博士学位論文

ピルビン酸脱水素酵素キナーゼ(PDHK)に対する

新規低分子阻害剤の創薬研究

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序論

　生物は食事などで得た栄養源から、生命活動に必要な様々な物質を産生している。三大栄養素（糖質・脂質・タンパク質）はグルコース・脂肪酸・アミノ酸に消化され、それぞれの代謝経路でエネルギー源が産生される。グルコースは解糖系の代謝経路でピルビン酸に分解され，その過程でエネルギー源となるアデノシン三リン酸（ATP）が合成される。ピルビン酸は更に，酸素が十分に供給される好気的条件下でミトコンドリア中のクエン酸回路に運ばれ，エネルギー産生に関与する。また，酸素が十分に供給されない嫌気的条件下では，ピルビン酸は細胞質内で乳酸に代謝される。

　ピルビン酸脱水素酵素（Pyruvate Dehydrogenase : PDH）は，ミトコンドリア内に存在し，ピルビン酸をアセチル CoA に変換する酵素である。PDH は解糖系と糖質酸化を繋ぐ，エネルギー代謝上重要な酵素である事から，PDH リン酸化 Kinase，脱リン酸化 Phosphatase により生体内で厳密に活性制御されている。PDH をリン酸化して不活性化する酵素が，ピルビン酸脱水素酵素キナーゼ（Pyruvate Dehydrogenase Kinase : PDHK）であり，4 種類のアイソフォームが存在する[1-3]。PDHK の誘導や活性化，及びそれに伴う PDH 活性の低下が，糖尿病，虚血性心疾患，末梢動脈疾患，肺高血圧症，癌など，様々な病態において確認されている[4-9]。この PDH 活性の低下が，解糖系と糖質酸化の連動不全状態を引き起こし，これらの疾患における糖代謝異常の要因となっている。

　本研究では，種々の病態における解糖系と糖質酸化の連動不全を是正する事を目的とし，PDHK に対する低分子阻害薬の創薬研究を実施した。この際，4 種の PDHK アイソフォームで構造が保存された ATP 結合サイトを標的部位に設定し，複合体構造情報を用いた化合物デザイン(Structure-based drug design : SBDD)を行った。分子サイズが小さい Fragment 化合物を起点化合物とし，SBDD 手法を用いたリード化合物の創出，並びに構造活性相関研究を行ったので，これを3 章にわたって論述する。

　第 1 章では，Fragment Screening によりリード化合物の起点化合物を探索した，Hit finding ステージの研究内容を報告する。ATP の Adenine 環が結合するポケット深部において，新規な化学構造と化学特性を併せ持つ新規ケモタイプの Hit 化合物を取得する事を目的として，PDHK4 に対して X-ray Fragment Screening を実施した。この際，得られた Fragment Hit の X 線複合体構造に対して，ポケット深部に存在する水分子のエネルギー解析を行う事で，既知阻害剤より活性・選択性プロファイルが優れた化合物の創出を試みた。

　第 2 章では，阻害活性向上を指向した Hit to Lead ステージの研究内容を報告する。Fragment Screening 由来の三環性化合物から，PDHK ATP 結合サイトのリボース・リン酸結合部位におけ

る SBDD を通じて，酵素阻害活性 IC_{50} 値を nM レベルに向上する事を検討した。既知阻害剤に比べて，分子サイズを抑えながらより強い阻害活性を有する化合物を見出すことで，経口でのPDHK 阻害薬開発に有利となるリード化合物の取得を目指した。

　第 3 章では，荷電性アミノ酸残基が複雑な水素結合ネットワークを形成する PDHK4 ATP 結合サイトに対して，相互作用エネルギー計算を通じた構造活性相関研究を行った内容を報告する。始めに，分子力学法を用いた種々の手法でエネルギー計算を実施したが，実測値との相関が得られなかった。このため，原子部分電荷を固定した分子力学法では精度を向上する事が難しいと考え，量子力学法であるフラグメント分子軌道法（Fragment Molecular Orbital Method : FMO 法）を用いた計算を検討した。この際，まずは効率よく FMO 計算を行うための計算条件を見出すことが重要であると考え， FMO 切り出しモデルの検討を行った。次に，見出した FMO 計算条件を用いた検討を PDHK4 に対して実施し，実測値との相関が高い結合自由エネルギー計算条件を探索した。また，量子力学計算結果の解析を通じて，PDHK ATP サイトでの水素結合ネットワークにおける電荷移動の詳細も解析した。

第 1 章　Fragment Screening による PDHK ATP 結合サイトでの新規リード化合物探索

第 1 節　背景と目的

　PDHK の活性中心である ATP 結合部位は C 末端領域に存在し，GHKL (Gyrase, Hsp90, Histidine Kinase, MutL) ATPase Kinase superfamily に属している[1, 2]。Protein kinase とは構造特徴が大きく異なり，　アスパラギン酸残基の側鎖を中心とした，水分子を介した水素結合ネットワークを活性中心で形成する。Figure 1-1a, 1-1b は基質ミミックである AMP-PNP と PDHK4 の X 線複合体構造を示しており，Adenine 環がポケット深部に存在する 4 つの水分子 (WAT1〜WAT4)と水素結合ネットワークを形成している[10]。GHKL family の既知阻害剤として Adenine 骨格や Resorcinol 骨格を有する化合物が知られており，何れもポケット深部に存在する水の水素結合ネットワークに介入する結合様式を X 線複合体構造で示している。Figure 1-1c に示す VER-24668 と PDHK2 との X 線複合体構造において，Resorcinol 骨格が 4 つの保存水の内 WAT4 を置換する結合様式となっている[11]。Adenine や Resorcinol 骨格は PDHK に対して一定の親和性を有するが，GHKL family に共通する水の水素結合ネットワークを保持する結合様式である事から，GHKL family に含まれる他のタンパク質に対しても同様の強度で結合する。このため，ポケット外側での構造変換を通じて選択性を獲得する必要があり，選択性を獲得した最終化合物は VER-24668 に示されるように分子構造が大きい（VER-24668 の分子量 = 553）。経口医薬品になりやすい化合物の特徴である Rule of five の分子量を超えており，経口での PDHK 選択的な阻害薬開発を狙うに際して，大きな課題となっている。
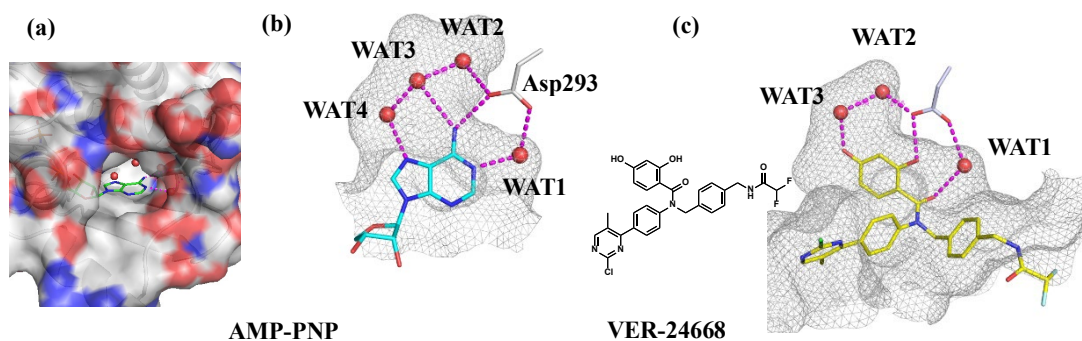

Figure 1-1. PDHK に対する X 線複合体構造。Red sphere は水分子，Dashed line は水素結合を表示

(a)　AMP-PNP (green color)と PDHK4 との X 線複合体構造(PDB ID = 2E0A)。PDHK4 のタンパク質表面を表示

(b)　(a)の構造の拡大図。タンパク質内部の表面を Gray mesh で表示

(c)　PDHK 既知阻害剤である VER-24668 (yellow color)と PDHK2 との X 線複合体構造(PDB ID = 4V25)

標的サイトに対して効率的に結合する化合物を見出す手法として，Fragment Screening が挙げられる[12]。High Throughput Screening (HTS)が，分子量 300〜500 位の化合物からリード候補化合物の取得を試みるのに対して、Fragment Screening では分子量 100〜300 位の複雑性の低い化合物をスクリーニングする。分子量の小さい化合物を対象とする事で，相対的に化合物の複雑性は低下し，親和性は弱いが標的に結合する可能性は高くなる。このため，Fragment Screening のヒット率は HTS よりも高い値が一般的に得られる（Figure 1-2）。　また、結合親和性を非水素原子数で割ることで計算される Ligand Efficiency (LE)値が、HTS で得られるヒットより良いものが得られることが多いのも特長である。課題は弱い親和性の Fragment Hit を、どのようにして LE 値を落とすことなく迅速にリード化合物にまで仕上げていくかであり、SBDD の手法を活用することが重要である。初期スクリーニングの Hit 率を高め、Hit の弱い親和性を SBDD の手法でフォローアップし、HTS とは異なる化学構造のリード化合物を探索する、というのがフラグメントスクリーニングの根本的な考えである。

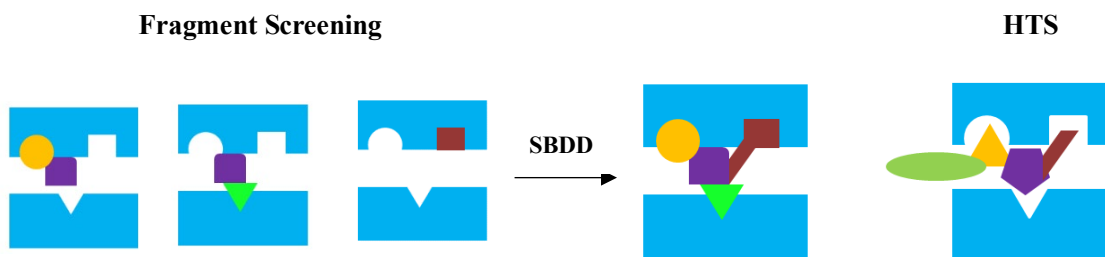**Figure 1-2.** Fragment Screening の概念図

HTS が分子量 300〜500 程度の化合物をスクリーニングするのに対して，Fragment Screening では分子量 100〜300 程度の複雑性の低い化合物をスクリーニングすることで，より多くのヒット化合物を取得する。Fragment Hit の複合体構造情報を用い，Fragment Growing, Merge, Linking 等の SBDD アプローチにより，HTS とは異なるリード化合物の取得を試みる。

　本研究では，PDHK に対する Fragment Screening を実施する事で，既知化合物よりも効率的に結合する，すなわち LE の高い新規なケモタイプを取得する戦略を立てた。Resorcinol と同等以上の LE で結合する新規ケモタイプの取得を目指し，Fragment Screening を実施した。

第2節　実験項

第1項　WaterMapを用いた水和領域の解析

　水分子のエネルギー評価を行うWaterMap計算を実施する目的で，Schrödinger Release version 2018-1を用いて計算のインプット作成を実施した。PDHK4と化合物 **1** のX線複合体構造（PDB ID = 7EAT）を初期構造として使用し，Maestroに含まれるProtein Preparation Wizardにより，分子への水素付加と互変異性状態を決定した。この際，化合物 **1** の周囲 8 Å 以内に含まれる水分子は保存し，デフォルトの計算設定で初期構造を作成した。WaterMap計算は，Binding siteの指定にFragment Hit **1** の結合座標を使用し，OPLS3e力場を用いて 2 ns の MD 計算を実施した。

第2項　吸光法による PDHK 酵素阻害活性測定

　PDHK反応後の残存 PDH 活性を測定する既報の手法に従い，酵素阻害活性評価を実施した[13]。まず，Sigma-Aldrichより購入した porcine PDH 複合体に対して，recombinant PDHK (1, 2, 3 or 4) を終夜 4 ℃でインキュベートすることで PDH/PDHK 複合体を形成した。その後，各 PDHK の K_m 値に相当する ATP と評価対象化合物を添加し，室温で 45 分インキュベート後，PDH の基質 (5.0 mM coenzyme A, 5.0 mM sodium pyruvate および 12 mM β-nicotinamide adenine dinucleotide)を加えて室温で 90 分 PDH 反応を進行させた。PDH 反応前後の NADH 量を，340 nm の吸光度測定で定量する事により，阻害%を算出した。得られた阻害%から，阻害活性 IC_{50} 値を TIBCO-Spotfire を用いて算出した。

第3項　X線複合体解析

　PDHK2, PDHK4 のタンパク質生産，精製は既報の手法に従い実施した[14, 15] (PDHK4：残基番号 10-411, PDHK2 :残基番号 16-407)。精製後の PDHK4 タンパク質を，50 mM KHPO₄ pH 7.5, 1.7 M ammonium sulfate, 4% (v/v) PEG400 および 5 mM ADP を用い，ハンギングドロップ法により結晶化することで，X線複合体解析で利用する PDHK4 タンパク質を取得した。

　X-ray Fragment screening は，JT 医薬総合研究所にて有する Fragment Library 638 化合物を用い，4 化合物をそれぞれ 12.5 mM の濃度で含む化合物溶液を PDHK4 に対して添加する事で実施した。24 時間 soaking 後に放射光施設 (Photn Factory (Japan), SPring-8 (Japan), Canadian Light Source (Canada) および Swiss Light Source (Switzerland))での結晶構造解析を実施した。PDHK2 に対する結晶化検討は，50 mM sodium acetate pH 5.5, 100 mM magnesium chloride および 8% (v/v) isopropanol を用いたハンギングドロップ法での共結晶化により実施した。

　PDHK4 複合体および PDHK2 複合体の初期モデルは，それぞれ Protein Data Bank (PDB) デー

タ（PDB ID：2ZKJ と 2BTZ）を用いた分子置換法で Coot ソフトウェア[16]を用いて構築し，CCP4 suite ソフトウェアの REFMAC5[17]を用いて精密化した。得られたデータは，次の PDB コードで登録した（PDHK4-化合物 **1**：7EAT, PDHK4-化合物 **2**：7EBB, PDHK2-化合物 **1**：7EA0, PDHK2-化合物 **2**：7EAS, PDHK2-化合物 **10**：7EBH, PDHK2-化合物 **13**：7VBU）。

第 4 項　HSP90 阻害活性測定

BPS Bioscience 社のアッセイキット (catalogue number：50293)を用いて，阻害活性を算出した。

第 5 項　BCKDK 阻害活性測定

Promega Corporation の ADP-GLO™ を用い，BCKDK (Branched Chain Keto Acid Dehydrogenase Kinase) の阻害活性評価系を構築した。Human recombinant BCKDK に 0.2 μM の ATP と化合物を添加し，37 ℃で 60 分インキュベートした。その後，ADP-GLO™ 試薬を添加して室温で 40 分インキュベーションを行い，kinase 反応を終了させた。その後，Kinase Detection 試薬を加えて室温で更に 40 分インキュベーションを行う事で，ADP を ATP に変換し，ATP 量を発光強度で定量した。

第 6 項　Protein Kinase Panel Assay

DiscoveRx 社の KINOMEscan™ アッセイサービスを用いて，59 種の Protein Kinase に対する選択性評価を実施した。

第3節　結果と考察

第1項　X-ray Fragment Screening による新規ケモタイプの取得

　PDHK ATP 結合サイトにおいて，新規な化学構造と化学特性を併せ持つ新規ケモタイプを取得する事を目的として，X-ray Fragment Screening を実施した。JT 医薬総合研究所で有する 638 種の Fragment ライブラリー化合物を用い， PDHK4 に対してソーキング法での X-ray Fragment Screening を実施し，17 種類の化合物が個別に結合した X 線複合体構造を取得した。17 化合物は全て ATP 結合サイトに結合しており，その中から 2 種の Fragment を起点化合物として選抜した (Figure 1-3)。第 1 節で記載したように，ATP サイトでの既知阻害剤はポケット深部に存在する 4 種の保存された水分子の内 3 種以上を活用し，水素結合ネットワークに介入する結合様式を示している。一方で，Fragment Hit **1** は保存水の内 2 種（WAT3, WAT4）を Phenyl 基で排斥し，既知化合物よりも ATP サイトの深部に結合する特徴が認められた。活性中心の Asp293 とは，骨格のシスアミド部分が WAT1 含めた水素結合ネットワークに介入する結合様式を与えた。ポケットに深く入り込む事で，既知阻害剤に比べて親和性・選択性を向上する事が期待できると考え，新規な結合様式を示す Fragment Hit **1** を起点化合物として選抜した。もう一方の Fragment Hit **2** は，シアノ基が WAT4 を置換し，残る 3 種の保存水と水素結合ネットワークを形成する結合様式が認められた。この化合物は，骨格部の結合様式が Fragment Hit **1** と良い重なりを示すことから，Fragment Merge による SBDD 最適化が可能になると期待し，選抜した。
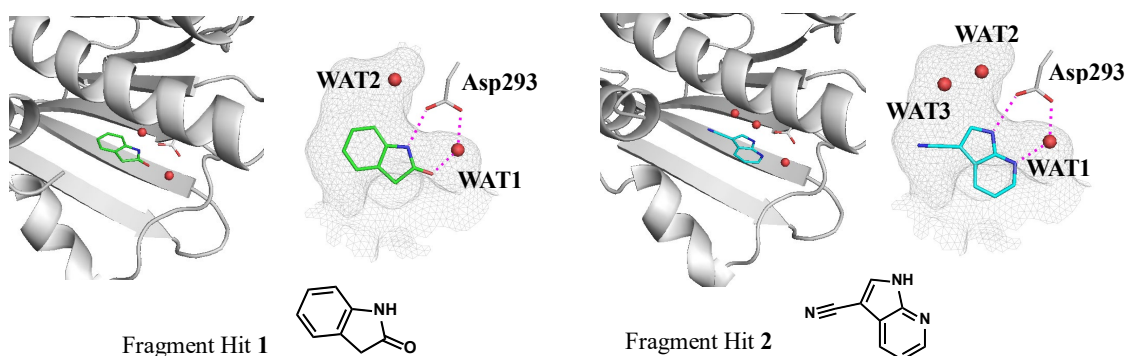

Figure 1-3. PDHK4 に対する X-ray Fragment Screening で選抜した 2 種の Fragment Hit の化学構造式と X 線複合体構造。PDHK4 の構造を Gray cartoon, ATP 結合サイトの表面を Gray mesh で表示。Red sphere は水分子，Dashed line は水素結合を表示。

　2 種の Fragment Hit に対して，PDHK4 酵素阻害活性の測定と，PDHK2 との X 線複合体構造取得を実施した。その結果，何れの化合物も 2.0 mM まで PDHK4 酵素阻害活性が認められなかった。また，PDHK2 との X 線複合体に関しては，ATP サイトでのアミノ酸配列は PDHK アイソフォーム間で保存されている事を反映し，PDHK4 と同様の結合様式が認められた。

第 2 項　Fragment Growing によるリード候補化合物の探索

　得られた Fragment Hit **1** から，SBDD による親和性向上を検討した。PDHK4 との X 線複合体構造を解析した所，carbonyl の α 位方向に Val298, Ile303, Leu306, Leu350 側鎖から構成される脂溶性サイトが認められた(Figure 1-4)。このサイトに向かって Me 基を付与した化合物 **3** を合成・評価した所，PDHK2, PDHK4 に対する阻害活性の向上が認められた。
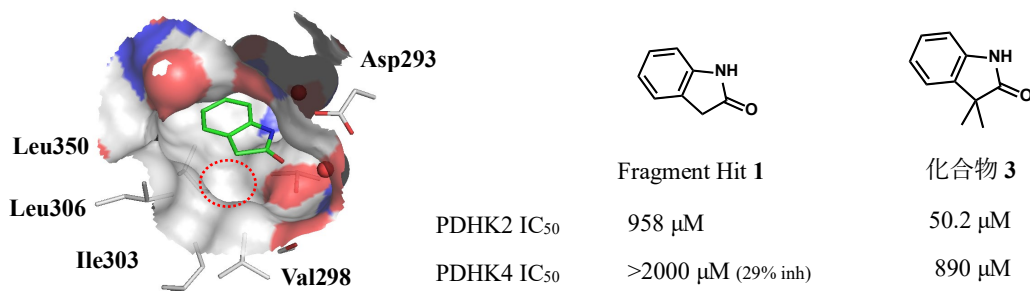

	Fragment Hit **1**	化合物 **3**
PDHK2 IC$_{50}$	958 μM	50.2 μM
PDHK4 IC$_{50}$	>2000 μM (29% inh)	890 μM

Figure 1-4. PDHK4 のタンパク質表面を原子種で色分けして表示（C：gray, O：red, N：blue）。

　Fragment Hit **1** から更に親和性を向上する方策を立てる目的で，WaterMap ソフトウェアを用いた水和領域の解析を実施した。WaterMap は，分子動力学（Molecular Dynamics：MD）計算で得られる計算トラジェクトリーから，水の存在密度が高い水和サイトの検出，並びにバルク水に比較したエネルギー状態を算出するプログラムである。バルク水は，水-水間で平均して 3〜3.5 本の水素結合を形成しており，エンタルピーとして安定な状態にある。また，構造自由度も高いことから，バルク水はエントロピーとしても安定な状態にある。このバルク水に比較したエンタルピー・エントロピー値，並びに自由エネルギー値を WaterMap 計算で算出し，どの水をどのように排斥すべきかの考察を行った。

　Fragment Hit **1** の PDHK4 との X 線複合体構造に対する WaterMap 計算結果を，Figure 1-5 に示す。WAT1 は周辺の PDHK4 残基，並びに Fragment Hit **1** と 4 点の水素結合を形成しており，エンタルピー値・自由エネルギー値がバルク水より安定な水和サイトとして検出された。置換することによるエネルギーの獲得が困難な構造水であると判断し，WAT1 は保存する事とした。一方，WAT2 はエンタルピー・エントロピー値が共にバルク水より不安定な水和サイトとして検出された。WAT2 は，Asp293 側鎖に加えて Ile291, Thr358 主鎖カルボニル基に近接するが，これら残基とは水素結合形成の理想角度から大きく離れている。この事を反映し，WAT2 におけるエンタルピー値はバルク水より不安定な値を与えている。同サイトでの極性基導入は，脱溶媒和のペナルティーを考慮するとエネルギー的に不利であると考えられる。そこで，脂溶性基による不安定水

の排斥が有利なアプローチであると判断し，WAT2 方向に対して脂溶性基の導入を検討した。
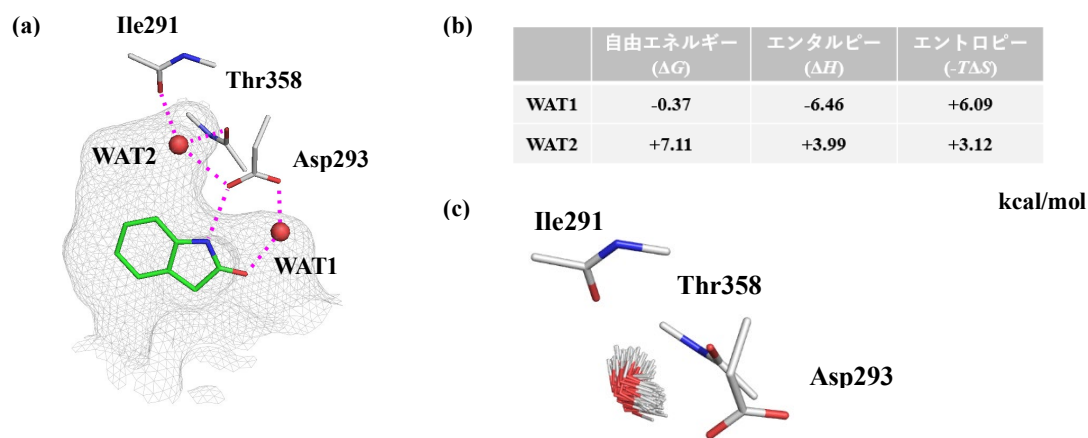
Figure 1-5. Fragment Hit **1** と PDHK4 の X 線複合体構造に対する WaterMap 計算結果

(a)　Fragment Hit **1** と PDHK4 の X 線複合体構造

(b)　2 種の水和サイトにおける熱力学エネルギーProfile。Bulk 水と比較した自由エネルギー (ΔG)，エンタルピー(ΔH)，エントロピー($-T\Delta S$)値を表示。

(c)　WAT2 水和サイトにおける水の MD Trajectory。

化合物 **3** の WAT2 方向に置換基導入を行った結果を Table 1-1 に示す。脂溶性基の導入による阻害活性値の向上が認められ，Cyclopropyl を導入した化合物 **5** において PDHK2 = 4.9 μM, PDHK4 = 43.6 μM の酵素阻害 IC_{50} 値が認められた。Methyl Amine を導入した化合物 **7** において更なる活性値の向上が認められ，PDHK2 IC_{50} = 0.59 μM, PDHK4 IC_{50} = 12.8 μM の阻害活性値を与えた。化合物 **7** の脱 Methyl 体である化合物 **8** においては，PDHK2 および PDHK4 に対する IC_{50} は共に 300 μM 以上であり，Me 基の有無で大きな阻害活性差が認められた。

Table1-1.　R1 置換化合物の PDHK2, PDHK4 阻害活性

	Compound	**3**	**4**	**5**	**6**	**7**	**8**
	R1	H	Et	*⟨▷	OMe	NHMe	NH₂
IC_{50}	PDHK2	50.2	11.0	4.9	Not tested	0.59	317
(μM)	PDHK4	890	304	42.6	842	12.8	455
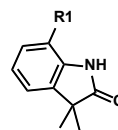
Figure 1-6 に，化合物 **7** と PDHK4 との X 線複合体構造を示す。不安定な水である WAT2 が占有していた領域を Methyl Amine が占めており，想定した結合様式が X 線複合体構造において認められた。化合物 **8** の Aniline 部位は，化合物 **7** と同様に Asp293 と水素結合を形成すると考えられるが，WAT2 を排斥するには距離が短く，WAT2 を排斥する化合物 **7** と大きな阻害活性差が生じたと考えられる。
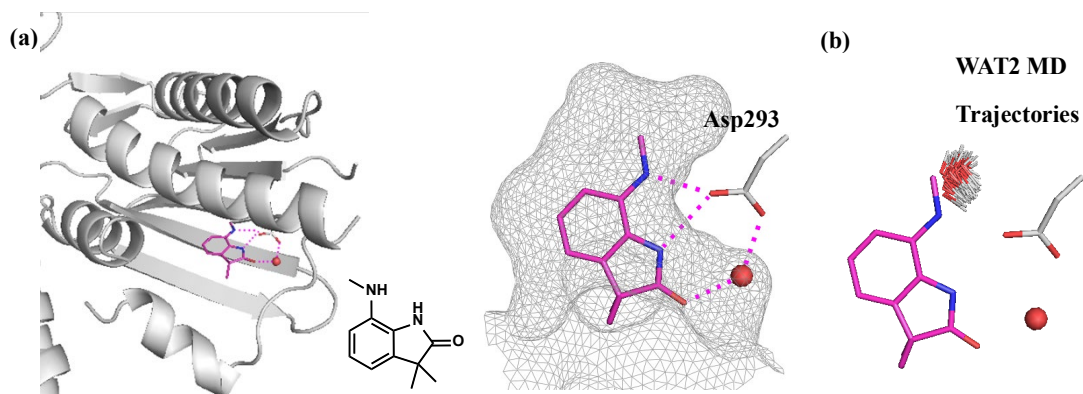
Figure 1-6. 化合物 **7** と PDHK4 の X 線複合体構造

