loaded into an FDSS4000 kinetic plate reader (Hamamatsu Photonics K.K., Shizuoka, Japan), and were pre-incubated for 180 s at 37 °C. Fluorescence intensity (excitation wavelengths 340 nm and 380 nm, measurement wavelength 500 nm) was measured at intervals of 2 s. After baseline measurements were taken for approximately 30 s, 20 μL of the test sample from the sample plate was added to the cell plate, and measurements were collected for a further 270 s. The Ca^{2+} flux caused by the test compound was calculated as the peak height, which represented the difference between the maximum value of the fluorescence intensity ratio at wavelengths 340 nm and 380 nm after addition of the test compound, and the fluorescence intensity ratio at baseline. Maximum response to the positive control, noradrenaline, was defined as 100%. The maximum response to each test compound was calculated as a percentage of the maximum response to noradrenaline, and is termed the Intrinsic Activity [IA (%)]. The compound's EC_{50} was also determined.

Human α_{1B} or α_{1D}-AR Agonist Assay. Human α_{1B} or α_{1D}-AR agonist activity was measured using transiently transfected HEK293 cells expressing recombinant human α_{1B} or α_{1D}-AR, and NFAT-Luciferase reporter gene

(Stratagene, CA, USA). Cells were cultured in DMEM containing 10% fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin. Cells were transfected using Lipofectamine 3000 Transfection Reagent (Life Technologies, CA, USA) according to the manufacture's protocol. Transfected cells were seeded on a 96-well white plate at a density of 1×10^5 cells/well and were cultured for 20 hours. Following test compound addition to the cells and culture for a further 6 hours, the medium was removed. The PicaGene LT2.0 Luciferase Assay System solution (30 μL, Wako, Osaka, Japan) was added to the cells, and chemiluminescence was analyzed using ARVOsx plate reader (PerkinElmer, MA, USA). The maximum response to the positive control noradrenaline was taken as 100%. The maximum response to each test compound was calculated as a percentage of the response of noradrenaline, and is termed the Intrinsic Activity [IA (%)]. The concentration of the compound solution that results in a response that was 50% of the maximum for that compound (EC_{50}) was determined The EC_{50} of noradrenaline for α_{1B}-AR and α_{1D}-AR was 359 nM and 101 nM, respectively.

Effects on Blood Pressure and HR in Rats. Male Sprague-Dawley rats (Japan SLC, Inc., Shizuoka, Japan) weighing 200-300 g were used. Rats were housed in an air-conditioned room (20-26 °C and 30-75% relative humidity with a 12-h light-dark cycle), fed a standard laboratory diet (CRF-1; Oriental Yeast, Tokyo, Japan), and given water ad libitum. Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p., and 25 mg/kg, s.c.). The left femoral vein and artery were exposed by a small incision. A polyethylene tube (SP10; Natsume Seisakusyo Co., Ltd., Tokyo, Japan) was inserted into the vein for injection of the compound. A polyethylene tube (SP31; Natsume Seisakusyo Co., Ltd.) was inserted into the artery and connected to a pressure transducer. Blood pressure was measured from the pressure transducer through a pressure amplifier (AP-641G; Nihon Koden Corp., Tokyo, Japan). HR was measured by the Heart Rate Counter (AT-601G; Nihon Koden Corp.), using the pulse wave of the blood pressure as the trigger. The blood pressure, MBP, and HR were recorded. Once the blood pressure and HR were almost constant, the test compound was administered through the left femoral vein and the parameters were recorded for 30 min. The maximum increase or decrease of HR and MBP is shown as a percentage change (%) relative to baseline.

Relaxant Activity on Isolated Marmoset Urinary Bladder Smooth Muscle. Three female common marmosets (*Callithrix jacchus*) aged 11-14 months were purchased from CLEA Japan Inc. (Tokyo, Japan) and were used between the ages of 19 and 20 months. Marmosets were housed in an air-conditioned room (27 ± 3 °C, 45 ± 15% humidity, 12-h light-dark cycle), fed 30 g daily of balanced marmoset food pellets (CMS-1; CLEA Japan), and given water ad libitum. Marmosets were anesthetized with an intramuscular injection of ketamine hydrochloride (15 mg/kg), and were sacrificed by exsanguination. The urinary bladder was carefully isolated and immersed in ice-cold Krebs–Henseleit solution that was gassed with 95% O_2 and 5% CO_2. The bladder was opened with a midline incision and six muscle strips of the bladder body, approximately 10-15 mm long and 2-5 mm wide, were prepared. The strips were suspended in a 10 mL glass organ bath filled with Krebs–Henseleit solution that was aerated with 95% O_2 and 5% CO_2 at 37 °C, and were allowed to equilibrate for over 30 min under a resting tension of 1 g. Strip tension was measured isometrically using a TB-612T force displacement transducer (Nihon Koden Corp.). Following the equilibration period, the strips were pre-contracted with KCl (40 mmol/L) three times and

strips that exhibited three equivalent contractions were used. These strips were again pre-contracted with 40 mmol/L KCl and, after the tension had stabilized, a test compound was added cumulatively at intervals of 20 min. When the relaxation response at the maximum concentration of the test compound was achieved, papaverine at a final concentration of 10^{-4} mol/L was added, and the maximum relaxation response of each strip was determined.