(a) PDHK4 の構造を Gray cartoon，タンパク質内部の表面を Gray mesh で表示。Red sphere は水分子，Dashed line は水素結合を表示。

(b) WaterMap 計算時の WAT2 MD Trajectory と化合物 **7** の X 線複合体結合様式の重ね合わせ図

　Fragment Growing の結果得られた化合物 **7** に対する，4 種の PDHK アイソフォームに対する酵素阻害活性の評価結果を Table 1-2 に記載する。PDHK ATP 結合サイトの構造はアイソフォーム内で保存されている事から，PDHK1～4 何れに対しても阻害活性が認められた。阻害活性値と非水素原子数から算出した Ligand Efficiency (LE)値が，0.48～0.63 と高い値を示しており，分子サイズに対して効率的な相互作用を取っている事が示唆される。

　次に，化合物 **7** の選択性プロファイルを Table 1-3 に示す。先行品である Resorcinol 化合物は，PDHK が含まれる GHKL kinase superfamily に共通する特徴であるポケット内部の水を保存した相互作用を形成しており，GHKL family 内での選択性をポケット内側の構造体では有していない。一方，化合物 **7** はポケット内部の構造水を排斥し，ポケット深部の領域を Me 基で占有している。ポケット深部の PDHK に特徴的な残基と直接相互作用を形成している事を反映し，他の GHKL family である HSP90・BCKDK に対する選択性が認められた。また，59 種の Protein Kinase に対する Panel 評価を実施した所，10 μM で 50%を超える酵素阻害作用を示した標的は MTOR と MAST1 の 2 種のみであり，良好な選択性プロファイルが確認された。

Table1-2. 化合物 **7** の PDHK1～4 に対する阻害活性値と Ligand Efficiency (LE)

Target	IC$_{50}$ (μM)	LE
PDHK1	0.37	0.63
PDHK2	0.59	0.61
PDHK3	8.4	0.50
PDHK4	12.8	0.48

Table1-3. 化合物 **7** の GHKL family, Protein Kinase に対する選択性

Family	Target	Inhibition (%) [a]
GHKL	HSP90	3.2 (30 μM)
	BCKDK	14 (500 μM)
Protein Kinase (10μM)	44 kinases	< 20
	13 kinases	20-50
	MTOR	58
	MAST1	60

a. カッコ内の値は化合物濃度

第 3 項　**Fragment Merge によるリード候補化合物のケモタイプ拡充**

　ケモタイプの拡充を目的として，次に Fragment Hit **2** との Merging を検討した。Figure 1-7 に，化合物 **7** と Fragment Hit **2** における PDHK4 X 線複合体の重ね合わせ図を示すが，骨格部が良い重なりを示している。重ね合わせ結果から，三環性骨格を Design し，周辺化合物の合成・評価を実施した。

Compound 7　　　**Fragment Hit 2**　　　**Tricyclic core**
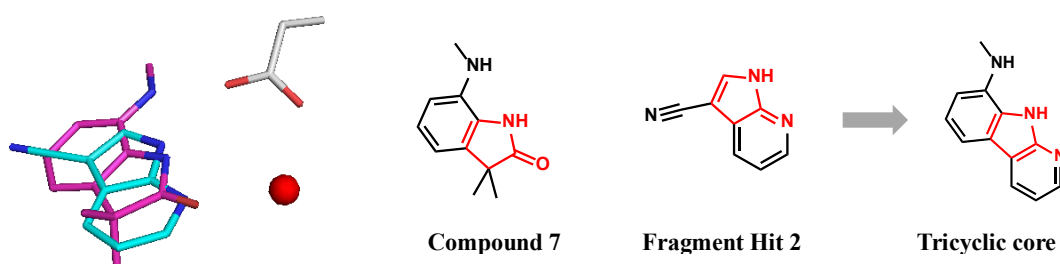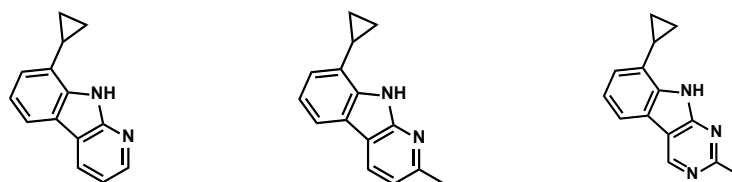
Figure 1-7. 化合物 **7** (purple)と Fragment Hit **2** (cyan)の重ね合わせ図

合成難易度を考慮し，Cyclopropyl 基をポケット深部に配置する化合物 **5** との Merging を実施した(Table 1-4)。Merge 化合物 **9** において，化合物 **5** と同等の PDHK2 阻害活性が認められた。PDHK4 に対する IC_{50} 値が減弱しているが，これは溶解度が低下した事に伴い高濃度での評価が実施できなかったためであると考えている。Pyridine 2 位の近傍に，2 種の Gly 残基 (PDHK4：Gly 295, Gly297)の主鎖カルボニル基が PDHK で保存されており，Me 基の導入による CH-O 相互作用形成を期待した化合物 **10** において，阻害活性が向上した。更に，骨格構造を Pyridine から Pyrimidine に変換した化合物 **11** において，PDHK2, PDHK4 両者に対する阻害活性の向上が認められた。骨格を Pyrimidine に変換する事で，誘起効果により Me 基の酸性度が向上し，CH-O 相互作用が強くなった影響であると考察している。

Table1-4. 三環性化合物の PDHK2, PDHK4 阻害活性

		9	**10**	**11**
Compound				
IC_{50}	PDHK2	3.5	2.0	0.71
(μM)	PDHK4	>300 (20%)	12.0	5.9

　化合物 **7**, **11** で得られた構造活性相関情報を Fragment Hit **2** の基本骨格である二環性骨格に転用し，更なるケモタイプ創出を実施した。Figure 1-8 で示すように，化合物 **11** の化学構造情報を活用し，Fragment Hit **2** の基本骨格を 2-Me pyrimidine に変換した。その上で，化合物 **11** の Cyclopropyl 基，化合物 **7** の Me-Amine と同様の領域を占有するために，R1 側鎖部に 3 原子分の長さに相当する置換基を導入する事を計画した。
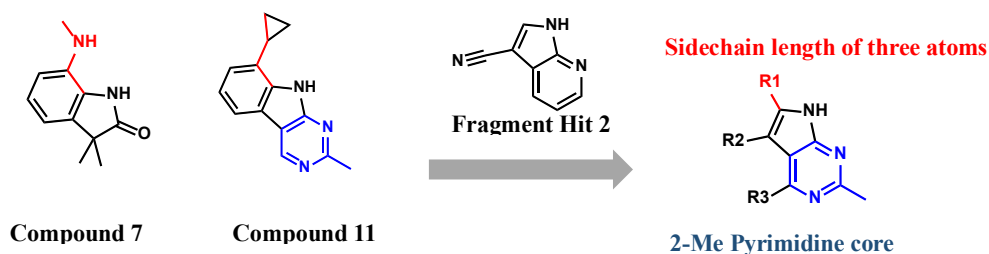
Figure 1-8. 化合物 **7** と化合物 11 の化学構造式の重ね合わせ結果から，二環性化合物をデザイン

二環性化合物の阻害活性結果を Table 1-5 に示す。3 重原子長の置換基として *n*-Pr 基を導入した化合物の合成を行い，化合物 **12** において PDHK2 阻害活性が弱いながら認められた。更に，化合物 **7** のベンゼン環が占有する領域を最適化する目的で R² 部位の置換基変換を行い，ハロゲン基を導入した化合物 **13, 14** において，PDHK2 に対する IC$_{50}$ 値が一桁 μM にまで向上した。

次に，PDHK 活性中心の Asp 側鎖と極性相互作用を形成する目的で，*n*-Pr 部位の置換基変換を実施した。化合物 **15** の Pyrimidine 4 位に Cl 基を導入した **16** において阻害活性の向上が認められたため，周辺化合物は Cl 基を導入した骨格で合成を行った。Amide NH による極性相互作用形成を指向した化合物 **17, 18** を合成したが，これらの化合物では PDHK に対する阻害活性が大きく減弱した。アルケン C-H による CH-O 相互作用形成を指向した化合物 **19** では，PDHK2 と PDHK4 の IC$_{50}$ 値が何れも 10 μM 以下にまで向上した。
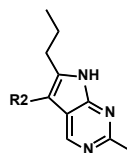
Table1-5. 二環性化合物の PDHK2, PDHK4 阻害活性

Compound		**12**	**13**	**14**	**15**
R2		CN	Cl	Br	Me
IC$_{50}$	PDHK2	>30 (34%)	1.8	2.2	14.7
(μM)	PDHK4	>30 (8.5%)	11.0	12.4	>30 (17%)

		16	**17**	**18**	**19**
Compound					
IC$_{50}$	PDHK2	9.9	42.8	>30 (21%)	1.5
(μM)	PDHK4	>30 (45%)	>100 (34%)	>30 (3.1%)	8.0

新たに取得したケモタイプである二環性化合物 **14** と三環性化合物 **10** に対して，X 線複合体構造の取得を検討した。PDHK2 との X 線複合体構造の方が高い分解能を与える事から，二種の化合物に対しては PDHK2 との X 線複合体構造解析を実施した。得られた X 線複合体構造を Figure 1-9 に示す。二環性化合物 **14** と三環性化合物 **10** は何れもポケット深部に存在した WAT2 を排斥し，脂溶性置換基がポケット深部を占有する想定した結合様式を与えた。また，Me 基が Gly292, 294 主鎖カルボニル基に近接する結合様式を与えており，指向した CH-O での相互作用

形成と合致する結合様式が X 線複合体構造において認められた。
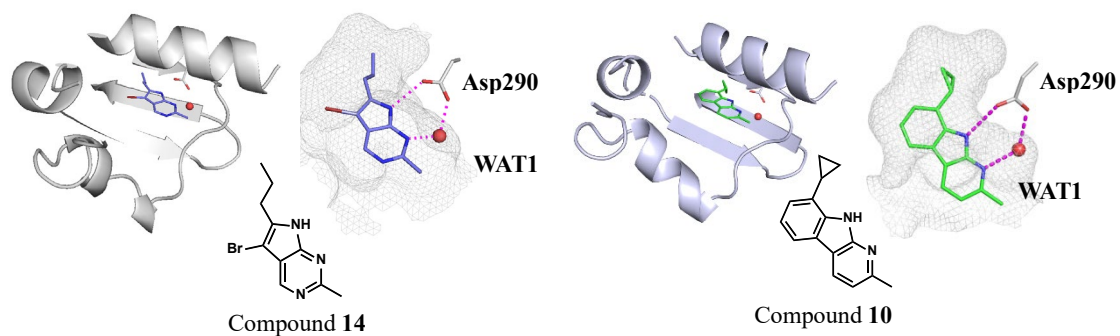
Figure 1-9. Fragment Merge により見出した，二環性化合物 14 と三環性化合物 10 の化学構造式と X 線複合体構造

PDHK2 の構造を Gray cartoon，タンパク質内部の表面を Gray mesh で表示

Red sphere は水分子，Dashed line は水素結合を表示。

小括

Fragment-based Drug Discovery の手法を用い，PDHK ATP サイトに結合する新規ケモタイプでのリード候補化合物を 3 種（化合物 **7, 11, 14**）を取得した。X-ray Fragment Screening Hit からの Growing および Merge により得られた，代表化合物の PDHK 阻害活性値と LE 値を Table 1-6 に示す。Fragment Screening により，先行品である VER-24668 と同じ Resorcinol 骨格を有する化合物 **20** も Hit として得られており，比較のためにデータを示す。化合物 **20** は，PDHK2 に対する阻害活性値が 192 μM，PDHK4 に対する阻害活性 IC$_{50}$ 値が 345 μM であり，LE 値はそれぞれ 0.42, 0.39 である。Fragment Hit **1** は Resorcinol 骨格よりもポケット深部に深く入り込む結合様式を示し，PDHK2 に対して同等の LE 値 (0.41)を示した。ここから，WaterMap 計算を用いた水和領域の解析を行い，ポケット深部に存在する WAT2 を脂溶性基で排斥する SBDD 戦略を立てて，周辺化合物の合成・評価を実施した。その結果，阻害活性値を向上しながら LE 値を改善した化合物 **5, 7** を取得することに成功し，化合物 **7** の X 線複合体構造において想定した結合様式を確認した。PDHK は GHKL family に属しており，ポケット深部に存在するアスパラギン酸残基周辺に水を介した水素結合ネットワークが存在する。この水素結合ネットワークは GHKL family 内で保存されており，水素結合ネットワークを保持しながら結合する Resorcinol タイプの既知化合物は，ポケット内側のみ占める構造では family 内での選択性が認められない。一方で，本研究で取得した化合物はポケット深部に入り込む結合様式をとっており，ポケット内のみ占める化合物 **7** の分子サイズで，GHKL family に属する HSP90, BCKDK との選択性を発現している点が特長として挙げられる。阻害活性が向上した化合物 **5** と Fragment Hit **2** との Merge を行う事で，三環性化合物 **11** と二環性化合物 **14** も併せて取得した。

Table1-6. 代表化合物の PDHK2, PDHK4 阻害活性 IC$_{50}$ 値（μM)と Ligand Efficiency

Compound	**20**	**1**	**5**	**7**	**11**	**14**
重原子数	12	10	15	14	17	14
PDHK2	192	958	4.9	0.59	0.71	2.2
(LE)	(0.42)	(0.41)	(0.48)	(0.61)	(0.49)	(0.55)
PDHK4	345	>2000	43.6	12.8	5.9	12.4
(LE)	(0.39)		(0.40)	(0.48)	(0.42)	(0.48)

第 2 章　PDHK 複合体構造情報を用いた分子設計による阻害活性向上

第 1 節　背景と目的

　第 1 章では，Fragment Screening により Lead 化合物の起点化合物を探索した，ヒット探索ステージの研究内容を報告した。阻害活性 IC_{50} 値が mM レベルの Fragment Hit 化合物から，PDHK との複合体結晶構造情報を活用した SBDD を実施し，μM レベルの阻害活性と GHKL-family 内での選択性を有する 3 種類のリード候補化合物を見出した。第 2 章では，得られたリード候補化合物から更なる SBDD を実施し，阻害活性 IC_{50} 値を nM レベルに向上した研究内容を報告する。

　3 種類のリード候補化合物の内，類縁体の合成難易度を考慮し，三環性化合物である化合物 **10** を代表化合物として選抜した。Figure 2-1 に，化合物 **10**，並びに VER-24668 と PDHK2 との X 線複合体構造[11]を示す。化合物 **10** は VER-24668 よりも深く ATP サイトに結合し，ポケット内側に存在するアデニン環結合部位は，空間的にほぼ満たされた状態にある。更なる活性向上を化合物 **10** から図るに際して，ATP サイトのポケット外側に向かって置換基を伸長する事とした。
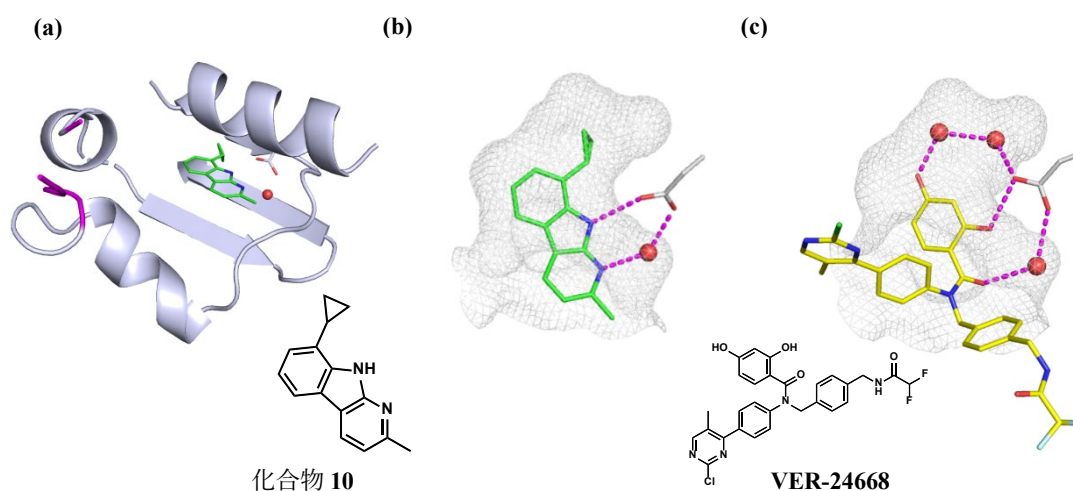
Figure 2-1. PDHK2 の X 線複合体構造。Red sphere は水分子，Dashed line は水素結合を表示
(a)　化合物 **10** との X 線複合体構造。PDHK2 の構造を Gray cartoon で，Pocket 外側に位置する ATP lid 領域 を magenta で表示。
(b)　化合物 **10** との X 線複合体構造の拡大図。タンパク質内部の表面を Gray mesh で表示
(c)　VER-24668 の X 線複合体構造

　PDHK ATP サイトのポケット外側は，ATP のリボースが結合する部位とリン酸が結合する部位から構成され，ATP のリン酸をトラップする役割を担う ATP-lid と呼ばれる領域が近傍に存在する。ATP-lid は構造変化が大きく，Figure 2-1 に示す X 線複合体構造においても，電子密度が

観察されず構造が一様でない。先行品である VER-24668 は，GHKL-family での選択性を有さない resorcinol 骨格からポケット外側への合成展開を行い，活性と選択性を向上している[11]。その結果分子サイズが大きくなっており（VER-24668 の分子量 ＝553），経口での PDHK 阻害薬を開発するに際して大きな課題となっている。このため，化合物 **10** のポケット外側での合成展開を進めるに際して，分子サイズを抑えながら阻害活性を向上する事が重要となる。そこで，合成展開に合わせて適宜複合体構造を解析し，PDHK タンパク質との相互作用を効率的に獲得する戦略を取ることにした。

第 2 節　実験項

第 1 項　**WaterMap を用いた水和領域の解析**

　　Schrödinger Release version 2019-1 を用いて，WaterMap 計算のインプット作成と計算を実施した。PDHK2 と化合物 **22** の X 線複合体 chain B 構造（PDB ID = 7VBV）を初期構造として使用し，Maestro に含まれる Protein Preparation Wizard により水素付加と互変異性状態を決定した。この際, Ligand 周囲 8 Å 以内に含まれる水は保存し, Default の設定で初期構造を作成した。WaterMap 計算は，Binding site の指定に化合物 **22** の結合座標を使用し，OPLS3e 力場を用いて 2 ns の MD 計算を実施した。

第 2 項　**PDHK2 に対する複合体モデル構築**

　　Schrödinger Release version 2019-1 を用いて，複合体モデル構築のインプット作成と計算を実施した。PDHK2 と化合物 **35** の X 線複合体構造（PDB ID = 7VBX）を初期構造として使用し，Protein Preparation Wizard により水素付加と互変異性状態を決定後，Glide を用いてドッキング計算の Grid ファイルを作成した（デフォルト計算設定値を利用）。Glide SP モードを用いて，ドッキング計算を実施後，OPLS3e 力場を用いた Macromodel での構造最適化計算を行い，複合体モデルを作成した。

第 3 項　**吸光法による PDHK 阻害活性測定**

　　第 1 章の第 2 項に示す手法と同様の手法で，阻害活性測定を実施した。

第 4 項　**X 線複合体解析**

　　第 1 章の第 3 項と同様の手法で，PDHK2 との X 線複合体解析を実施した。得られたデータは，次の PDB コードで Protein Data Bank に登録した（PDHK2-化合物 **22**：7VBV, PDHK2-化合物 **35**：7VBX）。

第 5 項　**BCKDK 阻害活性測定**

第 1 章の第 5 項と同様の手法で，阻害活性測定を実施した。

第3節　結果と考察

第1項　三環性化合物 C3 位からの SBDD による活性向上の検討

初めに，三環性化合物 C3 位に対して置換基導入を行い，Adenine 結合領域近傍での構造活性相関（SAR; Structure Activity Relationship）データを取得した（Table 2-1）。*N*-methyl Amide (**21**), phenyl (**23**), 2-Pyridine (**24**), 1,3,4-thiadiazole (**26**)の阻害活性値は，無置換体である化合物 **10** と同等の値を示した。一方，Methyl ester (**22**), Ester 等価体である oxadiazole (**25**)において，PDHK2/4 に対して 4 倍から 10 倍の阻害活性値の向上が認められた。

Table2-1. R1 置換化合物の PDHK2, PDHK4 阻害活性
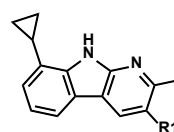
Compound		10	21	22	23	24	25	26
R1		H	CONHMe	CO₂Me	Phenyl	2-Pyridine		
IC$_{50}$	PDHK2	2.03	5.51	0.21	5.33	1.51	0.49	1.49
(µM)	PDHK4	11.87	>30 (35%)	1.54	7.49	2.95	1.51	6.62

Ester 基導入での活性向上要因を把握する目的で，化合物 **22** に対して PDHK2 との X 線複合体構造を取得した。得られた X 線複合体構造を Figure 2-2 に示すが，ATP-lid の配座が異なる 2 種類の構造を含む結晶が取得された。Figure 2-2a に示す複合体においては，ATP-lid の電子密度は不明瞭で，構造は一様でない。一方，もう一種類の複合体構造である Figure 2-2b においては，ATP-lid が化合物方向に倒れこむ配座で構造化し，Phe326 側鎖が化合物とスタッキングする新規な結合様式が認められた。該当する領域の拡大図を Figure 2-2c に示しており，Phe326 側鎖が Ester Carbonyl と π-π 相互作用，また Arg258 側鎖と cation-π 相互作用を形成し得る位置を占めていた。化合物 **22** と **25** は，その他の化合物に比べて三環性骨格に対して R1 部の置換基が平面的な構造を取りやすく，Figure 2-2b の相互作用形式の方が SAR との整合性が高い。この事から，Figure 2-2a より 2-2b での相互作用形式が活性発現状態を反映すると判断し，2-2b での結合様式を基に更なる SBDD の検討を行う事とした。

得られた X 線複合体構造を用い，第 1 章第 3 項で論述した WaterMap を用いた水和領域の解析を行った所，Lys299・Arg302・Leu303 に近接する領域において，不安定な水和領域が認められた（Figure 2-3）。該当する水和サイトは，エンタルピー・エントロピー値が共にバルク水より

不安定な水として検出されており，自由エネルギーとしてバルク水に比べて 5.4 kcal/mol 高い値を与えた。エンタルピー値が不安定な領域であることから，極性基導入はタンパク質とリガンドの脱溶媒和ペナルティーを補填する事が困難であると考えられ，脂溶性置換基による不安定水の排斥が有効な手法であると考察した。

Compound 22
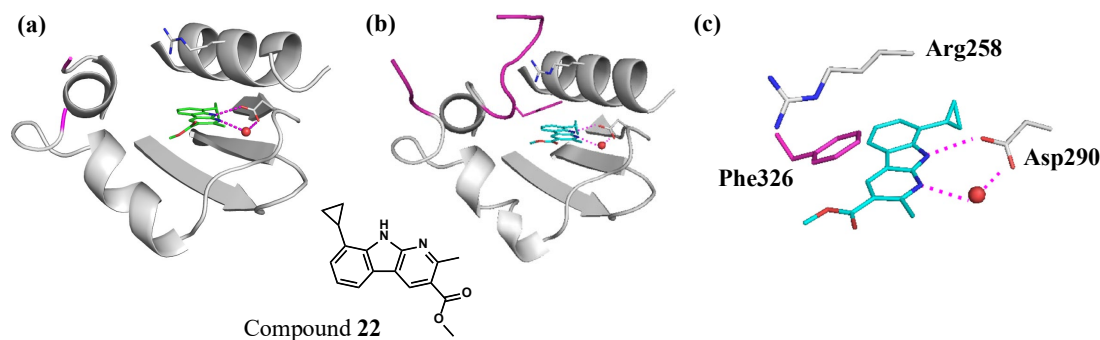
Figure 2-2. PDHK2 と化合物 **22** の X 線複合体構造。Pocket 外側に位置する ATP lid 領域 (magenta 表示) に関して，二種類の Conformation が認められた。Red sphere は水分子，Dashed line は水素結合を表示

(a)　X 線複合体 Chain A の構造。

(b)　X 線複合体 Chain B の構造。ATP lid 領域が構造化している。

(c)　(b) の拡大図。Phe326 が Arg258 と Ester 基の相互作用を仲介。

	ΔG	ΔH	$-T\Delta S$
5th	+5.4	+1.4	+4.0
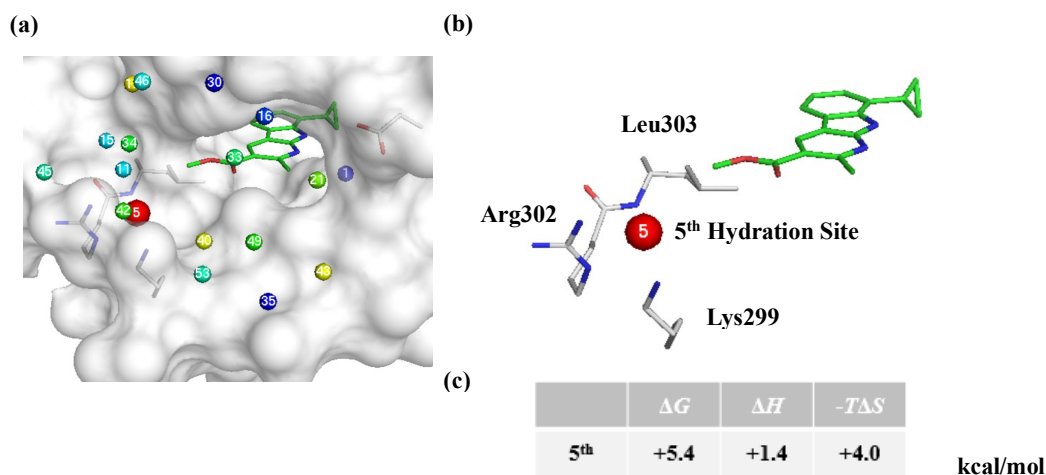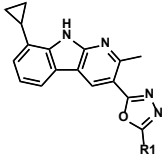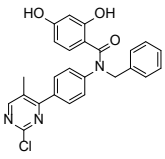
kcal/mol

Figure 2-3. 化合物 **22** と PDHK2 の X 線複合体構造に対する WaterMap 計算結果

(a)　WaterMap 計算で検出された水和サイト。自由エネルギー値に基づき色分け (-5.0 kcal/mol : blue ～ +5.0 kcal/mol : red)。球中の数字は，MD 計算での水の存在率の相対順位を示す。

(b)　最も不安定な自由エネルギー値を与えた，5 番目の水和サイト周辺の拡大図

(c)　5 番目の水和サイトにおける熱力学エネルギープロファイル。バルク水と比較した自由エネルギー (Δ*G*)，エンタルピー(Δ*H*)，エントロピー(-*T*Δ*S*)値を表示。

WaterMap で認められた不安定水の排斥を目的として，化合物 **25** の 1,3,4-oxadiazole 環 2 位に Alkyl 置換基を導入した化合物の SAR 結果を Table 2-2 に示す。Alkyl 鎖の伸長に伴い阻害活性値が向上し，化合物 **30** において PDHK2 と PDHK4 の IC_{50} がそれぞれ 0.056 μM と 0.462 μM であった。化合物 **31** は先行品である VER-24668 の部分構造であり，VER-24668 と同等の阻害活性を示すことが報告されている[11]。今回の評価系において，化合物 **31** の阻害活性値は化合物 **30** と同等の値が認められた。化合物 **31** の重原子数が 32 に対して，化合物 **30** の重原子数は 26 である事から，化合物 **30** は LE 値が改善し，より効率的に相互作用を取得している。

Table2-2. R_1 置換 1,3,4-oxadiazole 類縁体の PDHK2, PDHK4 阻害活性

	Compound	**25**	**27**	**28**	**29**	**30**	**31**
	R1	Me	Et	*i*-Pr	*n*-Pr	(isobutyl)	(構造式)
IC_{50}	PDHK2	0.494	0.190	0.130	0.091	0.056	0.035
(μM)	PDHK4	1.510	2.122	0.592	0.292	0.462	0.350

次に，更なる酵素阻害活性の向上を目的として，Arg258 と直接相互作用を形成する化合物を探索する事とした。ATP-lid は本来自由度の高い領域であるが，Figure2-2 で先述したように，Phe326 が化合物と π-π 相互作用，並びに Arg258 と cation-π 相互作用を形成して構造化している。化合物と Arg258 の相互作用を仲介するために構造化することで，エントロピーのペナルティーを伴っていると考えられる。リガンドの分子内で π-π 相互作用を形成して直接 Arg258 と cation-π 相互作用を取得することで，ATP-lid は自由度の高い構造に開放され，エントロピーの安定化効果が期待できると考えた。

化合物 **30** から，分子内で π-π 相互作用を形成する置換基をデザインする目的で，PDB database を対象とした三次元構造検索を実施した（Figure 2-4）。化合物 **30** の複合体モデルを基に，oxadiazole と Phe326 側鎖 Phenyl の二つの環の関係を三次元構造検索式に定義し，MOE/PSILO ソフトウェア[18]を用いた三次元構造検索を実施した。検索の際には，2 つの芳香環の中心環距離が 3.45 – 4.45 Å，芳香環の面角度が 5.2 – 24.8 度の範囲に絞り検索を実施した。得られた Hit 化合

物を目視で解析し，分子内 π-π 相互作用形成に活用しうる置換基の情報を抽出した。その結果，PDB ID が 1TCX の置換基情報が転用可能であると判断し，Merge 化合物である化合物 **32, 33** のデザイン・合成を実施した（Table 2-3）。Merge 化合物である **32, 33** は，ラセミ体で化合物 **30** に比べて PDHK2/4 の阻害活性値が何れも 10 倍程度向上する結果を与えた。また，GHKL family 内の BCKDK に対する選択性評価においても，良好な選択性プロファイルが確認された。

| Step1 : Generate Docking Pose | Step2 : Construct 3D search query | Step3 : Search PDB database and extract useful linker |

Aromatic centroid distance : 3.95 Å
Angle of planes : 9.80 degrees

PDB ID : 1TCX
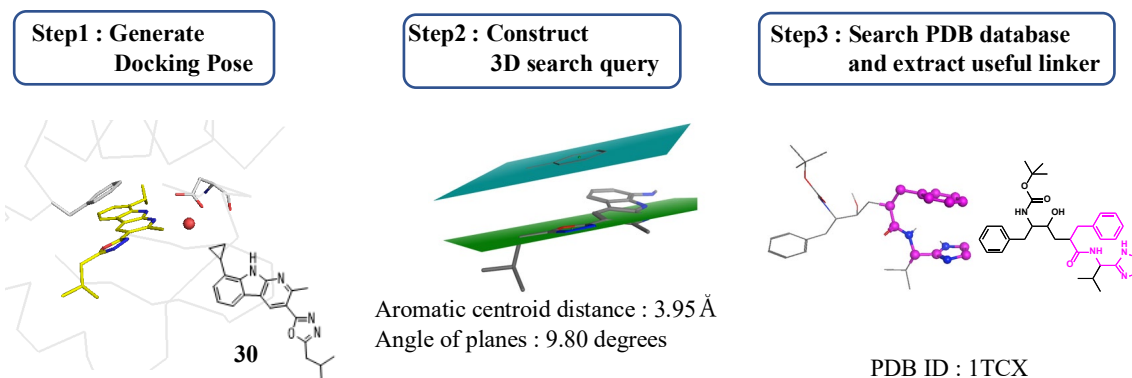
Figure 2-4.分子内 π-π 相互作用を形成する Ligand の検索手順。化合物 **30** の複合体モデルから三次元検索式を構築し，PSILO PDB データベースに対して分子内 pi-pi 相互作用を形成する Ligand を検索
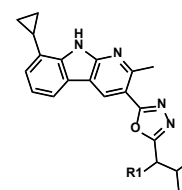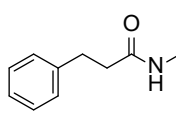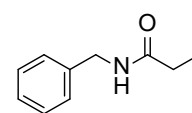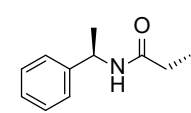
Table2-3. 1,3,4-oxadiazole 類縁体の PDHK2, PDHK4, BCKDK 阻害活性

Compound		**30**	**32**	**33**	**34**
R1		H			
IC$_{50}$ (μM)	PDHK2	0.0558	0.0042	0.0060	0.0171
	PDHK4	0.4624	0.0241	0.0329	0.0615
	BCKDK	---	>100 (3.0%)	>100 (18%)	---

キラル化合物である化合物 **34** と PDHK2 との X 線複合体構造を，Figure 2-5 に示す。デザイン方針を反映した複合体構造が認められ，Isoxazole と Phenyl 基が π-π スタックし，かつ Arg258 側鎖と cation-π 作用を形成しうる構造での結合が認められた（Figure 2-5a）。ATP-lid は，電子密度が不明瞭で一様な構造を取っておらず，エントロピー的に安定な自由度の高い構造に開放されたと考えられる。また，WaterMap で不安定な水和サイトが検出された Lys299・Arg302・Leu303 近

傍のサイトが，iso-butyl 基で占有されている事も確認された。更に，Asn255 側鎖と水を介した相互作用を Amide 部分が形成している事が，新たな情報として取得された。Figure 2-5c に，Merge 化合物の起点となった，Figure 2-4 PDB 検索時の Hit 構造との重ね合わせ図を示す。高い重なりを与える事から， PDB での結合様式を用いた三次元構造検索の有用性が示された。
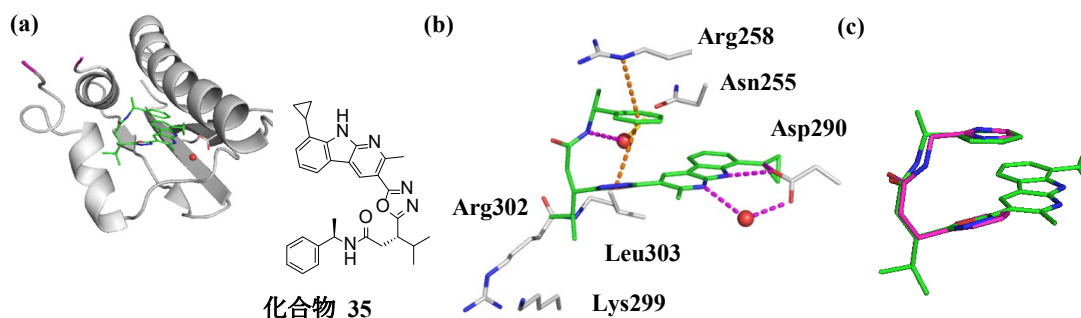
Figure 2-5. PDHK2 と化合物 **35** の X 線複合体構造。Pocket 外側に位置する ATP lid 領域を magenta 表示。Red sphere は水分子，Purple dashed line は水素結合，Orange dashed line は π 相互作用を表示

(d) ATP サイトの全体像。ATP lid 領域が，構造化しない Open 状態で存在

(e) 化合物 **35** 周囲の拡大図。**35** が分子内 π-π stucking を形成し，Arg258 と cation-π 相互作用を形成 Asn255 と水を介した相互作用も形成

(f) 化合物 **35** の PDHK2 結合 Form と，デザインの基となった Figure 4 の PDB 検索で Hit した Ligand 部分構造 (PDB ID = 1TCX)との重ね合わせ図

第2項　三環性化合物 C4 位への構造情報の転用

第 1 項で三環性化合物 C3 位からの SBDD を行い，以下に示す 3 つの相互作用を通じて，酵素阻害活性の向上が認められた。

- ✓ Arg258 との Cation-pi 相互作用
- ✓ Lys299・Arg302・Leu302 近傍の脂溶性サイトの占有
- ✓ Asn255 との極性相互作用

PDHK 酵素阻害活性 IC_{50} 値は nM レベルに到達したものの，分子量が 500 近くまで大きくなっており，経口剤を狙うに際して更なる分子サイズの低減が望まれる。これらの相互作用ポイントは，三環性化合物 C4 位からの方が距離的に近い位置に存在している。このため，C4 位から C3 位で認められた相互作用を再現することで,分子サイズを低減する事が期待できる。そこで次に，C4 位からの SBDD を検討した。

C4 位に種々の置換基を有する周辺化合物に対して複合体モデル構築を行い，先述した 3 つの相互作用を再現しうる置換基の探索を行った。その結果，sp3 性の高いピロリジン骨格において，Lys299・Arg302・Leu302 近傍の脂溶性サイトを占有することが認められた。ピロリジン骨格は，Asn255, Arg258 の方向に置換基を付与する事が可能であり，C3 位からの相互作用ポイントを再現する有望な置換基として考えられたため，周辺化合物の合成展開を実施した（Figure 2-6）。

化合物 11

PDHK2 IC_{50} = 0.71 μM
PDHK4 IC_{50} = 5.90 μM
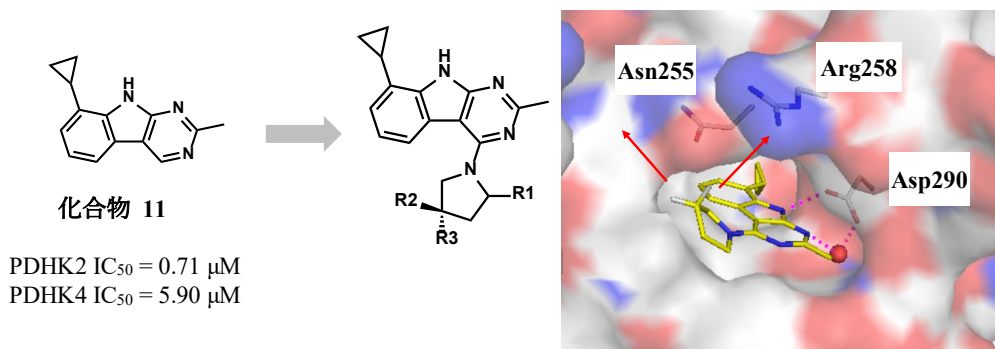
Figure 2-6. 化合物 **11** の Pyrimidoindole C4 部置換ピロリジン化合物の PDHK2 への複合体モデル

Table 2-4 に，ピロリジン誘導体の阻害活性評価結果を示す。ピロリジン導入体である化合物 **35** で若干の阻害活性の減弱が認められたが，R1 方向に置換基を導入することでピロリジンの配座規制を志向した **36, 37** において,化合物 **11** の阻害活性を保持する事が認められた。次に Arg258 との cation-π 相互作用形成を指向し，Phenyl 基をピロリジン R2 部位に付与した所，benzyl 体である化合物 **40** において阻害活性の向上が認められた。更に，R3 部位から Asn255 との極性相互

作用の形成を指向した化合物の合成を行い，Amide 基を導入した化合物 **42** において，PDHK2 で 0.0045 μM，PDHK4 で 0.0264 μM の IC$_{50}$ 値が認められた。
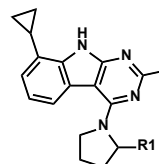
Table 2-4. Pyrolidine 化合物の PDHK2, PDHK4 阻害活性

Compound		**35**	**36**	**37**	**38**
R1		H	Ethyl (racemate)	(*S*)-CH$_2$OH	(*R*)-CH$_2$OH
IC$_{50}$	PDHK2	2.41	0.758	0352	8.52
(μM)	PDHK4	10.3	8.46	4.53	>30 (21%)

Compound		**39**	**40**	**41**	**42**
pyrrolidine					
IC$_{50}$	PDHK2	1.01	0.073	0.0146	0.0045
(μM)	PDHK4	7.70	1.12	0.292	0.0264

　Figure 2-7 に，得られた化合物 **42** の PDHK2 に対する複合体モデルを示す。モデル構造において，Benzyl 基が Arg258 と cation-π 相互作用を形成し，Amide 基が Asn255 と水素結合を形成している。Asn255 の側鎖は，周辺の PDHK 主鎖とも水素結合を形成しており，立体的
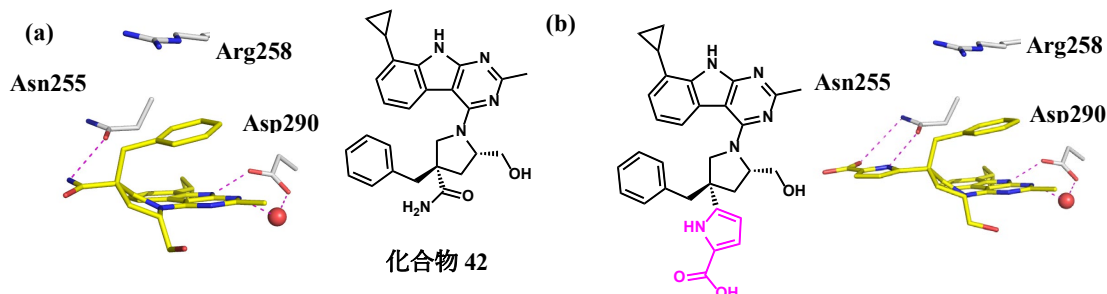
Figure 2-7. 化合物 **42** の PDHK2 に対する複合体モデル。Amide 部分が Asn255 と水素結合を形成 Asn255 と二点で水素結合を形成する目的で，2-carboxy pyrrole をデザインし，複合体モデルで相互作用形成を確認

に固定された側鎖となっている。Asn255 側鎖 NH_2 の片側のプロトンが，リガンド近傍に位置しながら水素結合パートナーが存在しない状態となっている事から，この部分への水素結合アクセプター導入で親和性を向上する事が考えられる。この目的でいくつかのデザイン化合物に対して複合体モデルを構築し，その中から Figure 2-7b に示す 2-carboxy pyrrole 体を有望な構造として選抜した。

　2-carboxy pyrrole 体の合成を行う際に，Benzyl 基を残したままであると分子量が 500 を超えてしまう事から，脱 Benzyl 体である化合物 **43－45** の合成・阻害活性評価を行った（Table 2-5）。PDHK2 の IC_{50} 値が 0.0051〜0.0092 μM，PDHK4 の IC_{50} 値が 0.0122〜0.0406 μM であり，Asn255 と二点で水素結合を形成する事の強い効果が示された。
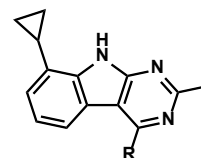
Table 2-5. Pyrrole カルボン酸化合物の PDHK2, PDHK4 阻害活性

Compound		**43**	**44**	**45**
R				
IC_{50}	PDHK2	0.0092	0.0051	0.0055
(μM)	PDHK4	0.0406	0.0122	0.0178

小括

　本章では, Fragment Screening 由来のリード候補化合物から SBDD を実施し, 酵素阻害活性 IC_{50} 値を nM レベルに向上した化合物のデザイン研究について詳述した（Figure 2-8）。具体的には, ATP サイトのアデニン環結合領域を占有する三環性化合物から, ポケット外側に存在するリボース・リン酸結合領域に対して合成展開を実施した。C3 位から合成展開を進める過程で, リン酸結合領域に存在する ATP-lid が構造化し, Arg258 との相互作用を仲介する新規な結合様式が化合物 **22** の X 線複合体構造で認められた。得られた複合体構造情報から, 不安定水の排斥, 分子内 π-π 相互作用の形成に着目した SBDD を行うことで, 酵素阻害活性が nM レベルに到達する化合物 **33** を見出した。この際, PDB 検索等の三次元構造情報を活用した分子設計を行う事で, 着目するコンセプトに合致する化合物を効率良く見出すことが出来た。

　次に, C3 位からの検討で得られた SBDD 情報（Arg258 との Cation-pi 相互作用, Lys299・Arg302・Leu303 近傍の脂溶性サイトの占有, Asn255 との極性相互作用）を C4 位に転用し, 分子サイズを低減した低分子阻害剤の創出を試みた。デザイン化合物との複合体モデルの検討結果から, ピロリジンを基本骨格として利用した合成展開を行い, 化合物 **33** の相互作用を再現する化合物 **42** を見出した。更に, Asn255 と二点で水素結合を形成する事を指向した化合物のデザインを行い, Arg258 との Cation-π 相互作用を除いた状態で同等の阻害活性作用を示す, 化合物 **44** も新たに見出した。本章で得られた SBDD 情報を用いる事で, ATP サイトのポケット外側において効率よく阻害活性を向上する事が可能となる。この事から, PDHK ATP サイトにおける低分子阻害薬の創薬研究の分野に対して, 重要な知見を新たに提供する事が出来たと考えられる。
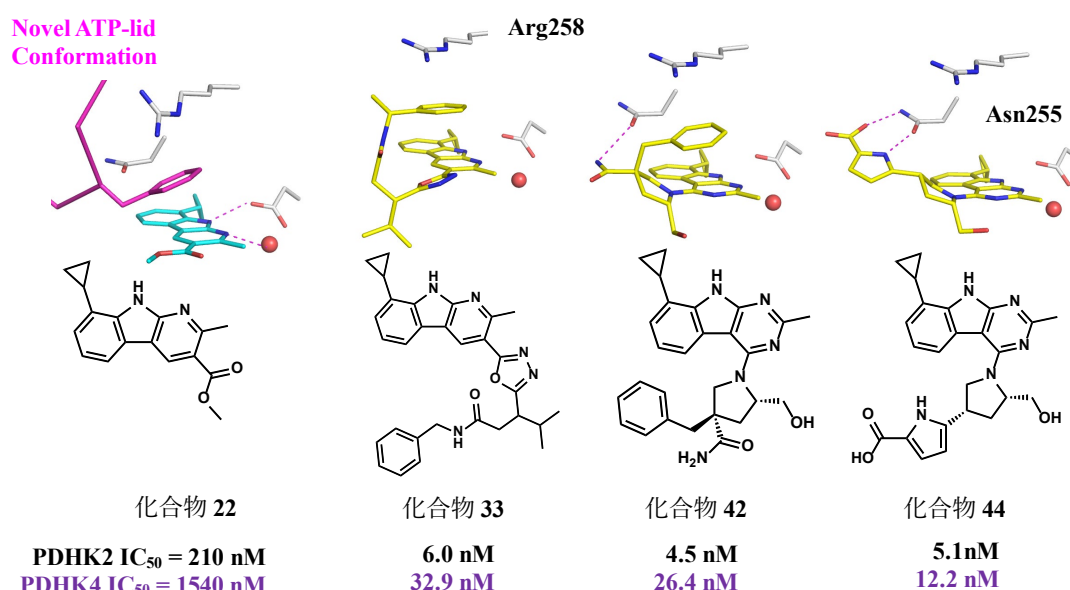
Figure 2-8. 三環性代表化合物の構造情報, PDHK 酵素阻害活性値

化合物 **22**
PDHK2 IC_{50} = 210 nM
PDHK4 IC_{50} = 1540 nM

化合物 **33**
6.0 nM
32.9 nM

化合物 **42**
4.5 nM
26.4 nM

化合物 **44**
5.1nM
12.2 nM

第3章　フラグメント分子軌道法を用いた構造活性相関の解析

第1節　背景と目的

　第1章，第2章において，PDHK 酵素阻害活性 IC_{50} 値が mM レベルの Fragment Hit から SBDD を実施し，nM レベルの阻害活性を有する化合物を見出した研究について記述した。PDHK との X 線複合体構造に基づき，デザインした化合物のドッキングモデルを構築する事で，活性が向上した化合物を効率的に取得する取り組みを実践した。この際，モデル構築に併せてドッキングスコアが適合性のスコアとして得られるが，一般的にドッキングスコアと結合親和性の相関は低いため，親和性の評価は目視評価で実施した。PDHK への結合親和性を精度よく予測する計算手法を見出すことで，より効率よく阻害活性が向上した化合物を創出する事が可能になると期待される。

　標的タンパク質に対する結合親和性の評価，すなわち結合自由エネルギー計算を行い化合物間の順位付けを精度よく行う事は，計算機化学の分野で重要な項目である。そのため，Linear Interaction Energy (LIE) 法[19]，Molecular Mechanics and generalized Born/surface area (MM-GBSA)法[20]，Free Energy Perturbation (FEP)法[21-25]などの，多くの手法の開発が進められている。これらの手法は，分子力場（MM; Molecular Mechanics）パラメータを利用しており，各原子に対して部分電荷を割り当てて計算を実行する。化学結合・原子種に基づき割り当てられた分子力場法での部分電荷は，分子の配座が変化しても計算中で一定の値を保つため，分極や電荷移動等の効果を十分に表現する事は出来ない。そのため，金属原子を含む計算系や，複雑な水素結合ネットワークを含む計算系に対しては，分子力場を用いて結合自由エネルギーを精度よく評価する事は困難である。一方，電子効果を取り込んだ計算を行う手法として，量子力学（QM; Quantum Mechanics）を用いた手法が挙げられる。QM 法を用いる事で，タンパク質とリガンドの結合自由エネルギーを精度よく計算する事が期待できるが，生体分子などの高分子系に量子力学計算を行う事は計算負荷が非常に高い。

　フラグメント分子軌道法（FMO; Fragment Molecular Orbital）は，タンパク質等の高分子系に対する量子力学計算を効率的に実施する手法である[26-36]。本手法では，計算対象はアミノ酸残基等のフラグメントに分割され，各フラグメントとフラグメントペアに対して量子力学計算を実施する。並列化効率が高い特長を有しており，量子力学法での計算結果を迅速に取得する事が可能である。QM 計算において，溶媒効果は polarizable continuum model (PCM)法[37]，conductor-like screening model (COSMO)法[38-40]，あるいは GBSA 法などで計算する対応が取られる。FMO 計算においても，これら溶媒効果を取り込んだ計算結果が幾つか報告されているが，各手法においてどのような差が生じるかは不明な点が多い。