Solubility Assay. Stock solutions (10 mM) of each compound were prepared in DMSO, and 5 μL/well of the 10 mM stock solution was transferred into a 96-well plate. The plate was freeze-dried overnight, and to each well was added a pH 1.2 buffer solution (167 μL of Dissolution Test Solution 1, 11500-76; Kanto Chemical Co., Inc., Tokyo, Japan) or a pH 6.8 buffer solution (167 μL, Dissolution Test Solution 2 11499-76; Kanto Chemical Co., Inc.). The plate was sealed and shaken for over 18 h at 37 °C. Samples were filtered through a 0.4-μm multiscreen HTS-PCF filter plate (Millipore/Merck, Darmstadt, Germany) mounted on 96-well plate by centrifugation at 2000 rpm for 1 min at room temperature. Standards were created by initially preparing a 5 μL solution from the combination of the 10 mM stock solution and aqueous acetonitrile (162 μL, acetonitrile/water = 95:5) in a 96-well plate. The sample was then analyzed by HPLC. Water solubility was calculated according to the equation: Solubility (μM) = peak $area_{sample}$ / peak $area_{standard}$ x 300.
The HPLC analysis was performed with the following conditions: Waters ACQUITY UPLC BEH C18 column (1.7 μm, 2.1 x 50 mm), 40 °C; 0.6 mL/min flow rate; photodiode array detection (254 nm); solvent A consisting of 10 mM ammonium acetate in water; solvent B consisting of acetonitrile; a linear gradient of 5-98% B (2.5 min), 98% B (0.5 min), 5% B (1.5 min).

Madin-Darby Canine Kidney (MDCK) Permeability Assay. *In vitro* membrane permeability was determined using the MDCK cell monolayer system. The MDCK cell line (CCL-34™) was obtained from ATCC (Manassas, VA, USA). The cells were seeded on 24-well cell culture insert plates (Transwell, #3495; Corning, NY, USA) at a density of 3.32 x 10^5 cells/cm^2 and cultured for 4 days in Modified Eagle's Medium (MEM; Gibco, Waltham, MA, USA) to form monolayers. The MEM was changed once every 2-3 days. A buffer of Hanks' Balanced Salt Solution (HBSS), pH 7.4, with 25 mM HEPES and 10 mM glucose at 37 °C was used as the medium in the permeability study. The monolayers were rinsed twice with HBSS in both the donor (upper) and receiver (lower) compartments, and incubated in HBSS with 0.005% HCO 60 for 10 min to stabilize physiological parameters. During the transmembrane permeability experiments, the integrity of cell monolayers was assessed by transepithelial electrical resistance (TEER) measurements at fixed times using a tissue resistance measurement chamber (EVOM; World Precision Instruments, Sarasota, FL, USA). MDCK cell monolayers (TEER ≥ 600 $\Omega \cdot cm^2$) were washed in HBSS with 0.005% HCO60, and then a solution containing the test compound (50 μM) was added to the donor compartment, followed by an incubation at 37 °C for 60 min. The flux of test compound into the receiver compartment was measured with LC-MS/MS (Sciex, Foster City, CA, USA), and the apparent permeability coefficient (P_{app}) was calculated with the following equation: P_{app} (cm/s) = (dQ/dt) × 1/A × $1/C_0$, where dQ/dt is the increase in the amount of drug in the receiver chamber per time interval, C_0 is the initial concentration in the donor compartment, and A is the permeability area of the cell culture insert. Three reference compounds were also screened: atenolol to represent paracellular transport; propranolol to represent efficient passive transcellular

transport; and methotrexate to represent poor passive transcellular transport. Atenolol and propranolol have a known human absorption of 50% and 90%, respectively, and can be used for ranking compounds with unknown characteristics.