本章では PDHK ATP サイトを対象とし，溶媒エネルギー項を含めた MM, FMO 計算を実施し，精度の高い結合自由エネルギー計算手法を探索した。Figure 3-1 に示すように，PDHK ATP サイトはアスパラギン酸残基を中心とした複雑な水素結合ネットワークを形成しており，複数の電子効果を含む対象であると考えられる。種々の計算条件で FMO 計算を検討する際に，計算系全てを対象として結合自由エネルギー計算を実施すると，計算負荷は FMO 法においても高くなる。仲西らは，リガンド周辺のアミノ酸残基を切り出した計算モデル（切り出しモデル）を用いて，全系でのエネルギー値を再現する FMO 計算条件の検討を実施してきた[41-46]。そこでまず第 3 節第 1 項で，切り出しモデルを用いた FMO 計算検討結果を横断的に解析し，適切な計算条件を探索した。続く第 3 節第 2 項で，切り出しモデルを用いた相互作用エネルギー計算を種々の MM, FMO 計算条件で実施し，実測値との相関解析を行った。また，FMO 法を用いた相互作用エネルギー計算の検討に加え，PDHK ATP サイトにおける電子効果を解析した。
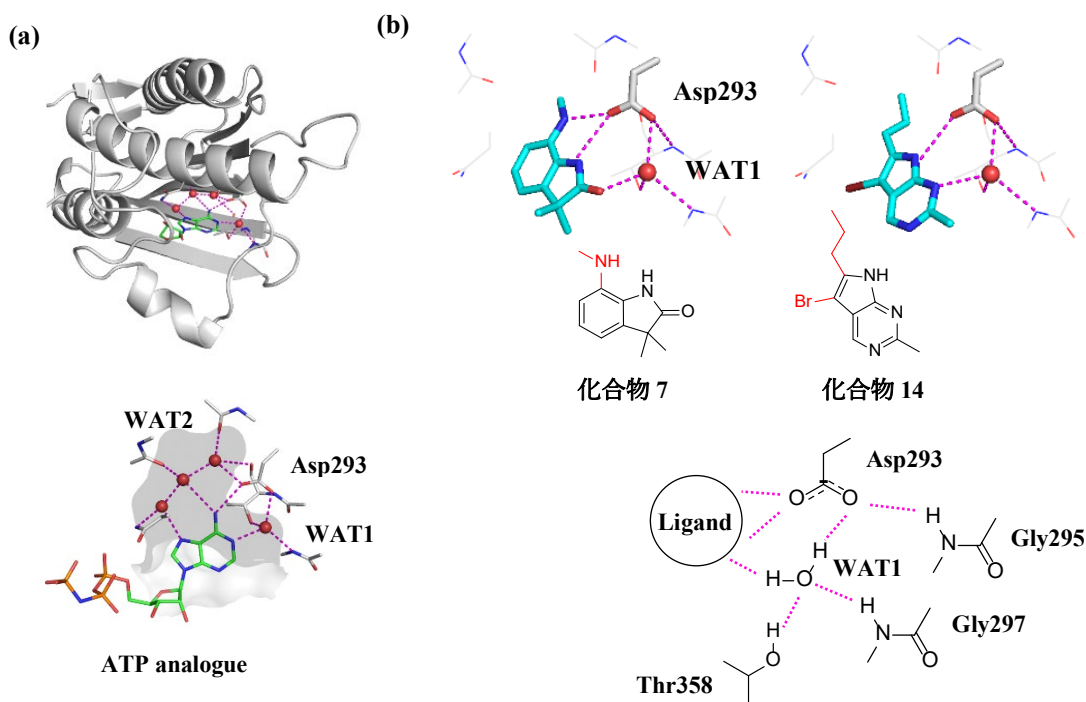
Figure 3-1 PDHK の X 線複合体構造。PDHK の構造を Gray cartoon，タンパク質内部の表面を Gray mesh で表示。Red sphere は水分子，Dashed line は水素結合を表示

(a) PDHK4 と AMP-PNP の X 線複合体構造(PDB ID: 3CRL)

(b) 化合物 **7, 14** と PDHK の X 線複合体構造(PDB ID: 7EBG, 7VBU)。水素結合ネットワークを概略図で示す

第 2 節　実験項

第 1 項　PDHK4 に対する複合体モデルの構築

Fragment Hit **1** の PDHK4 との X 線複合体構造（PDB ID = 7EAT）を初期構造に利用した。Schrödinger Release version 2021-2 を用い，Protein Preparation Wizard により水素付加と互変異性状態を決定した。この際，Asp293 の近傍に存在する 2 種の水（Figure 3-1 での WAT1 と WAT2）のみ保持し，他の水は除去した。Protein Preparation 計算では，水素原子のみ構造最適化計算を実施した。得られた複合体のリガンド構造に対して，計算対象化合物を重ね合わせる事で，続く FMO 構造最適化計算の構造を作成した。Dimethyl Indole 骨格化合物に関しては，Fragment Hit **1** の骨格部分で重ね合わせ作業を行った。Pyrimido Indole 骨格化合物に関しては，化合物 **14** の PDHK2 との X 線複合体構造（PDB ID＝7VBU）を 7EAT 構造に重ね合わせ，初期配座を構築した。その後，重ねあわせ後の化合物 **14** に残る化合物の骨格を重ね合わせた。

作成した構造に対して，General Atomic and Molecule Electrostatic Structure System (GAMESS)[47] の ver2018 パッケージを用いて FMO 構造最適化計算を実施した。FMO 計算時に，PDHK4 タンパク質は，Cα と隣接する主鎖カルボニル炭素でフラグメント分割した。リガンドから 8 Å 以内に存在するアミノ酸残基（Met251-His266, Ile276-Val278, Ile289-Phe307, Gly333-Ser337, Leu348-Ile362＋WAT1）を抽出し，リガンドとの切り出しモデルとし，FMO 構造最適化計算を実施した。この際，末端アミノ酸残基の N 末，C 末部分は，隣接するアミノ酸残基の立体配座を基にアセチル基，メチルアミド基でキャップした。FMO 構造最適化計算は，リガンド周囲 5 Å 以内に存在するアミノ酸残基側鎖とリガンド以外の構造を固定し，それ以外の構造を可動領域に設定して構造最適化を実施した。6-31G 基底関数，FMO2/RHF-D 手法を利用し，タンパク質は 1 アミノ酸残基単位で分割，最大エネルギーグラジエントが 3.0×10^{-4} Hatree/Bohr を満たす収束条件で最適化計算を実施した。構造最適化後の座標を，全体構造に埋め戻す事で，続く MM, FMO 一点計算の入力構造とした。

第 2 項　PDHK4 複合体モデルに対する結合自由エネルギー計算

Schrödinger Release version 2022-1 を用いた MM 計算，並びに GAMESS version 2018-1 を用いた FMO 計算を実施した。結合自由エネルギー（ΔG）は，エンタルピー（ΔH）とエントロピー（$-T\Delta S$）から構成される（$\Delta G = \Delta H - T\Delta S$）。今回，計算対象となる化合物は母核が共通し，かつ置換基の自由度も高くない事から，エントロピー項は化合物間で差が無いと仮定し，計算対象から除外した。リガンドから 12 Å 以内に存在するアミノ酸残基（Val204 - Leu227, Leu248 - His266, Thr274 - Leu282, Lys290 - Ser308, Gly333 - Ala341, Asp347 - Tyr363 と WAT1）を抽出し，リガンド

との切り出しモデルに対して結合自由エネルギー計算を実施した。この際，末端アミノ酸残基のN末，C末部分は，隣接するアミノ酸残基の立体配座を基にアセチル基，メチルアミド基でキャップした。

MM/GBSA での一点計算は，Schrödinger Macromodel の OPLS4 力場を用い，デフォルトの計算条件で実施した。MM/GBSA 法の結合自由エネルギー（$\Delta G_{\text{bind}}^{\text{MM/GBSA}}$）は，以下に示す式（1）に従い算出した。

$$\Delta G_{\text{bind}}^{\text{MM/GBSA}} = G_{\text{complex}}^{\text{MM/GBSA}} - G_{\text{receptor}}^{\text{MM/GBSA}} - G_{\text{ligand}}^{\text{MM/GBSA}} \quad (1)$$

FMO 計算は，FMO2-RHF/PCM[1(2)]/6-31G*の計算条件で，複合体・タンパク質・リガンドの三者に対して実施した。FMO 計算の際に，電荷移動の自由度を高める目的で，Asp293 フラグメントと WAT1 は一つのフラグメントにまとめ，その他のアミノ酸残基フラグメントは 1 残基単位でフラグメント化して計算を実施した。FMO/PCM 法での結合自由エネルギー（$\Delta G_{\text{bind}}^{\text{FMO/PCM}}$）は，以下に示す式（2）に従い算出した。溶媒和エネルギーに GBSA 法由来の値を用いたFMO/GBSA 結合自由エネルギー（$\Delta G_{\text{bind}}^{\text{FMO/GBSA}}$）は，式（3）に示すように真空中での FMO 相互作用エネルギー（$\Delta E_{\text{int}}^{\text{FMO/vacuo}}$）に GBSA 脱溶媒和エネルギー項を加える事で算出した。

$$\Delta G_{\text{bind}}^{\text{FMO/PCM}} = G_{\text{complex}}^{\text{FMO/PCM}} - G_{\text{receptor}}^{\text{FMO/PCM}} - G_{\text{ligand}}^{\text{FMO/PCM}} \quad (2)$$

$$\Delta G_{\text{bind}}^{\text{FMO/GBSA}} = \Delta E_{\text{int}}^{\text{FMO/vacuo}} + \Delta G_{\text{desolv}}^{\text{GBSA}} \quad (3)$$

第 3 項　PDHK4 複合体モデルにおけるリガンドの歪みエネルギー計算

リガンドの歪みエネルギーの算出には，二段階の計算手法を利用した。リガンド構造に対して，まずは Schrödinger Macromodel を用いた配座解析を実施した。OPLS4 力場を利用し，GBSA水モデルを用いたデフォルトの計算条件で配座発生を行った。続いて，得られた安定配座群に対して GAMESS を用いた構造最適化計算を実施した。MP2/PCM[1(2)]/6-31G*計算条件で，最大エネルギーグラジエントが 1.0 x 10^{-4} Hatree/Bohr を満たす収束条件で最適化計算を行った。得られた配座群の中から，最安定エネルギー値（$E_{\text{ligand min}}^{\text{MP2/PCM}}$）を決定し，式（4）より PDHK4 結合時の歪みエネルギー（$\Delta E_{\text{ligand deform}}^{\text{FMO/PCM}}$）を算出した。

$$\Delta E_{\text{ligand deform}}^{\text{FMO/PCM}} = E_{\text{ligand}}^{\text{MP2/PCM}} - E_{\text{ligand min}}^{\text{MP2/PCM}} \quad (4)$$

第 4 項　QM, MD 計算による化合物の二面角解析

　化合物 **17** に対して，GAMESS パッケージを用い，メチルアミド部分の母核に対する回転障壁の計算を実施した。二面角を 0-180 度の範囲で 10 度刻みで配座を発生し，二面角を固定したうえで MP2/PCM [1(2)]/6-31G* 条件で構造最適化し，回転障壁の計算を実施した。

　次に，Schrödinger Desmond プログラムを用い，化合物 **17** の MD 計算を実施する事で，二面角のポピュレーション解析を実施した。PDHK4 複合体およびリガンド単体（結合フォームと反転フォーム）に対する MD 計算を，25 ns の MD シミュレーション時間で 4 回実施し，後半 12.5 ns の MD トラジェクトリーを解析に使用した。

第 3 節 結果と考察

第 1 項 フラグメント分子軌道法による結合自由エネルギー計算の効率化

　仲西らは，リガンド結合部位周辺のアミノ酸残基を切り出した計算モデルを用いて，全系での結合自由エネルギー値を再現する FMO 計算条件の検討を実施してきた[41-46]。Figure 3-2 に，本項で解析対象とした標的タンパク質名と，リガンドの構造式を示す。

Neutral Ligands

A1 : 1FKF　　　**A2** : 1FKI

FKBP

B1 : 2ZYE　　　**B2** : 3O9A

HIV protease

Charged Ligands

C1 : Model on 3AXW

CK2 kinase

D1 : 3GY7　　　**D2** : 3GY3

β-trypsin

E1 : 4ZE6

Endothiapepsin
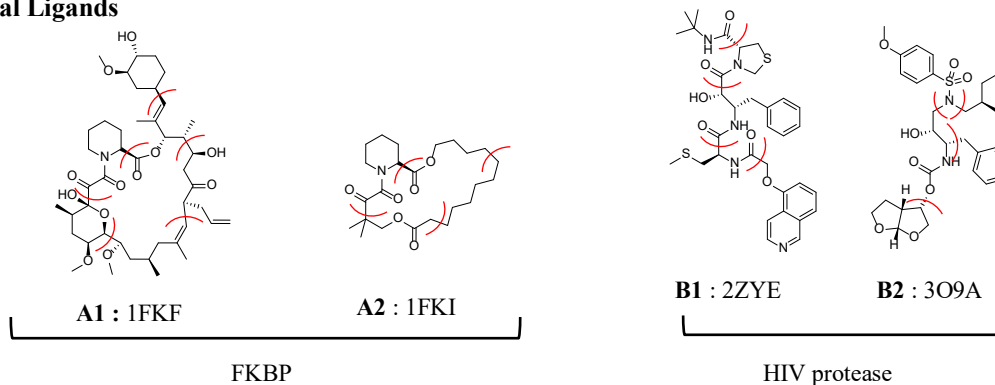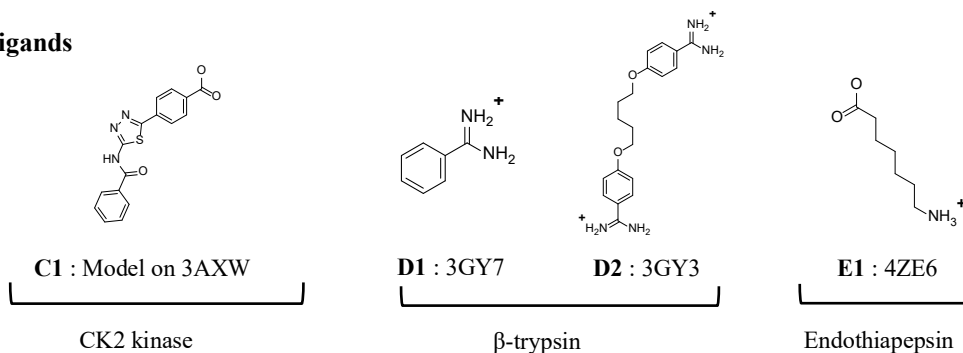
Figure 3-2 切り出しモデル計算系の PDB ID とリガンドの化学構造式
化合物 **A1-A2**, **B1-B2** は，FMO 計算時にリガンドもフラグメント化（赤色円弧部分でフラグメント化を行い，円弧の外側に電子対を配置）

　計算対象は 5 標的で，複合体構造に荷電状態が異なる化合物を含んでいる。FKBP に対しては，化合物の分子サイズによる切り出しモデルへの影響を検討するために 2 種の化合物を利用した。また，HIV Protease に関しては，アミノ酸側鎖の欠損による影響を検討する目的で，2 種の計算系を用いた。また，化合物の電荷の影響を探る目的で，アニオン性リガンドを含む CK2 kinase，カチオン性リガンドを含む β-trypsin，双性イオンリガンドを含む Endothiapepsin を対象とした。これらの計算系について，化合物周辺の残基を指定距離（5 Å, 8 Å, 12 Å, 15 Å）切り出したモデルに対して FMO 計算を行い，全系での FMO 計算で得られた結合自由エネルギー値との比較を行った。各 FMO 計算系におけるフラグメント総数と総電荷を，Table 3-1 に示す。切り出しモデルを利用する事でフラグメント総数が減少するが，計算系によっては総電荷の値も変動する。

Table 3-1. 各計算系における総フラグメント数と総電荷

(a) 総フラグメント数

Target	cmpd	Total number of protein fragments				
		Full	15 Å	12 Å	8 Å	5 Å
FKBP	**A1**	106	94	78	42	16
	A2	106	94	77	43	16
HIV Protease	**B1**	208	169	122	81	48
	B2	205	166	126	78	44
CK2 kinase	**C1**	330	129	95	51	21
β-trypsin	**D1**	224	134	94	47	23
	D2	221	144	112	64	33
Endo thiapepsin	**E1**	331	150	102	56	23

(b) 総電荷数。カッコ内数値は，左側がプラス電荷の総和，右側がマイナス電荷の総和。

Target	cmpd	Total charge of protein fragments				
		Full	15 Å	12 Å	8 Å	5 Å
FKBP	**A1**	+1 (+15, -14)	0 (+13, -13)	+1 (+10, -9)	+3 (+6, -3)	-1 (+1, -2)
	A2	+1 (+15, -14)	0 (+13, -13)	+1 (+10, -9)	+4 (+7, -3)	-1 (+1, -2)
HIV Protease	**B1**	+9 (+26, -17)	+1 (+16, -15)	+1 (+11, -10)	+3 (+8, -5)	-4 (+1, -5)
	B2	-1 (+16, -17)	-3 (+11, -14)	-2 (+9, -11)	0 (+5, -5)	-2 (+3, -5)
CK2 kinase	**C1**	-1 (+48, -49)	+6 (+21, -15)	+4 (+17, -13)	0 (+7, -7)	-2 (+2, -4)
β-trypsin	**D1**	+6 (+17, -11)	+5 (+10, -5)	+3 (+7, -4)	0 (+3, -3)	-1 (+1, -2)
	D2	+6 (+17, -11)	+4 (+10, -6)	+6 (+10, -4)	+1 (+4, -3)	-3 (0, -3)
Endo thiapepsin	**E1**	-18 (+8, -26)	-17 (+2, -19)	-13 (0, -13)	-8 (0, -8)	-4 (0, -4)

Table 3-2 に，各計算系の全体構造を用いた際に得られる，基準となる FMO-PCM, FMO/MP2 計算のエネルギー値を示す。FMO/PCM 計算では，HF 相互作用エネルギー（$\Delta E_{\mathrm{int}}^{\mathrm{HF}}$）と脱溶媒和エネルギー（$\Delta G_{\mathrm{desolv}}$）を計算し，その和として HF レベルでの水中結合自由エネルギー（$\Delta G_{\mathrm{bind}}^{\mathrm{HF}}$）を算出した。この際，脱溶媒和エネルギーは静電項（$\Delta G_{\mathrm{desolv}}^{\mathrm{ele}}$）と非静電項（$\Delta G_{\mathrm{desolv}}^{\mathrm{non\,ele}}$）に分割して算出した。また，FMO/MP2 計算で電子相関エネルギー（ΔE_{corr}）を計算し，HF レベルでの水中結合自由エネルギー（$\Delta G_{\mathrm{bind}}^{\mathrm{HF}}$）値に加えることで，FMO 相互作用エネルギー値を算出した。

Table 3-2. FMO-RHF/PCM，FMO-MP2 法で算出した，全系での FMO 相互作用エネルギー値 [a]

| cmpd | PCM | | | | MP2 | PCM + MP2 |
| | HF Interaction Energy | Desolvation Energy $\Delta G_{\mathrm{desolv}}$ | | Binding Energy | Correlation Energy | Total Binding Energy |
	$\Delta E_{\mathrm{int}}^{\mathrm{HF}}$	$\Delta G_{\mathrm{desolv}}^{\mathrm{ele}}$	$\Delta G_{\mathrm{desolv}}^{\mathrm{non\,ele}}$	$\Delta G_{\mathrm{bind}}^{\mathrm{HF}}$	ΔE_{corr}	$\Delta G_{\mathrm{bind}}^{\mathrm{MP2}}$
A1	-30.5	38.5	51.9	59.9	-68.9	-9.0
A2	-26.0	29.3	40.3	43.6	--	--
B1	-85.9	71.1	59.0	44.2	-105.7	-61.5
B2	-81.4	76.8	51.9	47.3	--	--
C1	-114.0	99.8	33.3	19.1	-57.7	-38.6
D1	-15.9	7.9	16.1	8.1	-31.9	-23.8
D2	4.3	-5.2	29.5	28.6	-78.6	-50.0
E1	-81.1	67.7	9.4	-4.0	-33.5	-37.5

a) エネルギー単位は全て kcal/mol

　　まずは FMO/PCM 計算で得られるエネルギー値に関して，切り出しモデルと全系とのエネルギー差を算出した（Table 3-3）。中性化合物の結果を Table 3-3a に示しており，HIV Protease 複合体 **B1** の 5Å 切り出しモデルにおいて，HF 相互作用エネルギー値（$\Delta\Delta E_{\mathrm{int}}^{\mathrm{HF}}$）で 5.0 kcal/mol を超えるエネルギー差（-20.4 kcal/mol）が認められた。Table 3-1 で示したように，切り出しモデルによっては総電荷の値が全系から変動している。HIV Protease 複合体 **B1** の切り出しモデルは，全系での総電荷が+9 であるのに対して，15Å モデルで+1，5Å モデルでは-4 と大きく異なっている。この結果，最大切り出しモデルである 15Å 系でも HF 相互作用エネルギー値（$\Delta\Delta E_{\mathrm{int}}^{\mathrm{HF}}$）で 2.5 kcal/mol，最小切り出しモデルである 5Å 系では-20.4 kcal/mol の大きなエネルギー差が認められている。同じ標的である HIV Protease 系であるにも関わらず，化合物 **B2** の計算系では **B1**

Table 3-3. 切り出しモデルと全系における FMO/PCM 計算値の差エネルギー

a) Neutral ligands

Size	cmpd	Interaction Energy $\Delta\Delta E_{int}^{HF}$	Desolvation Energy $\Delta\Delta G_{desolv}$		Binding Energy $\Delta\Delta G_{bind}^{HF}$
			$\Delta\Delta G_{desolv}^{ele}$	$\Delta\Delta G_{desolv}^{non\,ele}$	
15Å	**A1**	-0.1	0.1	0	0
	A2	-0.6	0.6	0	0
	B1	-2.5	2.4	0	-0.1
	B2	-1.4	1.4	0	0
12Å	**A1**	-0.1	0.3	-0.1	0.1
	A2	0	0.3	-0.1	0.2
	B1	-1.9	1.9	-0.1	-0.1
	B2	-1.8	2.2	-0.1	0.3
8Å	**A1**	1.1	-0.3	-0.7	0.1
	A2	-0.6	1.4	-0.5	0.3
	B1	-2.1	2.6	-0.8	-0.3
	B2	5.1	-3.8	-0.6	0.7
5Å	**A1**	-4.2	5.6	-5.0	-3.6
	A2	-0.9	3.6	-3.3	-0.6
	B1	-20.4	21.2	-3.0	-2.2
	B2	-1.7	3.2	-2.3	-0.8

b) Charged ligands

Size	cmpd	Interaction Energy $\Delta\Delta E_{int}^{HF}$	Desolvation Energy $\Delta\Delta G_{desolv}$		Binding Energy $\Delta\Delta G_{bind}^{HF}$
			$\Delta\Delta G_{desolv}^{ele}$	$\Delta\Delta G_{desolv}^{non\,ele}$	
15Å	**C1**	-75.6	75.4	0	-0.2
	D1	-6.0	5.7	0	-0.3
	D2	-22.0	21.2	0	-0.8
	E1	-20.9	20.3	0	-0.6
12Å	**C1**	-42.9	43.1	-0.2	0
	D1	-14.8	14.2	-0.1	-0.7
	D2	20.4	-21.0	-0.1	-0.7
	E1	-16.9	15.9	-0.1	-1.1
8Å	**C1**	72.0	-69.4	-0.6	2.0
	D1	-95.8	93.4	-0.4	-2.8
	D2	-183.7	180.3	0.5	-2.9
	E1	-27.7	27.0	-0.3	-1.0
5Å	**C1**	113.8	-108.4	-3.3	2.1
	D1	-126.9	124.7	-1.0	-3.2
	D2	-276.9	272.4	-1.8	-6.3
	E1	-61.3	57.0	-2.0	-6.3

エネルギー単位は全て kcal/mol

程大きなエネルギー差が認められていない。化合物 **B2** の初期構造となる PDB データ（PDB ID ＝3O9A）において，HIV Protease のカチオン性残基の側鎖が複数欠損しており，全系と切り出しモデルの総電荷値の差が **B1** に比べて小さくなっている（Table 3-1）。この事が，全系と切り出しモデルのエネルギー差が小さくなる要因として働いていると考えられる。脱溶媒和エネルギーの静電項（$\Delta\Delta G_{\mathrm{desolv}}^{\mathrm{ele}}$）に関しても，化合物 **B1** において最大切り出しモデルである 15 Å 系でも 2.4 kcal/mol のエネルギー差が認められており，HF 相互作用エネルギー値（$\Delta\Delta E_{\mathrm{int}}^{\mathrm{HF}}$）と同様に総電荷値の差の影響であると考えられる。脱溶媒和の非静電項（$\Delta\Delta G_{\mathrm{desolv}}^{\mathrm{non\ ele}}$）に関しては，切り出しモデルサイズが 8 Å を超えると，0.8 kcal/mol 以下の数値に収まる結果が得られた。

　興味深いことに，HF/PCM 計算で得られる 2 種の静電項（$\Delta\Delta E_{\mathrm{int}}^{\mathrm{HF}}$と$\Delta\Delta G_{\mathrm{desolv}}^{\mathrm{ele}}$）に関して，符号が常に逆で絶対値がほぼ同じ値を示すという特徴が認められた。これにより，エネルギー項の総和である HF 結合自由エネルギー（$\Delta\Delta G_{\mathrm{bind}}^{\mathrm{HF}}$）に関しては，誤差が単独でのエネルギー項より緩和されている。8 Å を超える切り出しモデル系であれば，$\Delta\Delta G_{\mathrm{bind}}^{\mathrm{HF}}$の誤差は 0.7 kcal/mol に収まる結果を与えた。

　次に，荷電性化合物に対する切り出しモデルの結果について考察する（Table 3-3b）。中性化合物の結果と同様に，溶媒和エネルギーの非静電項（$\Delta\Delta G_{\mathrm{desolv}}^{\mathrm{non\ ele}}$）の誤差は小さく，8 Å を超える切り出しモデル系であれば誤差が 0.7 kcal/mol に収まる結果が認められた。一方で，二種の静電項エネルギー項（$\Delta\Delta E_{\mathrm{int}}^{\mathrm{HF}}$と$\Delta\Delta G_{\mathrm{desolv}}^{\mathrm{ele}}$）の誤差は中性化合物の場合に比べてはるかに大きく，最大切り出しモデルである 15 Å 系でも 75.6 kcal/mol のエネルギー誤差が認められた。誤差エネルギーの正負の方向性も切り出しモデル毎に異なっており，切り出しサイズを大きくしても収束性は認められない。双性イオンリガンドである化合物 **E1** のトータル電荷は中性であるが，荷電性化合物と同様に大きなエネルギー誤差が認められた。中性化合物と荷電性化合物における誤差の要因を探る目的で，CK2 kinase と FKBP 全系での FMO ペア相互作用エネルギー（フラグメント間の相互作用エネルギーPIE; Pair Interaction Energy）計算を実施した（Figure 3-3）。CK2 kinase は化合物がアニオン性であり，カチオン性アミノ残基との間に強い静電相互作用が存在する。フラグメント残基番号 1-40, 200-330 のアミノ酸残基はリガンドから 15 Å 以上離れているが，PIE 値が 10.0 kcal/mol を超える強い値を示している。中性化合物の FKBP 計算系においては，PIE 値が 10.0 kcal/mol を超えるのは 5 Å 以内に存在する限られた残基のみであり，荷電性化合物を含むCK2 の結果とは大きく異なっている。また，先述したように CK2 kinase は切り出しモデル毎に総電荷の値が大きく異なっている(Table 3-1)。全系の総電荷値は-1 であるのに対し，15 Å 系では+6，12 Å 系では+4 となっており，切り出しサイズの大きい15 Å 系の方が総電荷値の差が大きくなっている。これにより，HF 相互作用エネルギー（$\Delta\Delta E_{\mathrm{int}}^{\mathrm{HF}}$）の誤差が，15