LC-MS/MS Analysis. The LC-MS/MS system consisted of an Agilent 1100 series gradient HPLC pump (Agilent Technologies, Palo Alto, CA, USA), a CTC HTS PAL Autosampler (AMR, Inc., Tokyo, Japan) and a Sciex API 3200 triple quadrupole mass spectrometer (Sciex) equipped with a turbo ionspray interface. The samples were separated by reverse phase HPLC using a Capcell Pak MG III RP C18 50 × 2.1 mm column (Shiseido Inc., Tokyo, Japan). The compounds were analyzed with an optimized protocol within the following range of conditions: flow rate of 0.4mL/min [range: 0-0.7 min]; injection volume of 20 µL; solvent A consisting of 0.1% formic acid in water; solvent B consisting of acetonitrile; a linear gradient of 5%-90% B, 90% B (1.3 min), a return to initial conditions, and equilibration (1.5 min).

Human and Rat Unbound Microsomal Intrinsic Clearance Determination.

Human Microsomal Stability Assay. The incubation mixtures were prepared in 96-well cluster tubes (1.4 mL PP; Micronic, Aston, PA, USA). The metabolic stability of the test compounds was determined with pooled, mixed-gender human microsomes (pool of n = 50; XenoTech, Kansas City, KS, USA). Each reaction mixture consisted of 100 mM potassium phosphate buffer (pH 7.4), liver microsomes (final concentration, 0.5 mg/mL), NADPH regenerating mix (final concentration, 1.0 mM NADP+, 4.0 mM glucose-6-phosphate, 3.0 mM $MgCl_2$, 0.4 U/mL of glucose-6-phosphate dehydrogenase, 5.0 mM UDP-GA, and 0.165 mM β-NAD), and the test compound (1 µM) in a total volume of 100 µL. The incubation mixture was prewarmed for 5 min. Liver microsomes were added to the mixture, and kept in a shaking water bath for 15 min at 37 °C. To the mixture was added chilled acetonitrile (200 µL) containing an internal standard to stop the reaction. The samples were centrifuged at 3500 rpm for 15 min at 4 °C. The supernatants were analyzed by LC-MS/MS. Controls were prepared by omitting NADPH regenerating mix from the reaction mixture, and adding microsomes after the reaction was terminated.

Human Liver Microsomal Binding Assay. Microsomal binding of the test compounds was determined by equilibrium dialysis. Dialysis mixtures contained the test compound (1 µM), microsomal solution (final concentration of microsomes, 0.5 mg/mL; ratio of components = 1 : 1 : 8 [microsomes at 5 mg/mL : PBS, pH 7.4 : 125 mM phosphate buffer, pH 7.4]), in a final volume of 150 µL. Duplicate mixtures were subjected to equilibrium dialysis against 150 µL of 100 mM potassium phosphate buffer (pH 7.4) using 96-well Micro-Equilibrium Dialysis Devices (HT Dialysis LLC, Gales Ferry, CT, USA). The dialyzing unit consisted of two chambers separated by an ultrathin membrane with a molecular weight cut-off of 12-14 kDa. The plate was rotated at 300 rpm for 6 h at 37 °C in the plate shaker (Taitec Inc., Japan). Upon completion of the dialysis, 50 µL samples were obtained from the microsome and buffer sides. For a matrix composition match, 50 µL of 100 mM phosphate buffer (pH 7.4) was added to the microsome side sample and the microsome mixture was added to the buffer side sample. To the mixture was added chilled acetonitrile (300 µL) containing an internal standard to stop the reaction; the samples were then analyzed by LC-MS/MS. Results are expressed as the area ratio of each sample versus control: human microsome fu = peak $area_{sample}$ / peak $area_{control}$.

Rat Microsomal Stability Assay. The incubation mixtures were prepared in Corning 96-well cluster plates. The

metabolic stability of the test compounds was determined in pooled liver microsomes from male Sprague-Dawley rats (XenoTech). The reaction mixture consisted of 100 mM Tris-HCl buffer (pH 7.4), liver microsomes (5 mg/mL), NADPH regenerating mix (final concentrations of 0.165 mM NADP+, 4 mM glucose-6-phosphate, 3.0 mM $MgCl_2$, 0.1 U/mL of glucose-6-phosphate dehydrogenase, 5.0 mM UDP-GA, and 0.165 mM β-NAD), and the test compound (0.5 μM) in a total volume of 100 μL. Reactions were initiated by the addition of liver microsomes (final concentration, 0.5 mg/mL) and incubated in a shaking water bath for 20 min at 37 °C. To the mixture was added chilled acetonitrile (200 μL) containing an internal standard to stop the reaction. A control sample was prepared by adding acetonitrile to a reaction mixture lacking microsomes, and then adding microsomes after the reaction was terminated. The samples were centrifuged at 3500 rpm for 15 min at 4 °C. The supernatants were analyzed by LC-MS/MS.

Rat Liver Microsomal Binding Assay. Microsomal binding of the test compounds was determined by ultracentrifugation. Each ultracentrifugation mixture contained the test compound (0.5 μM), microsomal solution (final concentration, 0.5 mg/mL; ratio of components = 1 : 9 [microsomes at 5 mg/mL : 100 mM Tris-HCl buffer, pH 7.4]), in a final volume of 100 μL. Aliquots of 100 μL were placed in polyallomer ultracentrifuge tubes (8 mm × 34 mm; Beckman Coulter, Fullerton, CA, USA) and centrifuged at 160,000*g* for 16 h at 37 °C in an Optima TL ultracentrifuge (Beckman Coulter). Upon completion of the ultracentrifugation, 50 μL of the supernatant was obtained. To the supernatant was added chilled acetonitrile (50 μL) containing an internal standard to stop the reaction; the samples were then analyzed by LC-MS/MS. Controls were prepared by adding the test compound to the ultracentrifugal supernatant of the microsomal solution. Results are expressed as the area ratio of sample versus control: rat microsome fu = peak $area_{sample}$ / peak $area_{control}$.

LC-MS/MS Analysis. The LC-MS/MS system consisted of an Agilent 1100 series gradient HPLC pump (Agilent Technologies, Palo Alto, CA, USA), a CTC HTS PAL Autosampler (AMR, Inc., Tokyo, Japan), and a Sciex API 3200 triple quadrupole mass spectrometer (Sciex, Foster City, CA, USA) equipped with a turbo ionspray interface. The samples were separated by reverse phase HPLC using an Inert Sustain RP C18 50 × 2.1 mm column (GL Science Inc., Tokyo, Japan) or Capcell Pak RP C18 50 × 2.1 mm column (Shiseido Inc., Tokyo, Japan). The compounds were eluted with an optimized gradient, which fell within the following method conditions: flow rate of 0.4 mL/min (range: 0-0.7 min); injection volume of 20 μL; solvent A consisting of 0.1% formic acid in water; solvent B consisting of acetonitrile or 0.1% formic acid in acetonitrile; the percentage of B was linearly increased from 5% to 90%, 90% B (1.3 min), returned to initial conditions, equilibrated (1.5 or 1.6 min). The area ratio of each test compound was calculated by comparing the peak area of the compound to the peak area of an internal standard.

Calculation of Human and Rat Unbound Microsomal Intrinsic Clearance ($hCL_{int,u}$ and $rCL_{int,u}$). Metabolic stability was determined by plotting the natural logarithm of the concentration of unchanged test compound as a function of time. The first-order rate constant was calculated using the equation $k = [Ln(C_0) – Ln(C)]$/incubation time, where C_0 was the initial concentration of the test compound, C was the concentration of the test compound remaining after incubation ($C = C_0$ × remaining ratio), and the incubation time was 15 min (human) or 20 min (rat). The half-life ($t_{1/2}$) was estimated using the equation $t_{1/2} = 0.693/k$. The $hCL_{int,u}$ was estimated using the equation $hCL_{int,u}$ = k/(microsomal protein concentration) × (microsomal protein per gram of liver) × (liver mass per kilogram of body

mass) / (human microsome fu), where k was the first-order rate constant, the microsomal protein concentration was 0.5 mg/mL, the microsomal protein per gram of liver was 48.8 mg, the liver mass per kilogram of body mass was 25.7 g, and the human microsome fu was determined experimentally from the human liver microsomal binding assay.[40] The $rCL_{int,u}$ was estimated using the equation $rCL_{int,u}$ = k / (microsomal protein concentration) × (microsomal protein per gram of liver) × (liver mass per kilogram of body mass) / (rat microsome fu), where k was the first-order rate constant, the microsomal protein concentration was 0.5 mg/mL, the microsomal protein per gram of liver was 44.8 mg, liver mass per kilogram of body mass was 40.0 g, and the rat microsome fu was determined experimentally from the rat liver microsomal binding assay.[40]

PK Study. Compounds **5**, **11**, and **56d** were subjected to PK studies in male Sprague-Dawley rats. Test compounds were administered orally to rats (n = 2 or 3) at a dose of 5 mg/kg in saline or water (dose volume 5 mL/kg). Blood samples were collected via the subclavian vein at 0.5, 1, 2, 4, 6, and 8 h post dose. Plasma was separated by centrifugation and stored frozen at -20 °C until analysis. Plasma compound concentrations were determined using LC-MS/MS. PK parameters were calculated using the non-compartmental analysis tool provided with WinNonlin® Enterprise software v6.4.0.768 (Certara, Princeton, NJ, USA).