Å 系では-75.6 kcal/mol，12 Å 系では-42.9 kcal/mol となっており，切り出しサイズの大きい 15
Å 系において誤差が大きくなる結果を与えたと考察される。
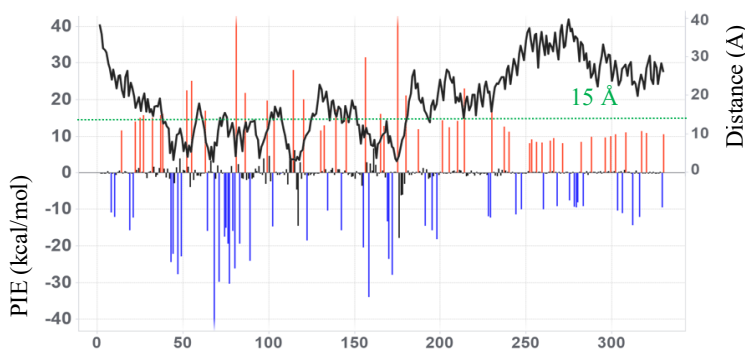
(a) CK2 system **(b) FKBP system**
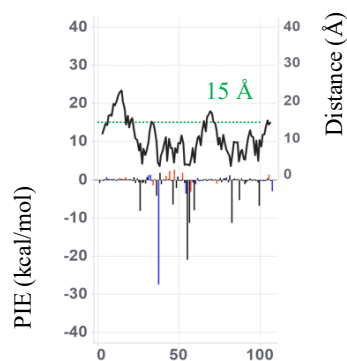
Figure 3-3 タンパク質構成フラグメントとリガンド間の，ペア相互作用エネルギーと最短距離
横軸はアミノ酸残基番号，左縦軸が相互作用エネルギー値，右縦軸が距離を示す。ペア相互作用
エネルギーを棒グラフで表示し，最短距離を折れ線グラフで表示。緑色点線は，15 Å の距離を示
す。ペア相互作用エネルギーに関して，カチオン残基のエネルギーは青色，アニオン残基のエネ
ルギーは赤色，中性残基のエネルギーを黒色で表示。

(a) アニオン性化合物 **C1** : CK2

(b) 中性化合物 **A1** : FKBP

　一方で，荷電性化合物に関しても，HF/PCM 計算で得られる 2 種の静電項（$\Delta\Delta E_{int}^{HF}$ と $\Delta\Delta G_{desolv}^{ele}$）
に関して，符号が常に逆で絶対値がほぼ同じ値を示すという特徴が認められた。この結果，エネ
ルギー項の総和である HF 結合自由エネルギー（$\Delta\Delta G_{bind}^{HF}$）の誤差は大きく緩和され，12Å を超
える切り出しモデル系であれば誤差が 1.10 kcal/mol に収まる結果が認められた。

　次に，各標的の代表構造（**A1**, **B1**, **C1**, **D1**, **D2**, **E1**）に対して FMO-MP2 計算を行う事で，相互
作用エネルギーの非静電項である電子相関エネルギー（Correlation Energy）を算出し，MP2 法で
の誤差を解析した（Table 3-4）。電子相関エネルギー（$\Delta\Delta E_{corr}$）に関して，中性化合物での誤差
は小さく，切り出しサイズが最も小さい 5 Å 系においても 0.4 kcal/mol の誤差に留まった。荷電
性化合物に対する電子相関エネルギー（$\Delta\Delta E_{corr}$）の誤差は若干大きな値が認められるが，12 Å
を超える切り出しモデル系であれば誤差が 0.9 kcal/mol に収まる結果が得られた。1.4 kcal/mol を
超えるエネルギー誤差を Table 3-3 中赤字でハイライトしており，中性化合物においては 8Å，荷
電性化合物においては 12 Å を，全系での数値を再現する妥当なカットラインとして設定する事
が提唱される。

Table 3-4. 切り出しモデルと全系における FMO/PCM, FMP-MP2 計算値の差エネルギー

| Size | ID | Compound | | MP2 | PCM | MP2+PCM |
| | | | | Correlation | Binding | Total |
		State	Charge	$\Delta\Delta E_{\text{corr}}$	$\Delta\Delta G_{\text{bind}}^{\text{HF}}$	$\Delta\Delta G_{\text{bind}}^{\text{MP2}}$
15 Å	**A1**	Neutral	0	0	0	0
	B1		0	-0.1	-0.1	-0.2
12 Å	**A1**	Neutral	0	-0.1	0.1	0
	B1		0	-0.1	0.1	0.2
8 Å	**A1**	Neutral	0	-0.2	0.1	-0.1
	B1		0	0	-0.3	-0.3
5 Å	**A1**	Neutral	0	0.4	**-3.6**	**-3.2**
	B1		0	0.1	**-2.2**	**-2.1**
15 Å	**C1**	Charged	-1	-0.9	-0.2	-1.1
	D1		+1	0.1	-0.6	-0.5
	D2		+2	0.3	-0.8	-0.5
	E1		±0	0.1	-0.6	-0.5
12 Å	**C1**	Charged	-1	-0.8	0	-0.8
	D1		+1	-0.2	-0.7	-0.9
	D2		+2	-0.1	-0.7	-0.8
	E1		±0	0	-1.1	-1.1
8 Å	**C1**	Charged	-1	**-2.7**	**2.0**	-0.7
	D1		+1	-0.3	**-2.8**	**-3.1**
	D2		+2	**-1.4**	**-2.9**	**-4.3**
	E1		±0	0.2	-1.0	-0.8
5 Å	**C1**	Charged	-1	**-2.0**	**2.1**	0.1
	D1		+1	**-2.6**	**-3.2**	**-5.8**
	D2		+2	**-3.4**	**-6.3**	**-9.7**
	E1		±0	1.0	**-6.3**	**-5.3**

エネルギー単位は全て kcal/mol

第 2 項　PDHK ATP サイトに対する結合自由エネルギー計算の検討

　Figure 3-4 に示す化合物群を対象として，相互作用エネルギーおよび結合自由エネルギー計算を種々の MM, FMO 計算条件で実施し，実測値との相関解析を実施した。これらの化合物は，第 1 章第 2 項で示した様に，PDHK ATP サイトのポケット深部に存在する構造水の排斥を指向した化合物である。実測値との相関が高い計算条件を見出すことで，より効率的に PDHK ATP サイトでの化合物最適化を進める事が可能になると期待される。Dimethyl Indole 系統，Pyrimido Indole 系統の化合物を，阻害活性順に化合物 ID を **F1-F5**, **G1-G6** で割り当て，**Reference** 化合物からの相対結合自由エネルギー値を解析した。この際，IC_{50} 値を結合親和性の指標となる乖離定数 Kd 値と近似して，実測結合自由エネルギーを算出した。

(a) Dimethyl Indole 系統

Reference	**F1**	**F2**	**F3**	**F4**	**F5**
(化合物 3)	(化合物 7)	(化合物 5)	(化合物 4)	(化合物 6)	(化合物 8)
890 μM	12.8 μM	43.6 μM	304 μM	455 μM	842 μM

(b) Pyrimido Indole 系統

G1	**G2**	**G3**	**G4**	**G5**	**G6**
(化合物 19)	(化合物 13)	(化合物 14)	(化合物 16)	(化合物 15)	(化合物 17)
8.0 μM	11.0 μM	12.4 μM	36.7 μM	144 μM	198 μM
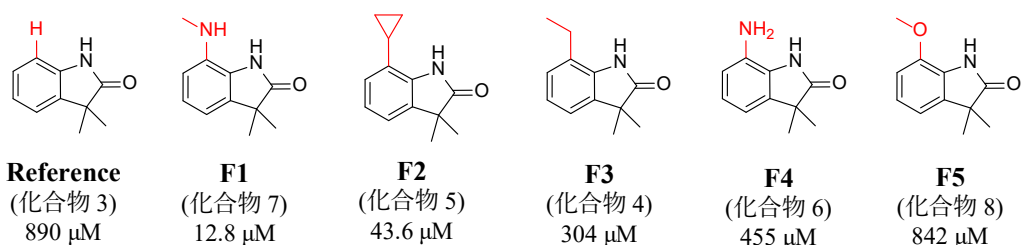
Figure 3-4. 計算を実施した 12 化合物の化学構造式と PDHK4 に対する IC_{50} 値
カッコ内に 1 章，2 章での化合物番号を示す。各系統骨格部の構造を黒色で表示し，
変換した置換基部分を赤色で示す

　第 1 項での FMO 検討結果から，中性化合物を対象とする本系においては，全系での相互作用エネルギー値を 8 Å の切り出しモデルで再現すると考えられる。今回，数値の精度を高めることを目的として，切り出しサイズを 12 Å に拡張して結合自由エネルギー計算を実施した。

　Table 3-5 に，IC_{50} 値から算出した実測相対結合自由エネルギーと，各種計算手法で得られた計算相対結合自由エネルギー値を示す。実測の相対結合自由エネルギー値は -2.8 kcal/mol までに収まるが，真空中での計算エネルギー相対値は，分子力場，フラグメント分子軌道法共に -30.0

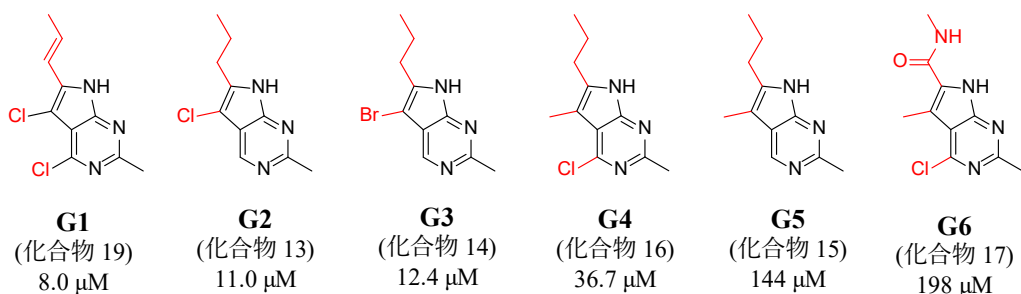kcal/mol を超える値が認められた。溶媒和エネルギーを加える事で，静電相互作用エネルギーの過剰評価が抑えられる方向に補正され，GBSA 法で-15.0 kcal/mol，PCM 法で-10.0 kcal/mol の値にまで改善する傾向が認められた。

Table 3-5. PDHK4 に対する 12 化合物のエネルギー値 [a]

ID	相対結合自由エネルギー						Ligand Deformation
	実測値	計算値					
		MM/vacuo	FMO/vacuo	MM/GBSA	FMO/GBSA	FMO/PCM	
	$\Delta\Delta G_{\text{exp}}$	$\Delta\Delta E_{\text{int}}^{\text{MM/vac}}$	$\Delta\Delta E_{\text{int}}^{\text{FMO/vac}}$	$\Delta\Delta G_{\text{bind}}^{\text{MM/GBSA}}$	$\Delta\Delta G_{\text{bind}}^{\text{FMO/GBSA}}$	$\Delta\Delta G_{\text{bind}}^{\text{FMO/PCM}}$	$\Delta E_{\text{lig deform}}^{\text{FMO/PCM}}$
ref.	0.0	0.0	0.0	0.0	0.0	0.0	3.1
F1	-2.5	-6.3	-11.5	-2.8	-8.1	-8.3	5.4
F2	-1.8	7.1	-4.1	12.1	0.9	-5.6	5.8
F3	-0.6	10.7	-0.9	13.0	1.4	-2.5	6.4
F4	-0.4	-5.1	-10.3	1.8	-3.4	-3.4	3.9
F5	-0.0	-3.8	-4.2	-4.9	-5.4	-4.4	7.8
G1	-2.8	-16.2	-19.3	-5.0	-8.1	-8.8	3.9
G2	-2.6	-10.7	-13.1	0.2	-2.2	-7.2	3.6
G3	-2.5	-11.1	-14.2	0.3	-2.8	-8.3	2.9
G4	-1.9	-11.6	-14.9	-1.5	-4.7	-6.4	5.5
G5	-1.1	-7.9	-11.0	1.9	-1.1	-4.9	3.9
G6	-0.9	-30.8	-31.0	-14.4	-14.6	-10.1	6.1

a) エネルギー値は全て kcal/mol。結合自由エネルギーに関しては，基準化合物からの相対値を表記
　　実測結合自由エネルギー値は，実測 PDHK4 IC_{50} 値より算出

相対結合自由エネルギーの計算値と実測値の相関を，ピアソン相関係数 R で解析した結果を Figure 3-5 に示す。真空中での相互作用エネルギー計算に関して，MM での相関は R 値で 0.28 と低く，FMO 法において R 値が 0.40 まで改善することが認められた。活性中心に存在する Asp293 と，複合体モデルにおいて Amide 部分で水素結合を形成する化合物 **G6** (Figure 3-6)が，回帰直線から大きく離れる結果となっている。化合物 **F4** も **G6** と同様に置換基部分で Asp293 と追加の水素結合を形成しており（Figure 3-6），化合物 **F2, F3** に比べて相互作用エネルギーが強く見積もられる結果を与えている。化合物 **G6** を除いた場合の R 値を $R_{\text{-G6}}$ として計算したところ，MM/vacuo において 0.55，FMO/vacuo において 0.76 となり相関が向上した。
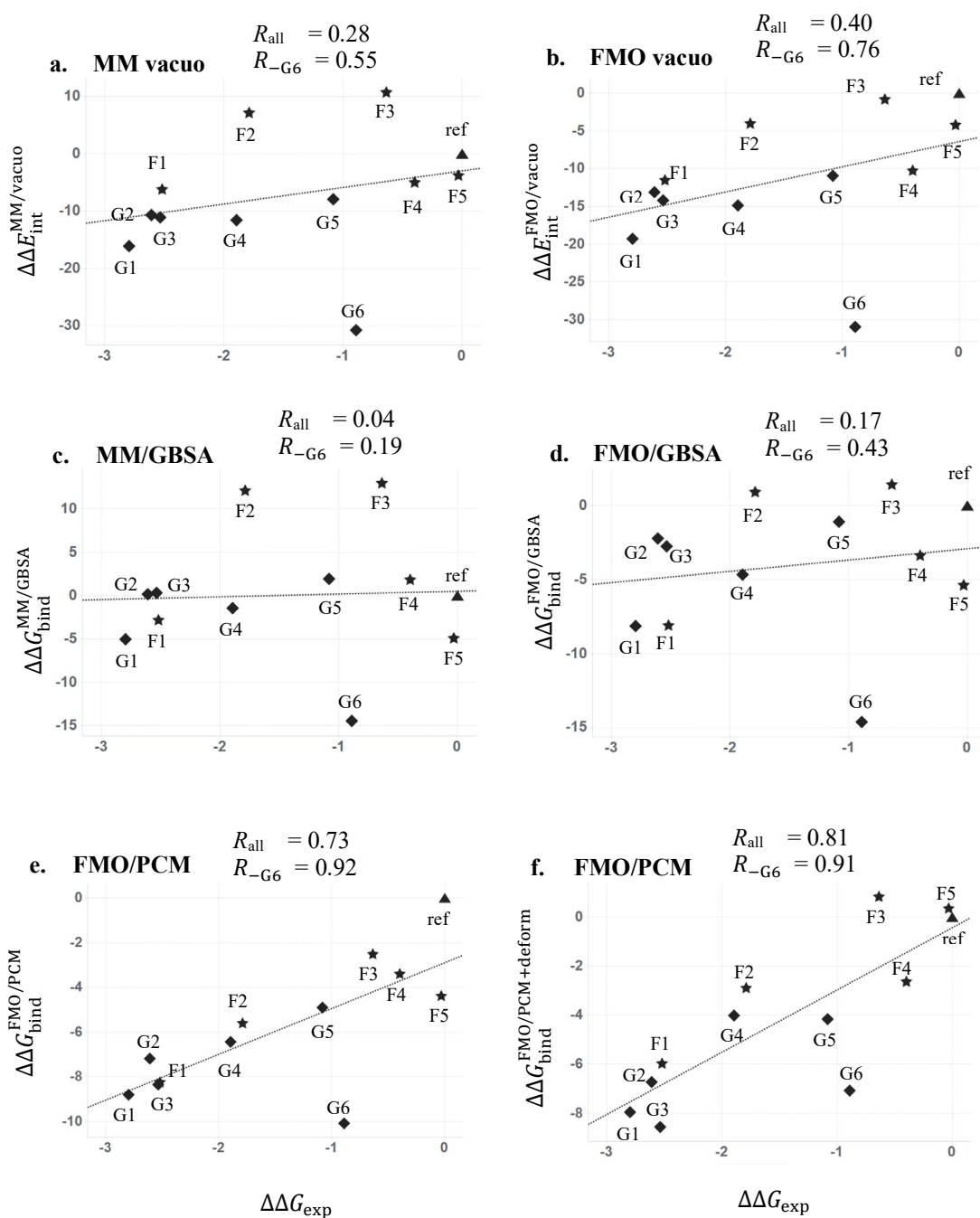
Figure 3-5. 実測相対結合自由エネルギー（横軸）と計算相対結合自由エネルギー（縦軸，a,b は相互作用エネルギー）の相関図

エネルギー単位は kcal/mol。R_{all} は全化合物のピアソン相関係数，R_{-G6} は化合物 G6 を除いたピアソン相関係数を表し，回帰直線を点線で示す

化合物番号，構造式は Figure 3-4 に示し，Dimethyl Indole 系統を星印，Pyrimido Indole 系統をひし形，基準化合物を三角形で表示
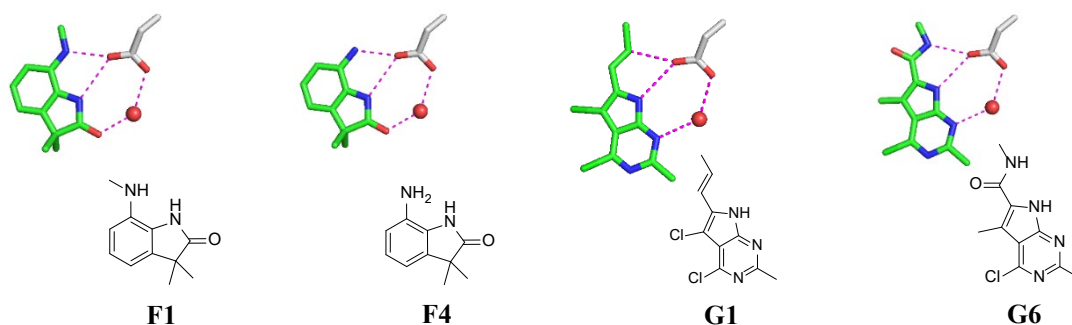
Figure 3-6. 化合物 **F1**, **F4**, **G1**, **G6** の PDHK4 に対する複合体モデル

化合物構造を緑色の炭素原子，Asp293 を白色の炭素原子，水分子は赤色の球で表示

　次に，溶媒和エネルギー項を加えた場合の相関関係を解析した。分子力場由来の GBSA エネルギー項を加えた場合，MM, FMO 法何れにおいても相関が大幅に悪化した。一方，量子力学由来の PCM 法による溶媒和エネルギー項を FMO 法に加える事で，全体での相関が R 値で 0.73 にまで向上した。

　溶媒和エネルギー項での補正効果に大きな差が認められた要因を把握する目的で，各化合物毎に算出された GBSA 法と PCM 法の溶媒和エネルギーを解析した（Table 3-6）。Figure 3-6 で示したように，化合物 **F1**, **F4**, **G1**, **G6** の 4 化合物は，Asp293 と置換基部分で極性相互作用を形成する。これら 4 化合物の脱溶媒和エネルギーは，GBSA 法と PCM 法で同等の値を示している。一方で，他の化合物は F5 を除き PCM 法において GBSA 法よりも小さな脱溶媒和エネルギーの値を示しており，結果として 4 化合物の相対的な脱溶媒和エネルギーが PCM 法において高くなっている。4 化合物の静電相互作用が，PCM 法において GBSA 法よりも強く脱溶媒和ペナルティーを課せられる事で，実測値との相関が改善したと考えられる。

Table 3-6. GBSA 法と PCM 法の脱溶媒和エネルギーの値 [a]

	F1	F2	F3	F4	F5	G1	G2	G3	G4	G5	G6
GBSA	3.4	5.0	2.3	6.9	-1.1	11.1	10.9	11.5	10.2	9.9	16.4
PCM	3.3	-1.6	-1.7	6.9	-0.2	10.4	5.9	5.9	8.4	6.0	20.9
PCM-GBSA	-0.2	-6.5	-3.9	0.0	1.0	-0.7	-5.0	-5.6	-1.8	-3.8	4.5

a) エネルギー値は全て kcal/mol。

　次に，PCM 法で 4 化合物に対する脱溶媒和ペナルティーが高くなった要因を把握する目的で，FMO/PCM 計算を行った際の電荷移動について解析した。Table-3-7 で，Asp293/WAT1 フラグメ

ントからの電荷移動値を示す。分子力場計算においては，Asp293/WAT1 フラグメントは-1*e* の全電荷を割り当てられ，計算中において電荷値が変化する事は無い。一方で，FMO/PCM 計算においては，強い電荷移動が起きている事が示された。複合体状態においては-0.27*e*，タンパク質単独状態においても-0.22*e* 程の電荷が周辺フラグメントに渡されている。供与電荷の多くが，水素結合ネットワークを形成するリガンドおよび Gly295, Gly297, Thr358 に移っており，MM での電荷状態とは大きく異なる電子状態で計算が行われている事が示された。また，Asp293/WAT1 からの電荷移動量は結合様式の異なるリガンド毎に異なり，置換基部分で水素結合を形成する **F1**，**F4**, **G1**, **G6** に対して，より大きな-0.050*e* を超える電荷が化合物に対して供与されていた。この事は，化合物毎に異なる分極状態を FMO/PCM 計算で与えている事を意味する。以上の様に，Asp293/WAT1 から電荷移動が水素結合ネットワーク内で起き，化合物毎に異なる分極状態を計算中に反映する事で，FMO/PCM 計算において実測値との相関が高くなる結果を与えたと考えられる。

Table 3-7. FMO/PCM 計算における Asp293/WAT1 から周辺フラグメントへの電荷移動値

(a) 複合体状態での電荷移動値

cmpd	F1	F2	F3	F4	F5	G1	G2	G3	G4	G5	G6
Asp293 /WAT1	0.265	0.272	0.272	0.268	0.279	0.276	0.273	0.274	0.274	0.269	0.273
ligand	-0.056	-0.048	-0.049	-0.056	-0.047	-0.050	-0.047	-0.049	-0.043	-0.039	-0.061
Gly295	-0.059	-0.060	-0.061	-0.057	-0.060	-0.058	-0.058	-0.058	-0.058	-0.059	-0.057
Gly297	-0.040	-0.041	-0.041	-0.041	-0.041	-0.042	-0.040	-0.040	-0.043	-0.041	-0.040
Thr358	-0.085	-0.088	-0.087	-0.089	-0.103	-0.096	-0.095	-0.095	-0.096	-0.096	-0.093

(b) レセプター単独状態での電荷移動値

cmpd	F1	F2	F3	F4	F5	G1	G2	G3	G4	G5	G6
Asp293 /WAT1	0.223	0.224	0.225	0.220	0.222	0.225	0.222	0.222	0.224	0.222	0.225
Gly295	-0.063	-0.062	-0.063	-0.060	-0.061	-0.061	-0.060	-0.060	-0.059	-0.060	-0.062
Gly297	-0.038	-0.039	-0.038	-0.038	-0.038	-0.039	-0.038	-0.038	-0.040	-0.038	-0.039
Thr358	-0.085	-0.086	-0.085	-0.088	-0.087	-0.090	-0.090	-0.090	-0.090	-0.090	-0.089

実測値との相関が改善された FMO/PCM 計算においても，引き続き化合物 **G6** の過剰評価が認められるため，誤差の要因を探る解析を次に実施した。Figure 3-6 に示したように，PDHK4 に対する複合体モデルにおいて，化合物 **G6** は 2 つの水素結合ドナーNH を Asp293 の方向に向けている。一方で，化合物単独の状態においては，化合物 **G6** の 2 つの水素結合ドナーNH は，反対方向を向いた反転状態でも存在すると考えられる。いずれの配座が安定かを把握する目的で，化合物 **G6** に対する配座解析計算を実施した（Figure 3-7）。Figure 3-7a に，量子力学計算での化合物 **7f** の二面角プロット図を示しており，反転状態が安定な配座として示された。**G6** の PDHK4 結合フォームと反転状態の間に，二面角のエネルギー障壁として 5.0 kcal/mol を超える値が認められている。この解析結果に基づき，各化合物の PDHK4 結合時における最安定配座からの歪みエネルギーを相対結合自由エネルギーに加えて相関解析を改めて実施した。その結果，Figure 3-6f に示すように，実測値との相関が *R* 値で 0.81 まで改善する効果が認められた（Figure 3-6f）。
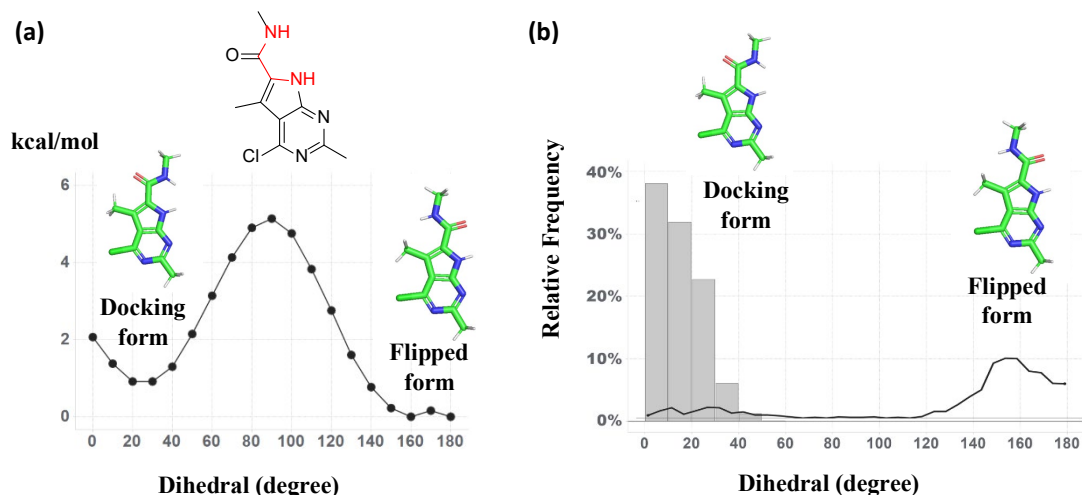
Figure 3-7. 化合物 **G6** の二面角計算プロット図

(a) QM（MP2/PCM¥6-31G*）による回転エネルギー障壁計算。対象二面角を赤字で表示

(b) MD 計算時の存在比率。黒色の折れ線グラフが化合物単体，灰色の棒グラフが複合体での存在比を示す

Figute 3-7b では，化合物 **G6** の水中および複合体中での MD 計算を行った際の二面角存在比を示している。PDHK4 結合時の MD 計算において，化合物 **G6** は二面角で 0-30 度の領域を占有し，平均値で 14.8 度の配座で存在している。一方で，化合物単独での水中 MD 計算を行った際には，Figure 3-7a で認められるエネルギー障壁を反映し，反転状態で大多数の配座が存在する結果を与えた。今回の計算検討においては，化合物の自由度が高くない事から，エントロピー項を無視してエネルギー計算を実施した。MD 計算結果からの考察として，エントロピー項を別法で計算する事により，更なる精度改善を行える可能性があると考えられる。

小括

本章では，PDHK ATP サイトを対象とした結合自由エネルギー計算の検討を行い，実測値との相関が高い計算条件を探索した。相関が高い計算条件を見出すことで，本標的に対する阻害活性を効率よく向上する事が可能となる。PDHK ATP サイトは，負電荷を有するアスパラギン酸を中心とした水素結合ネットワークを形成しており，電荷状態が特異な状況にあると考えられる。このため，量子力学法での検討が必要になると考え，フラグメント分子軌道法（FMO）を用いた計算検討を中心に実施した。

第 1 項では，仲西らが実施してきた，切り出しモデルを用いた FMO 結合自由エネルギー計算の効率化に向けた検討結果を横断的に解析した。荷電状態の異なる化合物を含む 5 つの標的に対して，切り出しモデルと全系とのエネルギー誤差を解析することで，中性化合物に対しては 8 Å，荷電性化合物に対しては 12 Å の切り出し距離が現実的な指標であることを明らかとした。この際，切り出しモデルの総電荷が全系と大きく異なる際には，切り出し距離に関わらず誤差が大きくなる事も併せて見出した。切り出しモデルを用いた計算は，大規模な計算系に対して適用する際に特に計算時間の短縮化が図れるため，本研究により見出された知見により FMO 計算の適用範囲を大きく拡張する事が可能であると期待される。

第 2 項では，PDHK ATP サイトの切り出しモデルに対して種々の計算条件で結合自由エネルギー計算を行い，実測値との相関が高い計算条件を探索した。溶媒モデルを含めた計算検討の結果から，量子力学法に基づく FMO 法と PCM 法を組み合わせた手法で結合自由エネルギーを計算する事が精度向上に重要であることを見出した。また，分子力場法由来の溶媒和エネルギー算出法である GBSA 法に比べ，PCM 法が何故精度を改善する結果を与えたかの解析も併せて行い，水素結合ネットワーク内で複雑な電荷移動が生じている事も明らかとした。更に，実測値との相関が低い化合物 **G6** に対する個別の解析を行い，化合物の歪みエネルギーを加える事で，全体のピアソン相関係数 R 値が 0.81 まで改善する計算条件を見出すことが出来た。

以上の研究から，切り出しモデルを用いた FMO 計算の効率化，並びに FMO 計算に PCM 法での溶媒和エネルギーを加える事での精度改善の知見を得た。これらの知見は，PDHK ATP サイトの様な複雑な水素結合ネットワークを形成する標的に対して，結合自由エネルギー計算を通じて有望な化合物を探索する際に有効な手法となる事が期待できる。

結論

本論文では，種々の病態における解糖系と糖質酸化の連動不全を是正する事を目的とし，PDHK に対する低分子阻害薬の創薬研究を実施した。

第 1 章では，Fragment Screening によりリード化合物の起点化合物を探索した。PDHK4 に対する X 線 Fragment Screening を実施する事で，既知化合物より ATP 結合ポケットに深く入り込む新規な結合様式を与える Fragment Hit 1 を見出した。阻害活性 IC_{50} が mM レベルの Fragment Hit 化合物から， X 線複合体構造を活用した Fragment Growing を実施する際に，ポケット深部の水素結合ネットワークに着目した水のエネルギー解析を実施した。WaterMap 計算より得られた結果から，ポケット深部の水を脂溶性基で排斥する事で，阻害活性を向上する事に成功した。また，Fragment Hit 2 との Fragment Merge を行う事で， µM レベルの IC_{50} を示す 3 種類のリード候補化合物を見出した。得られたリード候補化合物は，既知阻害剤よりポケット深部に深く入り込む特徴を有しており，選択性プロファイルで優れていることが明らかとなった。

第 2 章では，阻害活性向上を指向し，PDHK ATP サイトのリボース・リン酸結合部位における合成展開を実施した。三環性化合物の C3 位から合成展開を進める過程で，リン酸結合領域に存在するフレキシブルな ATP-lid が構造化し，Arg258 との相互作用を仲介する新規な結合様式が X 線複合体構造で認められた。得られた複合体構造情報から，不安定水の排斥，分子内 pi-pi 相互作用の形成に着目した SBDD を行うことで，IC_{50} が nM レベルに到達する化合物を見出した。更に，C3 位からの検討で得られた SBDD 情報を C4 位に転用し，分子サイズをより低減した阻害剤の創出を試みた。複合体モデルの重ね合わせ結果から，ピロリジンを基本骨格として利用した合成展開を行い， nM レベルの阻害活性をより小さい分子サイズで示す化合物を創出した。

第 3 章では，荷電性残基が複雑な水素結合ネットワークを形成する PDHK ATP サイトに対して，相互作用エネルギー計算を通じた構造活性相関研究を行った。計算検討を行うに際して，量子力学法であるフラグメント分子軌道法を用いた計算検討が必要になると考えた。そこで，まずは効率よく FMO 計算を行うための計算条件を見出すことが重要であると考え，仲西らが実施してきた切り出しモデルの検討結果を横断的に解析した。その結果，中性化合物に対しては 8 Å，荷電性化合物に対しては 12 Å の切り出し距離が現実的な切り出しサイズの指標であることを見出した。次に，見出した FMO 計算条件を用いた検討を PDHK4 に対して実施した。種々の計算検討を行い，脱溶媒和エネルギーを量子力学法である PCM 法で計算する事が，実測値との相関を高めるうえで重要であることを見出した。また，量子力学計算結果の解析を通じて，PDHK ATP サイトでの水素結合ネットワークにおける電荷移動の詳細を解析した。

以上，1 章から 3 章の検討を通じて，PDHK ATP サイトにおける新規低分子阻害剤の創薬研究

について論述した。ポケット深部に存在する水の排斥効果，ポケット外側での親和性向上に繋がる SBDD 情報を明らかとした事で，今後 PDHK ATP サイトにおいて効率よく阻害活性を向上する事が期待できる。また，脱溶媒和エネルギー項も量子力学法で扱う FMO/PCM 法での計算が，PDHK ATP サイトでの親和性予測計算の精度向上に重要であることも見出した。得られた結果は，同様の水素結合ネットワークを形成する標的に対して結合自由エネルギー計算を行う際に，重要な知見となることが期待できる。

　今回，PDHK ATP サイトでの SBDD 研究を進める上で，構造の深い理解が重要であることを再認識した。複合体構造を取得するだけでは不十分で，相互作用の本質を深く理解し，その理解に基づく分子設計と in silico 評価を行う事が，独創的な新薬候補化合物を創出する上で重要である。計算機化学者として今後の創薬研究を進めるに際し，構造の本質を理解するというスタンスを強く意識したい。

　革新的な新薬の創出は，人の命を守り，生活の質を改善する。一方で，創薬研究は長い時間とコストを要し，平均的な数値として一つの薬の創出に 22749 化合物の探索合成，10 年以上の開発期間，数千億円規模の開発費用を要する[48]。近年創薬標的の難易度は向上しており，この数値を改善する事は，多くの人々に必要な薬を届けるために重要な項目である。今回 PDHK ATP サイトでの研究を通じて得られた知見が，革新的な新薬の創出を効率よく進めるための礎となることを願う。

合成実験項

Synthetic Schemes

Compounds **4-8** were synthesized as shown in Scheme 1.　After protecting NH moiety of 7-bromo isatin **46** with PMB, the Wolff–Kishner reduction gave lactam **48**. Methylation of **48** with methyl iodide produced the key intermediate **49**. Palladium-catalyzed boration of **49** with vinyl pinacol diborane gave **50**. **50** was hydrogenated with palladium hydroxide on carbon, and subsequent PMB deprotection gave compound **4**. PMB deprotection of **52** followed by palladium-catalyzed boration afforded compound **5**. Palladium catalyzed carbonylation of **49** gave **53**, and subsequent Curtius rearrangement produced the Boc-protected **55**. Deprotection of PMB and Boc with HBr-AcOH produced compound **6**, and **7** was prepared in the same procedure after methylation. Compound **8** was prepared by methylating the lactam **57**.

Scheme 1
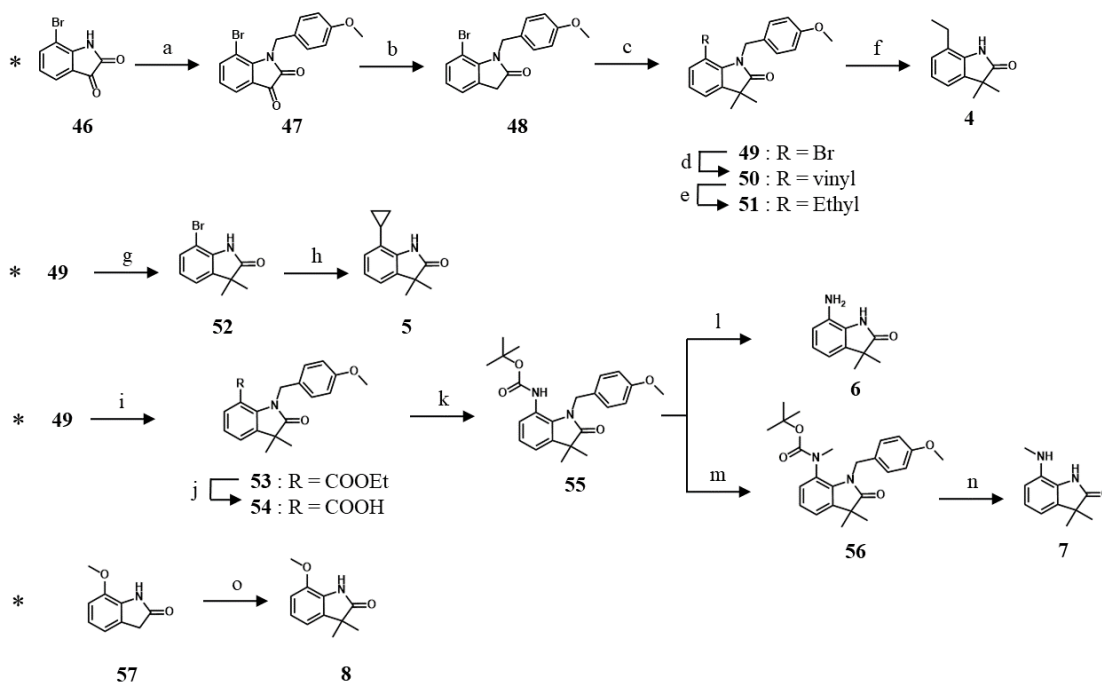
Reagents and conditions: (a) NaH, PMB-Cl, DMF, 0°C; (b) N_2H_4-H_2O, EtOH, 95°C; (c) NaH, MeI, DMF, 0°C; (d) vinyl-Bpin, $PdCl_2$(dppf)-CH_2Cl_2, Na_2CO_3, DME, H_2O, reflux; (e) Pd(OH)$_2$, H_2, MeOH, r.t.; (f) TFA, 60°C; (g) TFA, 50°C ; (h)cyclopropyl-B(OH)$_2$, $PdCl_2$(dppf)-CH_2Cl_2, Na_2CO_3, DME, H_2O, reflux; (i) CO,$PdCl_2$(dppf)-CH_2Cl_2, Et_3N, EtOH, 80°C; (j) 2N NaOH, MeOH, THF, 60°C; (k) DPPA, Et_3N, toluene, 85 °C then tBuOH, 60°C ; (l) TFA, CHCl$_3$, r.t.;(m) NaH, MeI, DMF, r.t. ; (n) TFA, 80°C; (o) n-BuLi, MeI, THF, r.t.

Compounds **9-11** were synthesized as shown in Scheme 2. The palladium-catalyzed Suzuki-Miyaura cross-coupling reaction of 2-bromoaniline **58** with cyclopropylboronic acid gave the cyclopropylbenzene **59**, and conversion of the amino group to the iodine via the Sandmeyer reaction provided the iodobenzene **60**. The palladium-mediated Buchwald-Hartwig type amination of the iodobenzene **60** and the aminopyridine **61** gave the compound **63**, and the subsequent intramolecular Heck-type cyclization provided the pyridoindole **9**. Pyridoindole **10** was prepared with the same procedure from **60** and **62**. The synthesis of compound **11** was started from 1-bromo-3-fluoro-2-nitrobenzene **65**. The fluorine in compound **66**, which was prepared from **65** and cyclopropylboronic acid by conducting the Suzuki-Miyaura cross-coupling reaction, was substituted by ethyl cyanoacetate to obtain **67**. Iron-mediated reduction of the nitro group in **67** was accompanied with direct cyclization and gave the indole **68**. The reaction of **68** and acetonitrile, treated under acidic conditions of 4N-HCl/dioxane first, then under basic conditions of saturated NaHCO₃ solution, gave the pyrimidoindole intermediate **69**. The hydroxyl group in **69** was converted to the chlorine **70** by treating with thionyl chloride, and subsequent hydrogenation with palladium hydroxide on carbon gave pyrimidoindole **11**.

Scheme 2
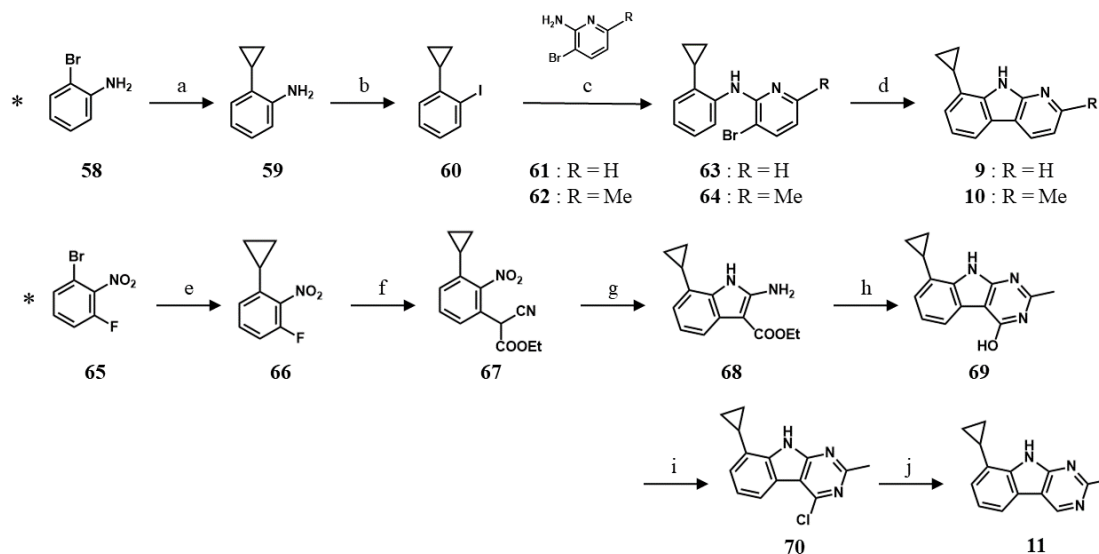
Reagents and conditions: (a) cyclopropylboronic acid, PdCl₂(dppf)-CH₂Cl₂, K₃PO₄, toluene, H₂O, 90°C, 6 h; (b) HCl, H₂O, NaNO₂, 0°C, 15 min, then NaI, 0°C, 30 min, then rt, 2 h; (c) Pd(OAc)₂, Xantphos, sodium t-pentoxide, toluene, reflux, 4 h; (d) Pd(OAc)₂, CyJohnphos, DBU, DMA, 130 °C, 4 h; (e) cyclopropylboronic acid, PdCl₂(dppf)-CH₂Cl₂, K₃PO₄, DME, H₂O, 110 °C; (f) ethyl cyanoacetate, K₂CO₃, DMF, 90°C; (g) Fe, AcOH, 100°C; (h) MeCN, 4N-HCl in dioxane, r.t., then sat. NaHCO₃ aq, MeOH,75°C; (i) SOCl₃, CHCl₃, DMF, 60°C; (j) Pd(OH)₂/carbon, H₂, K₂CO₃, THF, MeOH, r.t.

Compounds **12-16** were synthesized as shown in Scheme 3. For the synthesis of **12**, 4-chloro-2-methyl-7*H*-pyrrolo[2,3-d]pyrimidine **71** was protected by SEM to give **72**. Next, the 6-position of pyrrolopyrimidine was propylated and converted into **73**, and the chloro was removed reductively to obtain **74**. **74** was brominated with NBS to give 5-bromo compound **75**. Finally, cyanation of **75** and deprotection of SEM of **75** with BF$_3$-Et$_2$O yielded **12**. In the synthesis of **13** and **14**, compound **78** was synthesized by the Sonogashira reaction of 5-iodo-2-methylpyrimidin-4-amine **77** with 1-penthyne, and then pyrrole cyclization under basic conditions gave **79**. NCS-Chlorination and NBS-bromination of **79** gave **13** and **14**, respectively. The synthesis of **15** and **16** was started from **73** and bromination at the 5-position led to **80**. Conversion of the bromo of **80** to a methyl and then removal of SEM of **81** using TFA gave compound **16**. Subsequent reductive dehalogenation of chlorine gave **15**.

Scheme 3
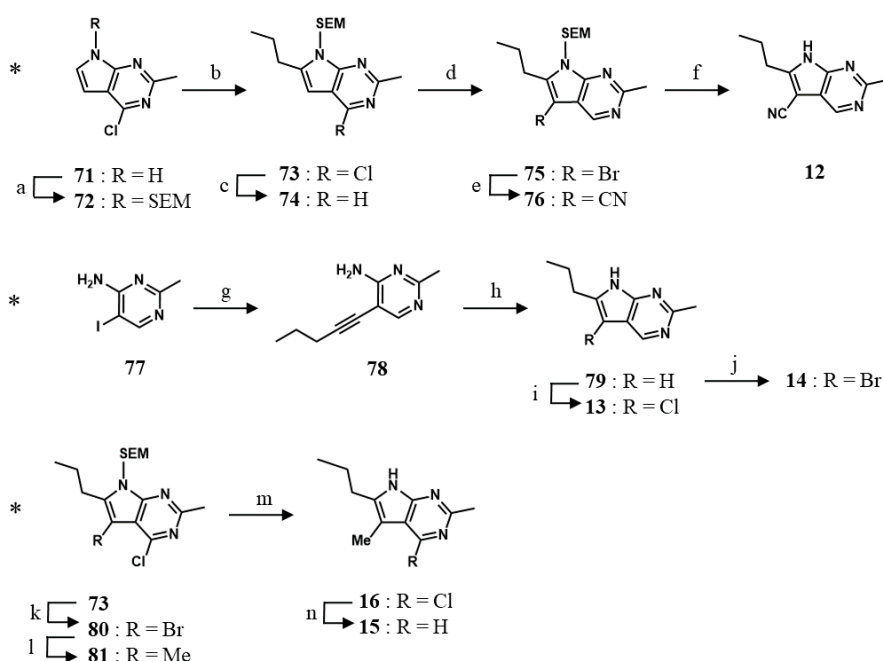
Reagents and conditions: (a) SEMCl, NaH, DMF, rt, 1.5 h; (b) 1-Iodopropane, *n*-BuLi, THF, rt, 1.5h; (c) 1 atm-H$_2$, 10% Pd/C, K$_2$CO$_3$, rt, overnight; (d) NBS, DMF, rt, 1 h; (e) TsCN, n-BuLi, THF, rt, 3 h; (f) BF$_3$-Et$_2$O, CHCl$_3$, rt, overnight; (g) 1-Penthyne, PdCl$_2$(dppf), CuI, Et$_3$N, DMF, 70°C, 2 h; (h) t-BuOK, NMP, 100°C, 1 h; (i) NCS, DMF, rt, 5 h; (j) NBS, CHCl$_3$, rt, 15 h; (k) NBS, DMF, rt, overnight; (l) MeI, *n*-BuLi, THF, rt, 2 h; (m) TFA, rt, 1 h; (n) 1 atm-H$_2$, 10% Pd/C, K$_2$CO$_3$, rt, 2 h

Compounds **17-19** were synthesized as shown in Scheme 4. Methylamide **17** was synthesized from 4-chloro-2-methyl-7*H*-pyrrolo[2,3-d]pyrimidine **71**. Similar to the synthesis of **81**, compound **71** was brominated to **82** and subsequently methylated to **83**. After Ts protection of the pyrrole nitrogen, introduction of the ethyl ester to the 6-position of **84** by anion reaction with ethyl carbonochloridate and *n*-BuLi gave compound **85**. After hydrolysis of the ester and deprotection of Ts with lithium hydroxide at the

Scheme 4
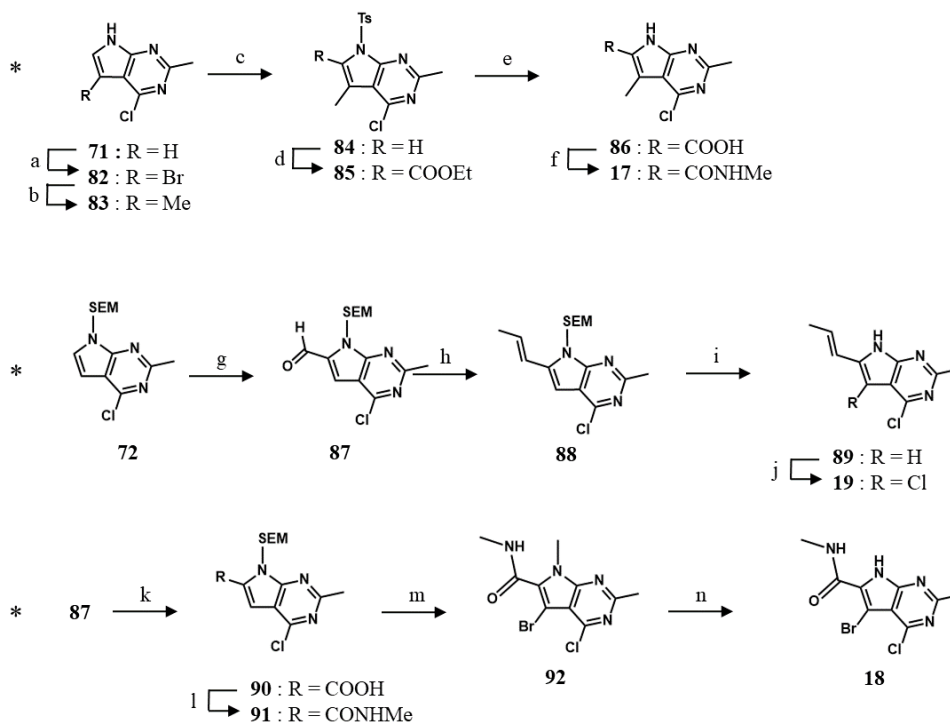
Reagents and conditions: (a) NBS, CHCl₃, rt, overnight : (b) MeI, n-BuLi, THF, 0°C, 1 h : (c) TsCl, NaH, DMF, 0°C, 0.5 h; (d) ClCOOEt, n-BuLi, THF, rt, overnight; (e) LiOH aq, THF, 50°C, overnight; (f) NH₂Me, WSC, DMAP, THF-CHCl₃, rt, overnight; (g) DMF, n-BuLi, THF, rt, 1 h; (h) EtPPh₃Br, PhLi, THF, -78°C, 1.5 h; (i) TFA, CHCl₃, rt, 1 h; (j) NCS, DMF, rt, overnight; (k) NaClO₂, NaH₂PO₄, 2-methylbut-2-ene, t-BuOH, H₂O, rt, overnight; (l) NH₂Me, WSC, DMAP, CHCl₃, rt, overnight; (m) NBS, CHCl₃, rt, 2.5 h; (n) BF₃-Et₂O, CH₂Cl₂, rt, 2 h.

same time, to obtain carboxylic acid **86** was obtained.　Methylamine, WSC, and DMAP were used to give methylamide **17**. Compounds **18** and **19** were both synthesized from **87** obtained by formylation of **72**. The Wittig reaction of the aldehyde **87** yielded trans-butene **88**, which was converted to **19** by SEM deprotection and NCS-chlorination. Carboxylic acid intermediate **90** was obtained from aldehyde **87** by the Pinnick oxidation. Subsequent amidation of **90** gave methyl amide **91**. NBS-Bromination gave 5-bromo compound **92** and SEM removal gave compound **18**.