Cytochrome P450 Inhibition Assay. CYP inhibition assays (CYP1A2, 2B6, 2C19, 2C8, 2C9, 2D6, and 3A4) were performed with Promega assay kits (P450-Glo™ Assays; Madison, WI, USA) according to the manufacturer's instructions.

In silico

Homology Modeling of the β_3-AR. The β_3-AR sequence was aligned with the human β_2-AR sequence.[35b] A three-dimensional model of the β_3-AR was constructed using Prime in the Maestro software (Schrödinger, LLC). Because it is very long and was predicted to be disordered, the third intracellular loop was truncated by five residues leading into TM6.

Docking Study. Energy minimization and protonation of all compounds was performed with LigPrep in the Maestro software (Schrödinger, LLC). Docking of the compounds into the β_3-AR model utilized three main steps that take into account several levels of structural flexibility and scoring criteria: (1) molecular modeling of compound-bound β_3-AR by docking compound **56f**, with consideration of both ligand and receptor flexibility; (2) rigid receptor docking of eight indazole analogues and four other series into the active site of the compound **56f**-bound β_3-AR model generated in the previous step; (3) rescoring according to the calculated binding score (Glide extra precision, XP; Schrödinger, LLC).

In order to account for both compound and receptor flexibility in the first step, the Glide "Induced Fit Docking (IFD)" protocol (Schrödinger, LLC) was utilized, followed by iteratively combining rigid receptor docking (Glide) and protein remodeling by side chain searching and minimization (Prime) techniques. Hydrogen-bonding constraints between the side chain COO^- group of Asp117 and the side chain C=O group of Asn332 were introduced, because this hydrogen-bonding formation is highly conserved in almost all known complexes formed

between members of the β-AR family bound to isoproterenol and a wide variety of agonists. In the protein remodeling stage, all residues within a 5 Å radius of each initial docked compound were refined using Prime. The compound was then redocked into the refined receptor structure using Glide in the standard precision (SP) mode. All the docked structures were then ranked according to Glide score. After modeling the compound-β_3-AR complex using the IFD protocol, grid generation and rigid receptor docking of the eight indazole analogues and four other series using Glide (SP mode) was conducted, using hydrogen-bonding constraint to connect the side chain oxygen atom of Ser208. The best orientation for each docked compound was rescored according to its binding score (Glide XP; Schrödinger, LLC).

MD simulation. The compound **56d**-bound structure of human β_3-AR was subjected to 20 ns MD simulations using Desmond version 2.3[41] with OPLS3 force field[42]. The initial model structure was placed into a large palmitoyloleoylphosphatidylcholine (POPC) bilayer at a depth consistent with the structure of human β_2-AR with BI-167107 (PDB entry 3P0G) from the Orientation of Proteins in Membranes (OPM) database[43] and TIP3P water molecules solvated with 0.15 M NaCl. After minimization and relaxation of the model, the production MD phase was performed for 20 ns in the isothermal-isobaric (NPT) ensemble at 300 K and 1 bar using Langevin dynamics. Long-range electrostatic setups were performed using Maestro (Schrödinger, LLC).

略語表

ADME	absorption, distribution, metabolism and excretion
AR	adrenergic receptor
AUC	area under the curve
Boc	*tert*-butoxycarbonyl
CHO	chinese hamster ovary
CYP	cytochrome P
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DHP	3,4-dihydro-2*H*-pyran
DMAP	4-(*N*,*N*-dimethylamino)pyridine
DMEAD	di-2-methoxyethylazodicarboxylate
DMF	dimethylformamide
ECL	extracellular loop
Glide XP	Glide extra precision
$hCL_{int,u}$	human unbound microsomal intrinsic clearance
HR	heart rate
IA	intrinsic activity
IFD	induced fit docking
MBP	mean blood pressure
MD	molecular dynamics
MDCK	madin-darby canine kidney
NBS	*N*-bromosuccinimide
NCS	*N*-chlorosuccinimide
OAB	overactive bladder
PDB	Protein Data Bank
PK	pharmacokinetics
QSAR	quantitative structure–activity relationship
$rCL_{int,u}$	rat unbound microsomal intrinsic clearance
RMSD	root mean square deviation
SD	standard deviation
SEM	standard error of mean
TBAF	tetrabutylammonium fluoride
TBDPS	*tert*-butyldiphenylsilyl
TBS	*tert*-butyldimethylsilyl
TES	triethylsilyl
THF	tetrahydrofuran
THP	tetrahydropyran-2-yl
TMAD	*N*,*N*,*N'*,*N'*-tetramethylazodicarboxamide

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