　　The synthetic methods of C3-position substituted pyridoindole **21 – 24** are illustrated in Scheme 5. The bromopyridine **96** was synthesized from the cyanide **93**, conducted the hydrolysis of the nitrile to the carboxylic group, the esterification of **94** to the methyl ester **95**, and the NBS-bromination of **95**. The palladium-mediated Buchwald-Hartwig type amination of the iodobenzene **60** and the aminopyridine gave the compound **97** and **100**, and the subsequent intramolecular Heck type cyclization provided the pyridoindole **22** and **101**. The ester of pyridoindole **22** was hydrolyzed to give the carboxylic acid **98**, and the amidation with methyl amine using HATU gave the compound **21**. The compound **23** was prepared

Scheme 5
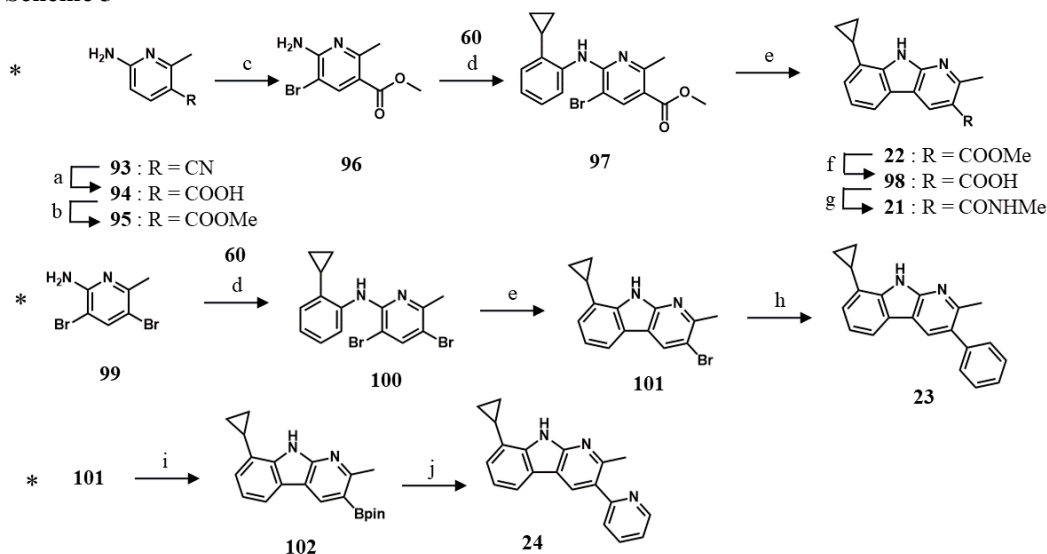
Reagents and conditions: (a) KOH, H$_2$O, 100 °C, 8 h; (b) SOCl$_2$, MeOH, 80 °C, 15 h; (c) NBS, THF, rt, 2 h; (d) Pd(OAc)$_2$, Xantphos, sodium t-pentoxide, toluene, 140 °C 4 h; (e) Pd(OAc)$_2$, CyJohnphos, DBU, DMA, 130 °C, 4 h; (f) NaOH, H$_2$O, THF, EtOH, 60 °C, 4.5 h.; (g) NH$_2$Me, HATU, Et$_3$N, DMF, rt, 2 h; (h) phenyl boronic acid, PdCl$_2$(dppf)-CH$_2$Cl$_2$, K$_2$CO$_3$, 1,4-dioxane, H$_2$O, 80 °C, 4 h; (i) (BPin)$_2$, PdCl$_2$(dppf)-CH$_2$Cl$_2$, KOAc, DMSO, 100 °C, 2 h; (j) 2-bromopyridine, PdCl$_2$(dppf)-CH$_2$Cl$_2$, K$_2$CO$_3$, 1,4-dioxane, H$_2$O, 100 °C, 2 h.

from **101** by conducting the Suzuki-Miyaura cross-coupling reaction with the phenyl boronic acid. The compound **101** was borated with bis(pinacolato)diboron to give **102**, and the subsequent cross-coupling reaction with 2-bromopyridine gave the compound **24**.

The synthetic methods of C3-position substituted pyridoindole **25 – 30** and **32** are illustrated in Scheme 6. The acylhydrazine **103** was prepared from the carboxylic acid **98** by the amidation with *tert*-butyl carbazate and the deprotection of *N*-Boc. In the next step, the compound **103** was reacted with the carboxylic acid by using the HATU condensation to obtain the intermediate diacylhydrazine, which was converted to the 1,3,4-oxadiazole **25** and **27– 30** by addition of the Burgess reagent. The thiadiazole **26** was synthesized from corresponding diacylhydrazine using the Lawesson reagent. The compound **104** was reacted with the carboxylic acid **105** using WSC/HOBt condensation to obtain the diacylhydrazine **106**, which was converted to the 1,3,4-oxadiazole **107** by addition of the Burgess reagent. *N*-Boc of **107** was deprotected to the target amine **108** by treating with HCl in dioxane solvent. Finally, the **108** was treated with 3-phenylpropanoyl chloride (**109**) under a basic condition to give the compound **32**.

Scheme 6
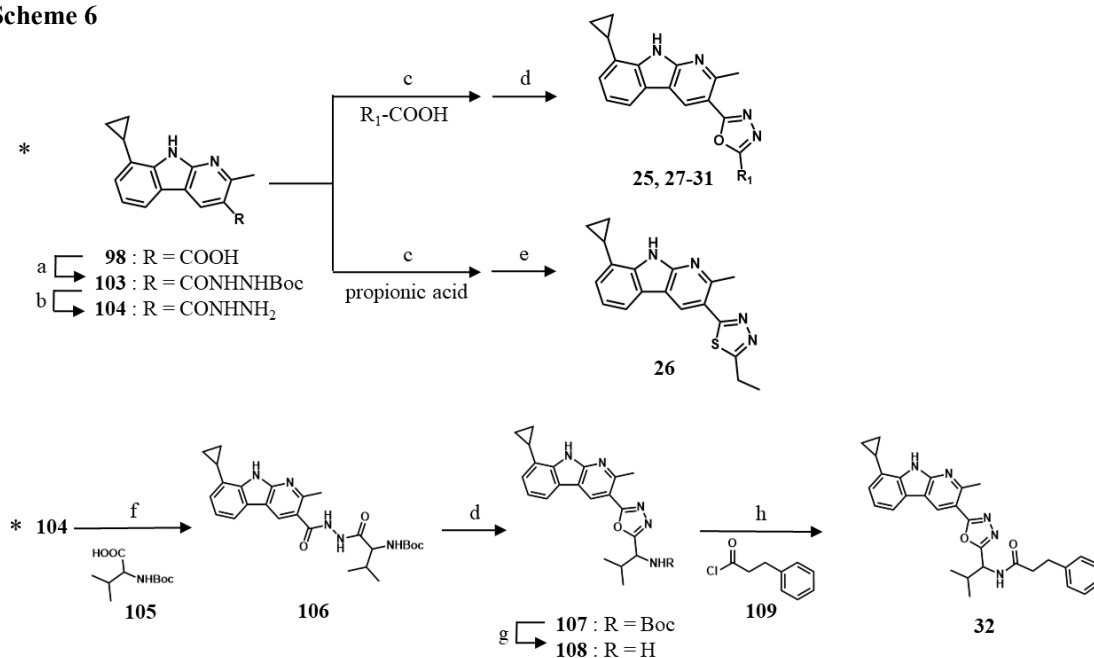
Reagents and conditions: (a) *t*-butyl carbazate, WSC, HOBt, DMF, rt, 4 h; (b) HCl, 1,4-dioxane, 70 °C, 3 h; (c) HATU, Et₃N, DMF, rt, 16 h; (d) Burgess reagent, THF, rt, 3 h; (e) Lawesson reagent, THF, 80 °C, 19 h; (f) WSC, HOBt, Et₃N, DMF, rt, 3 h; (g) HCl, 1,4-dioxane, rt, 1 h; (h) Et₃N, CHCl₃, 1 h. R₁ substituents of **25** and **27 - 30** are listed in Table 2-2 in page 19.

The synthetic methods of C3-position substituted pyridoindole **33** are illustrated in Scheme 7. 2-Isopropylmalonate (**110**) was reacted with the bromide **111** in the presence of NaH to give **112**, and the compound **112** was decarboxylated using KOH, and the subsequent amidation with *tert*-butyl carbazate gave the compound **115**. *N*-Boc of **115** was deprotected to the acylhydrazine **116** by treating with HCl in dioxane solvent. The carboxylic acid **98** was treated with **116** under the WSC/HOBt condensation to obtain the diacylhydrazine **117**. Subsequent cyclization of **117** in the presence of POCl₃ gave the 1,3,4-oxadiazole derivative **118**. Deprotection of the benzyl group of **118** led to the alcohol **119**, followed by Dess-Martin oxidation and Pinnick oxidation to the aldehyde **120** and the carboxylic acid **121** in that order. And then, the target compound **33** was obtained by the amidation with **121** and benzyl amine.

Scheme 7
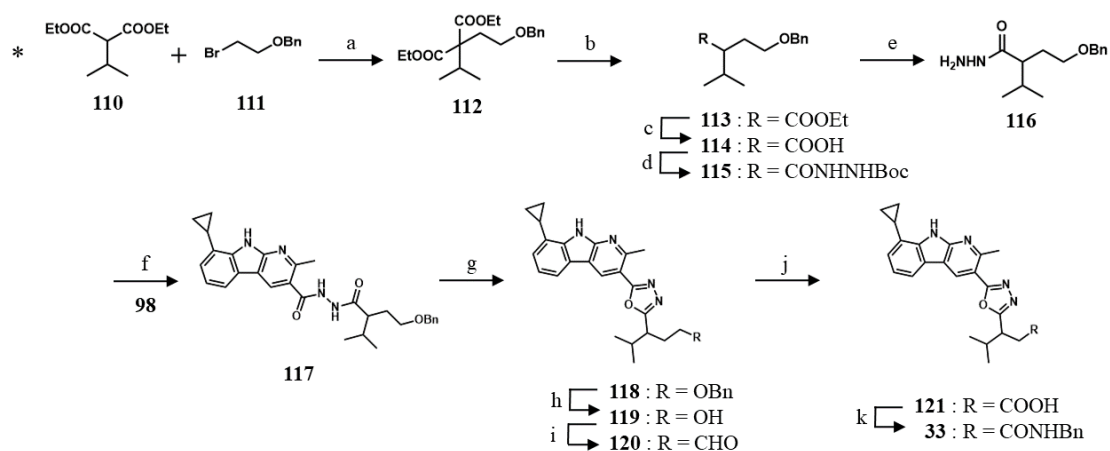
Reagents and conditions: (a) NaH, DMSO, 60 °C, 3 h; (b) KOH, EtOH, reflux, 17 h; (c) KOH, H_2O, EtOH, reflux, 8 h, (d) BocNHNH$_2$, WSC, HOBt, DMF, rt, 20 h.; (e) HCl, 1,4-dioxane, rt, 3.5 h; (f) WSC, HOBt, i-Pr$_2$EtN, DMF, rt, 1 h; (g) POCl$_3$, 110 °C, 2 h; (h) Pd/C (ASCA-2), H$_2$, rt, 2 days; (i) Dess-Martin Periodinane, CHCl$_3$, rt, 4 h; (j) NaClO$_2$, NaH$_2$PO$_4$, 2-methyl-2-butene, t-BuOH, H_2O, THF, rt, 1.5 h; (k) benzylamine, WSC, HOBt, CHCl$_3$, rt, 1 h

The synthetic methods of C3-position substituted pyridoindole **34** are illustrated in Scheme 8. The isovaleric acid **122** was reacted with the chiral oxazolidinone **123**, and the obtained compound **124** was reacted with *tert*-butyl bromoacetate in the presence of LiHMDS to give compound **125**. The chiral carboxylic acid **126** was obtained from **125** by removing oxazolidinone using H_2O_2. The compound **104** was reacted with **126** by using the WSC/HOBt condensation to obtain the diacylhydrazine **127**, which was converted to the 1,3,4-oxadiazole **128** by addition of the Burgess reagent. Then, the *tert*-butyl group in **128** was deprotected by treating with an excess amount of TFA to give the carboxylic acid **129**. Finally, the amidation of **129** with (*R*)-1-phenylethiamine gave the corresponding amide compound **34**.

Scheme 8
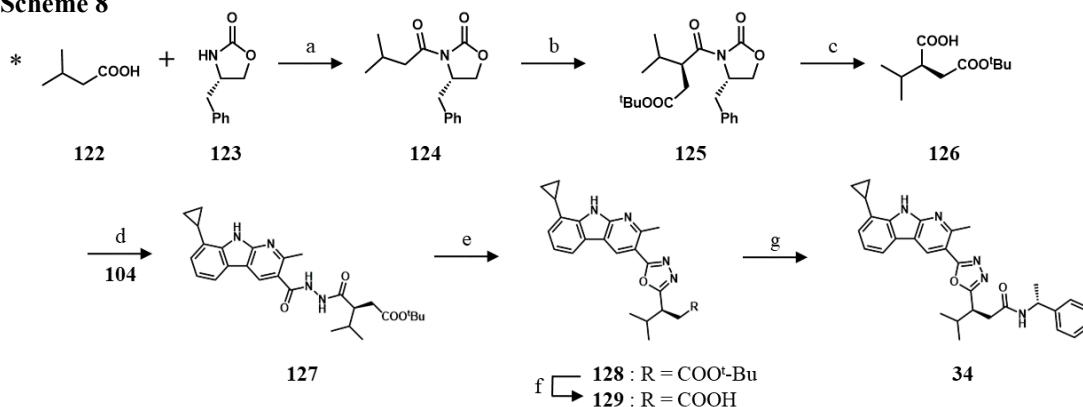
Reagents and conditions: (a) WSC, DMAP, MeCN, rt, 19 h; (b) *t*-butyl 2-bromoacetate, LiHMDS, THF, rt, 3.5 h; (c) H_2O_2, THF, 0 °C, 1 h then LiOH, H_2O, rt, 1 h; (d) WSC, HOBt, i-Pr$_2$EtN, DMF, rt, 2.5 h; (e) Burgess reagent, THF, rt, 0.5 h; (f) TFA, rt, 1 h; (g) WSC, HOBt, DMF, rt, 1.5 h.

The C4-position substituted pyrimidoindole **35 – 42** were prepared by the methods as detailed in Scheme 9. The pyrrolidine compounds were synthesized by substitutions of the chlorine atom of **70** by corresponding pyrrolidines using a Micro-Wave equipment. The cyano group of compound **133** was hydrolyzed during the reaction to give the compound **42** as the second major component.

Scheme 9

130 : R$_1$ = (S)-CH$_2$OH, R$_2$ = Ph, R$_3$ = H
131 : R$_1$ = (S)-CH$_2$OH, R$_2$ = Bn, R$_3$ = H
132 : R$_1$ = (S)-CH$_2$OH, R$_2$ = Ph, R$_3$ = CH$_2$OH
133 : R$_1$ = (S)-CH$_2$OH, R$_2$ = Bn, R$_3$ = CN

35 : R$_1$ = H, R$_2$ = H, R$_3$ = H
36 : R$_1$ = Et, R$_2$ = H, R$_3$ = H
37 : R$_1$ = (S)-CH$_2$OH, R$_2$ = H, R$_3$ = H
38 : R$_1$ = (R)-CH$_2$OH, R$_2$ = H, R$_3$ = H
39 : R$_1$ = (S)-CH$_2$OH, R$_2$ = Ph, R$_3$ = H
40 : R$_1$ = (S)-CH$_2$OH, R$_2$ = Bn, R$_3$ = H
41 : R$_1$ = (S)-CH$_2$OH, R$_2$ = Ph, R$_3$ = CH$_2$OH
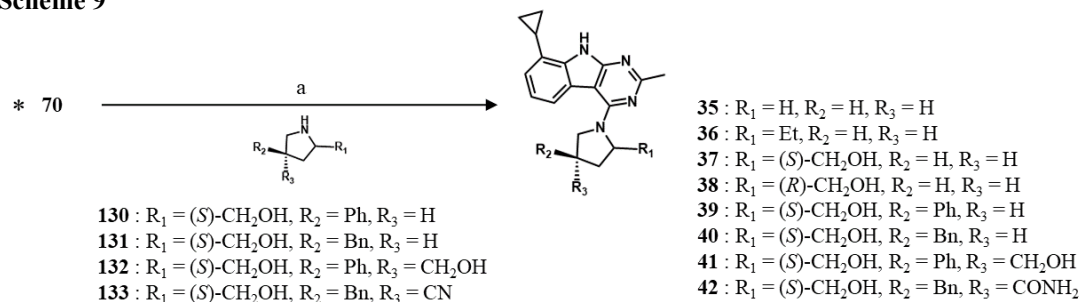**42** : R$_1$ = (S)-CH$_2$OH, R$_2$ = Bn, R$_3$ = CONH$_2$

Reagents and conditions: (a) corresponding pyrrolidine, *i*-PrNEt$_2$, NMP, Micro-Wave 140 °C, 2 h.

The synthetic methods of pyrrolidine compounds **130 – 133** are illustrated in Scheme 10. The carboxylic acid **134** was reduced to alcohol **135** using BH$_3$, and the subsequent *N*-Boc deprotection by treating HCl in dioxane solvent gave the target pyrrolidine **130**. The pyrrolidone **138** was synthesized from the pyrrolidone **136** by benzylating 3-position after protecting lactam NH with Boc group. The pyrrolidine **131** was obtained by reducing the pyrrolidone **138** using BH$_3$ and removing the Boc/Trityl protecting group by HCl in dioxane. The pyrrolidine **141** was synthesized in the similar synthetic procedure of **139**, except using methyl chloroformate as an alkylating reagent. The alcohol group of pyrrolidine **141** was oxidized to the carboxylic acid **142** using NaClO, which was then converted to the benzyl ester **143**. After benzylating 4-position of the pyrrolidine **143**, the ester was reduced to the alcohol **145** using LAH, and the removal of the Boc/Trityl protecting group gave target pyrrolidine **132**. The pyrrolidine **148** was synthesized from **146** by mesylating the 3-hydroxy with MsCl after protecting the 2-hydroxymethyl with trityl group. The methylsulfonyl group of compound **148** was converted to cyano group using the tetraethylammonium cyanide, and the pyrrolidine **150** was synthesized by benzylating 4-position. The pyrrolidine **133** was obtained after removing the Boc/Trityl protecting group of **150**.

Scheme 10
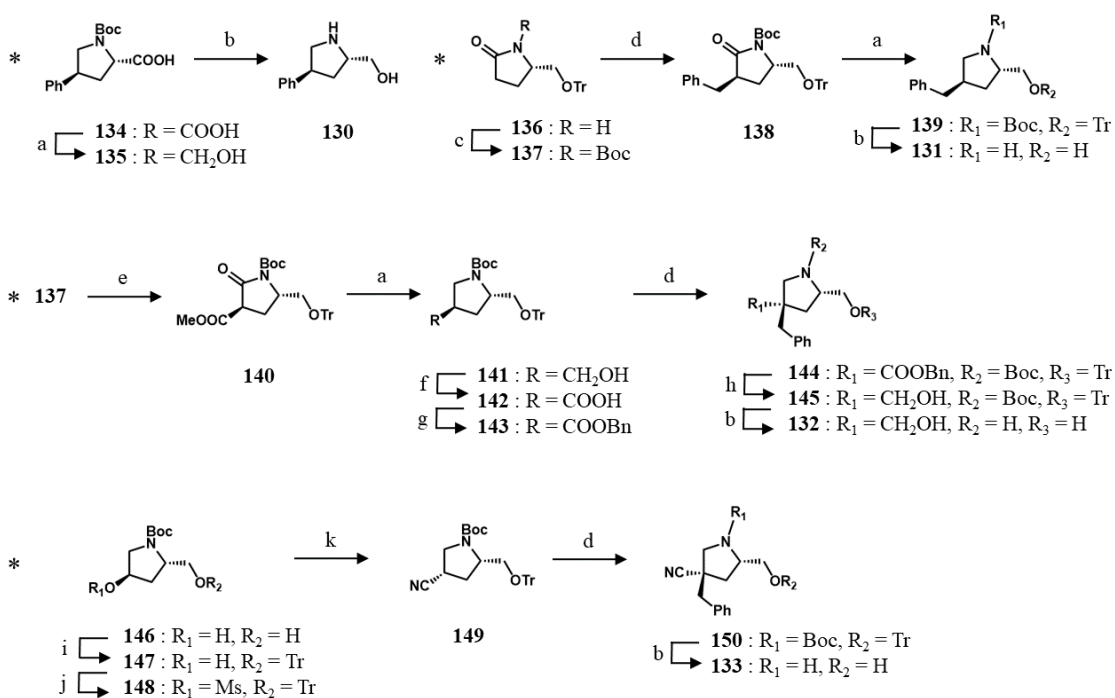
Reagents and conditions: (a) BH$_3$, THF, 80 °C, 20 min; (b) HCl, dioxane, rt, 1.5 h; (c) Boc$_2$O, DMAP, DMF, 70 °C, 4 h; (d) BnBr, LiHMDS, THF, rt, 1 h; (e) Methyl chloroformate, LiHMDS, THF, CHCl$_3$, -30 °C, 1 h; (f) 1-Me-AZADO, NaClO, NaClO$_2$, t-BuOH, H$_2$O, rt, 1.5 h; (g) BnBr, K$_2$CO$_3$, DMF, rt, 1.5 h; (h) LAH, THF, 0 °C, 30 min; (i) TrCl, NEt$_3$, DMAP, DMF, 60 °C, 4 h; (j) MsCl, Et$_3$N, CHCl$_3$, 0 °C, 30 min; (k) Et$_4$NCN, MeCN, 100 °C, 4 h.

The C4-position substituted pyrimidoindole **43 – 45** were prepared by the methods as detailed in Scheme 11. The pyrrolidine **152** was synthesized from **151** by reducing carboxylic acid to alcohol using BH$_3$, which was then oxidized to aldehyde using Dess-Martin Periodinane. The pyrrolidine **153** was reacted with Ph$_3$PMeBr to give **154**, which was then hydrogenated to give the pyrrolidine **155**. The pyrrolidine **157** was synthesized by reacting Tf$_2$NPh after oxidizing the hydroxyl group of compound **155** with Dess-Martin Periodinane. The palladium-mediated coupling reaction with pyrrole boronic acid gave the compound **158**, which was then hydrogenated to give the pyrrolidine **159**. After removing N-Boc group by HCl in dioxane, the pyrrolidine **160** was reacted with **70** using a Micro-Wave equipment to give **161.** The pyrimidoindole **43** was obtained by hydrolyzing methyl ester with NaOH.   The pyrimidoindole **44** was synthesized in the similar synthetic procedure of **43**, which was then amidated with ammonium chloride using HATU to give the compound **45.**

Scheme 11
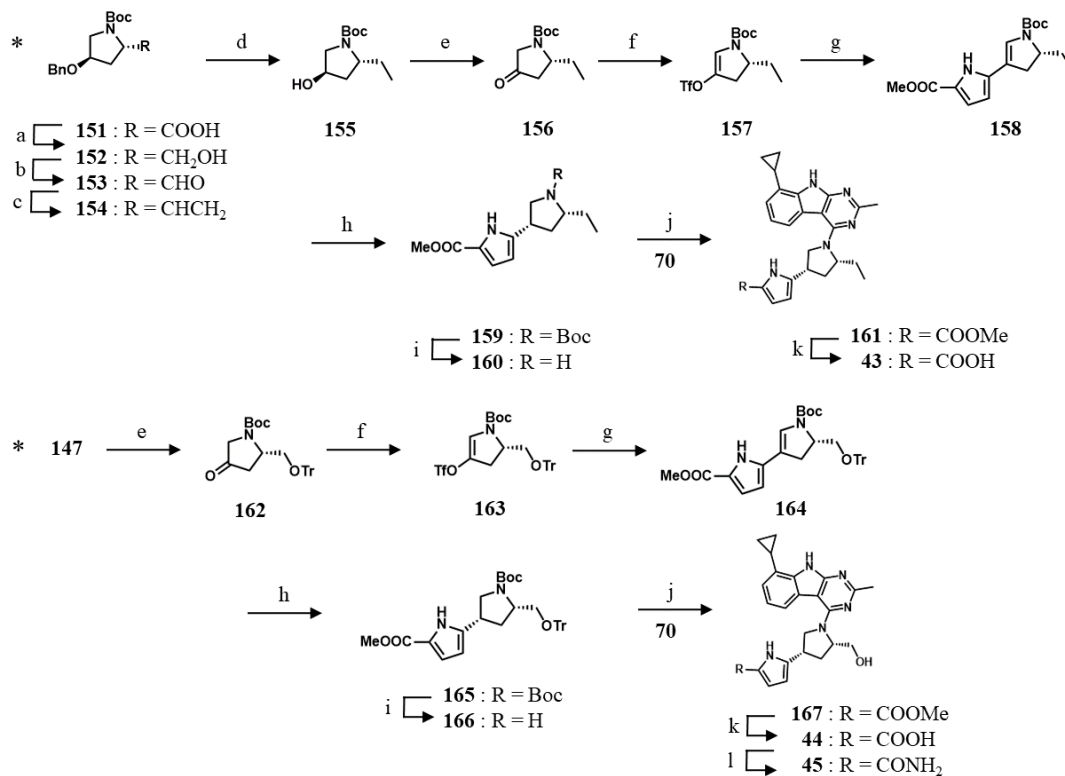
Reagents and conditions: (a) BH₃-THF, THF, 50 °C, 2 h; (b) Dess-Martin Periodinane, CH₂Cl₂, rt, 1.5 h; (c) Ph₃BMeBr, *t*-BuOK, THF, rt, 5.5 h; (d) H₂, Pd/C, MeOH, THF, rt, 24 h; (e) Dess-Martin Periodinane, CH₂Cl₂, rt, 1 h; (f) Tf₂NPh, LiHMDS, THF, -78 °C to rt, 1 h; (g) (5-(methoxycarbonyl)-1H-pyrrol-2-yl)boronic acid, PdCl₂(dppf)-CH₂Cl₂, Na₂CO₃,toluene, H₂O, 90 °C, 2 h; (h) H₂, Pd/C, MeOH, THF, rt, 24 h; (i) HCl, 1,4-dioxane, rt, 1.5 h; (j) *i*-PrNEt₂, NMP, Micro-Wave 140 °C, 2 h; (k) NaOH, H₂O, MeOH, THF, 80 °C; (l) NH₄Cl, HATU, NEt₃, CHCl₃, rt, 18 h

Synthetic Details

Solvents and reagents were obtained from commercial suppliers and used as received. Flash column chromatography was performed using Merck 230–400 mesh silica gel 60. Proton nuclear magnetic resonance (1H NMR) spectra were recorded on a Varian MERCURYplus-AS400, JEOL RESONANCE Inc. JNM-AL400, Bruker BioSpin K.K. AV400 or AVANCE III 400, or Agilent Technologies Inc. 400-MR spectrometer in the indicated solvent. Chemical shifts (δ) are reported in parts per million relative to internal standard tetramethylsilane. High-resolution mass spectra (HRMS) analyses were performed on an LC-MS system composed of Agilent 1290 Infinity LC and Thermo Fisher Orbitrap ID-X.

Preparation of compound 1, 2, 3 and 20

1, **2**, **3** and **20** were obtained from commercial suppliers and used without further purification.

Synthesis of compound 31

Compound **31** was synthesized based on the reported synthetic procedure of this compound reference.

Synthesis of compound 4

Step 1: 7-Bromo-1-(4-methoxybenzyl) indoline-2,3-dione (**47**)

7-Bromo isatin (**46**; 2.5g, 11.1mmol) in DMF (10mL) was added by drops, using a dropping funnel, into a solution of 60% sodium hydride (66 8mg, 16.7 mmol) in DMF (20 mL) at 0°C. *Para*- Methoxy benzyl chloride (2.3mL, 16.7mmol) was added dropwise at 0 °C, and the mixture was stirred at room temperature for 1.5 h. The reaction mixture was quenched with methanol (2 mL) at 0 °C and was diluted with H_2O and EtOAc. The organic layer was washed with H_2O and brine, and dried over Na_2SO_4. After filtration and concentration, the residue was purified by flash chromatography (solvent: EtOAc/CHCl$_3$ = 1/30 to 1/20) to give the title compound **47** (1.75 g, 46% yield).

^{1}H-NMR (400MHz, CDCl$_3$) δ: 3.78 (s, 3H), 5.38 (s, 2H), 6.86 (d, *J* =8.8 Hz, 2H), 6.99 (dd, *J* =8.0, 7.2 Hz, 1H), 7.23 (d, *J* =8.8 Hz, 2H), 7.61 (dd, *J* =7.2, 1.2 Hz, 1H), 7.67 (dd, *J* =8.0, 1.2Hz, 1H)

Step 2: 7-Bromo-1-(4-methoxybenzyl)indolin-2-one (**48**)

Hydrazine monohydrate (2 mL) was added to **47** (1.75 g, 5.06 mmol) in EtOH (20 mL) and stirred at 95 °C for 12 h. After cooling to room temperature, H_2O and EtOAc were added to the reaction mixture. The organic layer was washed with H_2O and brine, and dried over Na_2SO_4. After filtration and concentration, the residue was purified by flash chromatography (solvent: EtOAc/Hexane = 1/5, 1/4 to 1/3) to give the title compound **48** (876 mg, 52% yield).

^{1}H-NMR (400MHz, $CDCl_3$) δ: 3.77 (s, 3H), 5.34 (s, 2H), 6.84 (d, J =8.8 Hz, 2H), 6.86-6.92 (m, 2H), 7.16-7.21 (m, 3H), 7.28-7.32 (m, 1H), 7.35 (dd, J =8.4, 1.2 Hz, 1H)

Step 3: 7-Bromo-1-(4-methoxybenzyl)-3,3-dimethylindolin-2-one (**49**)

Compound **48** (876 mg, 2.64 mmol) in DMF (6 mL) was dropped into the solution of 60% sodium hydride (232 mg, 5.81 mmol) in DMF (6 mL) at 0 °C. Methyl iodide (489μL, 7.92 mmol) was added dropwise, and the mixture was stirred at room temperature for 2 h. The reaction mixture was quenched with water at 0 °C, and extracted with EtOAc. The organic layer was washed with H_2O and brine, and dried over Na_2SO_4. After filtration and concentration, the residue was purified by flash chromatography (solvent: EtOAc/Hexane = 1/6 to 1/5) to give the title compound **49** (889 mg, 93% yield).

^{1}H-NMR (400MHz, $CDCl_3$) δ: 1.42 (s, 6H), 3.77 (s, 3H), 5.34 (s, 2H), 6.83 (d, J =8.8 Hz, 2H), 6.90 (dd, J =8.4, 7.2Hz, 1H), 7.12-7.16 (m, 3H), 7.31 (dd, J =8.4, 1.2 Hz, 1H)

Step 4: 1-(4-methoxybenzyl)-3,3-dimethyl-7-vinylindolin-2-one (**50**)

A mixture of **49** (100 mg, 0.28 mmol), vinyl pinacol diborane (71 μL, 0.42 mmol) and Na_2CO_3 (89.0 mg, 0.84 mmol) in DME (2 mL) and H_2O (1 mL) was treated with $PdCl_2$(dppf)-CH_2Cl_2 (11.0 mg, 0.014 mmol) and stirred at 95 °C for 3 h. After cooling to room temperature, H_2O and EtOAc were added to the reaction mixture. The organic layer was washed with H_2O and brine, and dried over Na_2SO_4. After filtration and concentration, the residue was purified by flash chromatography (solvent: EtOAc/Hexane = 1/5) to give the title compound **50** (80.0 mg, 93% yield).

^{1}H-NMR (400MHz, $CDCl_3$) δ: 1.44 (s, 6H), 3.78 (s, 3H), 5.08 (s, 2H), 5.20 (dd, J =10.8, 1.2Hz, 1H),5.48 (dd, J =17.2, 1.2Hz, 1H), 6.81-6.88 (m, 3H), 7.03 (dd, J =7.6, 7.6Hz, 1H), 7.09 (d, J =8.8Hz, 2H), 7.15-7.18 (m, 2H)

Step 5: 7-Ethyl-1-(4-methoxybenzyl)-3,3-dimethylindolin-2-one (**51**)

To a solution of **50** (80.0 mg, 0.26 mmol) in MeOH (3 mL) was added Pd(OH)$_2$ (10 mg) and stirred under 1.0 atm of hydrogen at room temperature for 12 h. After removal of the catalyst by Celite® filtration, the filtrate was evaporated *in vacuo* to give the title compound **51** (73.0 mg, 91% yield).

^{1}H-NMR (400MHz, CDCl$_3$) δ: 1.10 (t, *J* =7.6 Hz, 3H), 1.44 (s, 6H), 2.39 (q, *J* =7.6 Hz, 2H), 3.77 (s, 3H), 5.10 (s, 2H), 6.83 (d, *J* =8.8 Hz, 1H), 6.99-7.01 (m, 2H), 7.04 (d, *J* =8.8Hz, 2H), 7.10 (dd, *J* =6.2, 2.2Hz, 1H)

Step 6: 7-Ethyl-3,3-dimethylindolin-2-one (**4**)

TFA (2 mL) was added to **51** (73.0 mg, 0.24 mmol) and stirred at 60 °C for 2 h. The reaction mixture was evaporated *in vacuo* and quenched with saturated aqueous NaHCO$_3$. EtOAc was added, and the organic layer was separated. The organic layer was washed with H$_2$O and brine, and dried over Na$_2$SO$_4$. After filtration and concentration, the residue was purified by preparative TLC (solvent: EtOAc/Hexane = 1/3) to give the title compound **4** (28.0 mg, 62% yield).

^{1}H-NMR (400MHz, CDCl$_3$) δ: 1.26 (t, *J* =7.6 Hz, 3H), 1.40 (s, 6H), 2.60 (q, *J* =7.6 Hz, 2H), 6.98-7.07 (m, 3H), 7.91 (brs, 1H)

HRMS (ESI, *m/z*) calced for C$_{12}$H$_{14}$ON (M-H)$^-$ 188.1081, found 188.1082

Synthesis of compound 5

Step 1: 7-Bromo-3,3-dimethylindolin-2-one (**52**)

TFA (2 mL) was added to **49** (310 mg, 0.86 mmol) and stirred at 50 °C for 2 h. The reaction mixture was evaporated *in vacuo* and quenched with saturated aqueous NaHCO$_3$. EtOAc was added, and the organic layer was separated. The organic layer was washed with H$_2$O and brine, and dried over Na$_2$SO$_4$. After filtration and concentration, the residue was purified by preparative TLC (solvent: EtOAc/CHCl$_3$ = 1/10) to give the title compound **52** (155 mg, 75% yield).

^{1}H-NMR (400MHz, CDCl$_3$) δ: 1.39 (s, 6H), 6.92 (dd, *J* =8.2, 7.2 Hz, 1H), 7.10 (dd, *J* =7.2, 1.0Hz, 1H), 7.31 (dd, *J* =8.2, 1.0Hz), 7.47 (brs, 1H)

Step 2: 7-Cyclopropyl-3,3-dimethylindolin-2-one (**5**)

A mixture of **52** (80.0 mg, 0.33 mmol), cyclopropyl boronic acid (43.0 mg, 0.50 mmol) and Na_2CO_3 (140 mg, 1.32 mmol) in DME (3 mL) and H_2O (1 mL) was treated with $PdCl_2$(dppf)-CH_2Cl_2 (24.0 mg, 0.03 mmol) and stirred at 95 °C for 7 h. After cooling to room temperature, H_2O and EtOAc were added to the reaction mixture. The organic layer was washed with H_2O and brine, and dried over Na_2SO_4. After filtration and concentration, the residue was purified by preparative TLC (solvent: EtOAc/Hexane = 1/2) to give the title compound **5** (18.0 mg, 27% yield).

^{1}H-NMR (400MHz, $CDCl_3$) δ: 0.61-0.73 (m, 2H), 0.88-1.00 (m, 2H), 1.40 (s, 6H), 1.73-1.80 (m, 1H), 6.95-7.04 (m, 3H), 7.75 (brs, 1H)

HRMS (ESI, *m/z*) calced for $C_{13}H_{14}ON$ (M-H)$^-$ 200.1081, found 200.1081

Synthesis of compound 6

Step 1: Ethyl 1-(4-methoxybenzyl)-3,3-dimethyl-2-oxoindoline-7-carboxylate (**53**)

To a solution of **49** (400 mg, 1.11 mmol) in EtOH (10 mL) was added $PdCl_2$(dppf)-CH_2Cl_2 (180 mg, 0.22 mmol) and Et_3N (1.5 mL, 11.1 mmol), and stirred under 1.0 atm of carbon monoxide at 80 °C for 12 h. After cooling to room temperature, H_2O and EtOAc were added to the reaction mixture. The organic layer was washed with H_2O and brine, and dried over Na_2SO_4. After filtration and concentration, the residue was purified by flash chromatography (solvent: EtOAc/Hexane = 1/6 to 1/4) to give the title compound **53** (374 mg, 95% yield).

^{1}H-NMR (400MHz, $CDCl_3$) δ: 1.20 (t, *J* =7.2 Hz, 3H), 1.45 (s, 6H), 3.73 (s, 3H), 4.14 (q, *J* =7.2 Hz, 2H), 5.22 (s, 2H), 6.75 (d, *J* =8.8 Hz, 2H), 6.92 (d, *J* =8.8Hz, 2H), 7.02 (dd, *J* =7.8, 7.2Hz, 1H), 7.32 (dd, *J* =7.2, 1.4 Hz, 1H), 7.35 (dd, *J* =7.8, 1.4Hz, 1H)

Step 2: 1-(4-Methoxybenzyl)-3,3-dimethyl-2-oxoindoline-7-carboxylic acid (**54**)

To a solution of **53** (374 mg, 1.06 mmol) in MeOH (4 mL) and THF (2 mL) was added aqueous 2N NaOH (1.1 mL, 2.12 mmol) at room temperature. The reaction mixture was stirred at 60 °C for 2 h. After cooling to 0 °C, aqueous 2N HCl (1.1 mL, 2.12 mmol) was added. After evaporating *in vacuo*, H_2O and $CHCl_3$ were added to the residue. The organic layer was washed with H_2O and brine, and dried over Na_2SO_4.

After filtration, the solvent was evaporated *in vacuo* to give the title compound **54** (360 mg, overweight) as a crude product. This was used in the next step without further purification.

^{1}H-NMR (400MHz, CDCl$_3$) δ: 1.47 (s, 6H), 3.71 (s, 3H), 5.28 (s, 2H), 6.73 (d, *J* =8.6 Hz, 2H), 6.95 (d, *J* =8.6Hz, 2H), 7.08 (dd, *J* =8.0, 7.2Hz, 1H), 7.38 (dd, *J* =7.2, 1.2 Hz, 1H), 7.56 (dd, *J* =8.0, 1.2Hz, 1H)

Step 3: *tert*-Butyl (1-(4-methoxybenzyl)-3,3-dimethyl-2-oxoindolin-7-yl) carbamate (**55**)

To a solution of **54** (360 mg, approx.1.06 mmol) in toluene (5 mL) were added Et$_3$N (233 μL, 1.67 mmol) and DPPA (310 μL, 1.44 mmol) and stirred at 85 °C for 10 min. *t*BuOH (1 mL) was added at room temperature and stirred at 60 °C for 3 h. After cooling to room temperature, H$_2$O and EtOAc were added to the reaction mixture. The organic layer was washed with H$_2$O and brine, and dried over Na$_2$SO$_4$. After filtration and concentration, the residue was purified by flash chromatography (solvent: EtOAc/Hexane = 1/6 to 1/4) to give the title compound **55** (290 mg, 19% yield for 2 steps).

^{1}H-NMR (400MHz, CDCl$_3$) δ: 1.47 (s, 6H), 1.50 (s, 9H), 3.78 (s, 3H), 5.04 (s, 2H), 6.87 (d, *J* =8.8 Hz, 2H), 6.99-7.08 (m, 2H), 7.13-7.18 (m, 3H)

Step 4: 7-Amino-3,3-dimethylindolin-2-one (**6**)

HBr-AcOH (1mL) was added to **55** (50.0 mg, 0.169 mmol) and stirred at r.t. for 16 h. After cooling to 0 °C, the reactant mixture was diluted with sat. aqueous NaHCO$_3$, and extracted with AcOEt. The organic layer was washed with H$_2$O and brine, dried over Na$_2$SO$_4$. After filtration and concentration, the residue was purified by preparative TLC (solvent: EtOAc/CHCl$_3$ = 1/1) to give the title compound **6** (4.0 mg, 13% yield).

^{1}H-NMR (400MHz, CDCl$_3$) δ: 1.38 (s, 6H), 6.63 (dd, *J* =8.2, 1.4 Hz, 1H), 6.68 (dd, *J* =7.2, 1.4 Hz), 6.90 (dd, *J* =8.2, 7.2 Hz, 1H), 9.81 (brs, 1H)

HRMS (ESI, *m/z*) calced for C$_{10}$H$_{11}$ON$_2$ (M-H)$^-$ 175.0877, found 175.0878

Synthesis of compound 7

Step 1: *tert*-Butyl (1-(4-methoxybenzyl)-3,3-dimethyl-2-oxoindolin-7-yl)(methyl)carbamate (**56**)

55 (145 mg, 0.37 mmol) in DMF (2 mL) was dropped into a solution of 60% sodium hydride (18.0 mg, 0.44 mmol) in DMF (1 mL) at 0 °C. Methyl iodide (35.0μL, 0.56 mmol) was added dropwise at 0 °C, and the mixture was stirred at room temperature for 3 h. H_2O and AcOEt were added to the reaction mixture, and the organic layer was separated. The organic layer was washed with H_2O and brine, dried over Na_2SO_4. After filtration, the solvent was evaporated *in vacuo* to give the title compound **56** (172 mg, overweight) as a crude product. This was used in the next step without further purification.

Step 2: 3,3-Dimethyl-7-(methylamino)indolin-2-one (**7**)

TFA (2 mL) was added to **56** (172 mg, approx. 0.37 mmol) in $CHCl_3$ (0.5 mL) and stirred at 80 °C for 24h. The reaction mixture was evaporated *in vacuo* and quenched with saturated aqueous $NaHCO_3$. $CHCl_3$ was added, and the organic layer was separated. The organic layer was washed with H_2O and brine, dried over Na_2SO_4. After filtration and concentration, the residue was purified by preparative TLC (solvent: $EtOAc/CHCl_3 = 1/5$) to give the title compound **7** (50.0 mg, 70% yield for 2 steps).

^{1}H-NMR (400MHz, $CDCl_3$) δ: 1.38 (s, 6H), 2.92 (s, 3H), 3.94 (brs, 1H), 6.59 (d, J =8.0 Hz, 1H), 6.64 (d, J =7.2 Hz, 1H), 7.00 (dd, J =8.0, 7.2 Hz, 1H)

HRMS (ESI, *m/z*) calced for $C_{11}H_{15}ON_2$ (M+H)$^+$ 191.1179, found 191.1175

Synthesis of compound 8

To a solution of 7-methoxyindolin-2-one (**57**; 300 mg, 1.84 mmol) and LiCl (195 mg, 4.60 mmol) in THF (5.0 mL), 1.60 M *n*-butyllithium in hexane (2.4 mL, 3.86 mmol) was added at 0 °C. After stirring for 1 h at 0 °C, methyl iodide (240 μL, 3.86 mmol) was added to the reactant mixture, and then the mixture was stirred at r.t. overnight. The mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with H_2O and brine, and dried over $MgSO_4$. After filtration and concentration, the residue was purified by flash chromatography (solvent: EtOAc/Hexane = 1/9 to 1/2) to give the title compound **8** (186 mg, 53% yield).

^{1}H-NMR (400MHz, DMSO-d_6) δ: 1.22 (s, 6H), 3.81 (s, 3H), 6.89-6.96 (m, 3H), 10.3 (brs, 1H)

^{13}C-NMR (400 MHz, DMSO-d_6) δ: 24.0 (2C), 44.1, 55.5, 110.7, 114.9, 122.1, 129.1, 136.8, 143.4, 182.0

HRMS (ESI, *m/z*) calced for $C_{11}H_{13}O_2N$ (M+H)$^+$ 192.1019, found 192.1014

Synthesis of compound 9

Step 1: 2-Cyclopropylaniline (**59**)

A mixture of 2-bromoaniline (**58**; 32.0 g, 186 mmol), cyclopropylboronic acid (23.9 g, 278 mmol), and K_3PO_4 (113 g, 532 mmol) in toluene (350 mL) and H_2O (100 mL) was treated with $PdCl_2$(dppf)-CH_2Cl_2 (5.66 g, 6.93 mmol) and stirred at 90 °C for 5 h under argon atmosphere. The mixture was cooled to 0 °C, ammonium pyrrolidinedithiocarbamate (APDTC, 5.06 g, 30.8 mmol) was added and the mixture was stirred at room temperature for 1 h. The reactant mixture was filtered through Celite®, and the organic layer was separated and washed with H_2O and brine. The resultant organic layer was loaded on silica gel column chromatography (eluted with n-hexane/EtOAc = 80/20 (v/v), approximately 1.6 L) for elimination of the polar component. The eluate was acidified by adding 4 M HCl in AcOEt (50 mL) without concentration. The precipitated crystals were collected by filtration, washed with EtOAc and *n*-hexane, and dried to give the hydrochloride salt of the title compound **59** as a white crystalline (28.6 g, 90% yield).

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.69-0.73 (m, 2H), 0.95-1.00 (m, 2H), 1.97-2.06 (br m, 1H), 7.09-7.05 (m, 1H), 7.22-7.26 (m, 1H), 7.31-7.39 (br m, 1H), 9.90 (br s, 3H).

Step 2: 1-Cyclopropyl-2-iodobenzene (**60**)

Sodium nitrate (11.5 g, 167 mmol) in H_2O (70 mL) was dropped into the mixture of the hydrochloride salt of **59** (25.6 g, 151 mmol) and 1 M aqueous HCl solution (20 mL) at 0 °C. After stirring at 0 °C for 15 min, to the reaction mixture was added sodium iodide (24.9 g, 166 mmol) in H_2O (70 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min and then room temperature for 2 h. The reactant mixture was quenched with aqueous NaS_2O_3, and the mixture was extracted with EtOAc. The extract was washed with H_2O and brine, and dried over Na_2SO_4. The solvent was evaporated *in vacuo*. The residue was purified by flash chromatography (Biotage-SNAP Ultra 100 g, eluted with *n*-hexane/EtOAc = 100/0 to 95/5 (v/v)) to give the title compound **60** (31.9 g, 86% yield).

^{1}H-NMR (400 MHz, CDCl$_3$) δ: 0.63-0.68 (m, 2H), 0.99-1.05 (m, 2H), 1.98-2.06 (m, 1H), 6.88 (t, *J* =7.7 Hz, 1H), 6.92 (d, *J* =7.7 Hz, 1H), 7.24 (t, *J* =7.7 Hz, 1H), 7.83 (d, *J* =7.7 Hz, 1H)

Step 3: 3-Bromo-*N*-(2-cyclopropylphenyl)pyridin-2-amine (**63**)

A mixture of **60** (1.00 g, 4.10 mmol), 3-bromopyridin-2-amine (**61**; 709 mg, 4.10 mmol), and sodium *tert*-pentoxide (902 mg, 8.19 mmol) in toluene (10 mL) was treated with Pd(OAc)$_2$ (92.0 mg, 0.41 mmol) and Xantphos (237 mg, 0.41 mmol) and stirred at 140 °C for 4.5 h under argon atmosphere. After cooling to room temperature, the mixture was diluted with EtOAc and H$_2$O. The insoluble solid was filtered through Celite®. The organic layer was separated from the filtrate and concentrated. The residue was purified by flash chromatography (Biotage-SNAP Ultra 25 g, eluted with *n*-hexane/EtOAc = 99/1 to 90/10 (v/v)) to give the title compound **63** (945 mg, 80% yield).

^{1}H-NMR (400 MHz, CDCl$_3$) δ: 0.67-0.78 (m, 2H), 0.97-1.12 (m, 2H), 1.80-1.91 (m, 1H), 6.63 (dd, *J* =7.7, 4.9 Hz, 1H), 6.97 (td, *J* =7.7, 1.2 Hz, 1H), 7.19 (dt, *J* =7.7, 1.2 Hz, 1H), 7.26 (td, *J* =8.1, 1.2 Hz, 1H), 7.75 (dd, *J* =7.7, 1.6 Hz, 1H), 7.78 (br s, 1H), 8.18 (dd, *J* =4.9, 1.6 Hz, 1H), 8.39 (dd, *J* =8.1, 1.2 Hz, 1H)

Step 4: 8-Cyclopropyl-9*H*-pyrido[2,3-b]indole (**9**)

A solution of **64** (945 mg, 3.27 mmol) and DBU (1.48 mL, 9.80 mmol) in DMA (19 mL) was treated with Pd(OAc)$_2$ (73.4 mg, 0.327 mmol) and CyJohnPhos (229 mg, 0.65 mmol) under argon atmosphere and stirred at 140 °C for 2 h. After cooling to room temperature, the mixture was diluted with H$_2$O and extracted with EtOAc. The organic layer was washed twice with H$_2$O and brine, and dried over Na$_2$SO$_4$. After filtration and concentration, the residue was purified by flash chromatography (Biotage-SNAP Ultra 25 g, eluted with *n*-hexane/EtOAc = 92/8 to 34/66 (v/v)). After concentration, for further purification, the residue was slurried in *n*-hexane/AcOEt (2/1 (v/v)). The precipitated solid was collected by filtration, washed with *n*-hexane, and dried to give the title compound **9** (251 mg, 37% yield).

^{1}H-NMR (400 MHz, DMSO-d$_6$) δ: 0.76-0.80 (m, 2H), 1.03-1.08 (m, 2H), 2.38-2.45 (m, 1H), 6.99 (d, *J* =7.6 Hz, 1H), 7.13 (t, *J* =7.6 Hz, 1H), 7.20 (dd, *J* =7.8, 4.8 Hz, 1H), 7.95 (d, *J* =7.6 Hz, 1H), 8.43 (dd, *J* =4.8, 1.5 Hz, 1H), 8.48 (dd, *J* =7.8, 1.5 Hz, 1H), 11.92 (s, 1H)

HRMS (ESI, *m/z*) calced for C$_{14}$H$_{13}$N$_2$ (M+H)$^+$ 209.1073, found 209.1072

Synthesis of compound 10

Step 1: 3-Bromo-*N*-(2-cyclopropylphenyl)-6-methylpyridin-2-amine (**64**)

A mixture of **60** (150 mg, 0.615 mmol), 3-bromo-6-methylpyridin-2-amine (**62**; 138 mg, 0.738 mmol), and cesium carbonate (300 mg, 0.921 mmol) in toluene (1.5 mL) was treated with Pd(OAc)$_2$ (28.0 mg,

0.125 mmol) and Xantphos (72.0 mg, 0.139 mmol) and stirred at 130 °C for 3 h under argon atmosphere. After cooling to room temperature, the mixture was diluted with EtOAc and H_2O. The insoluble solid was filtered through Celite®. The organic layer was separated from the filtrate and concentrated. The residue was purified by flash chromatography (Biotage-SNAP Ultra 10g, eluted with *n*-hexane/EtOAc = 99/2 to 85/25 (v/v)) to give the title compound **64** (64.0 g, 34% yield).

^{1}H-NMR (400 MHz, CDCl$_3$) δ: 0.70-0.75 (m, 2H), 1.03-1.09 (m, 2H), 1.80-1.89 (m, 1H), 2.45 (s, 3H), 6.51 (d, *J*=7.9 Hz, 1H), 6.94 (td, *J*=7.6, 1.3 Hz, 1H), 7.19 (dt, *J*=7.6, 1.3 Hz, 1H), 7.25 (td, *J*=8.1, 1.3 Hz, 1H), 7.62 (d, *J*=7.9 Hz, 1H), 7.82 (s, 1H), 8.58 (dd, *J*=8.1, 1.3 Hz, 1H)

Step 2: 8-Cyclopropyl-2-methyl-9*H*-pyrido[2,3-b]indole (**10**)

A solution of **64** (64 mg, 0.211 mmol) and DBU (63.0 μL, 0.421 mmol) in DMA (1.0 mL) was treated with Pd(OAc)$_2$ (14.0 mg, 6.24 x 10-2 mmol) and CyJohnPhos (22.0 mg, 6.28 x 10-2 mmol) under argon atmosphere and stirred at 140 °C for 20 h. After cooling to room temperature, the mixture was diluted with H_2O and extracted with EtOAc. The organic layer was washed twice with H_2O and brine, and dried over Na$_2$SO$_4$. After filtration and concentration, the residue was purified by flash chromatography (Biotage-SNAP Ultra 10g, eluted with n-hexane/EtOAc = 90/10 to 0/100 (v/v)). After concentration, for further purification, the residue was purified by preparative TLC (n-hexane/AcOEt = 1/1 (v/v)) to give the title compound **10** (35 mg, 75% yield).

^{1}H-NMR (400 MHz, CDCl$_3$) δ: 0.77-0.81 (m, 2H), 1.01-1.05 (m, 2H), 2.06-2.14 (m, 1H), 2.69 (s, 3H), 7.05 (d, *J*=7.8 Hz, 1H), 7.16-7.22 (m, 2H), 7.85 (dd, *J*=7.2, 1.8 Hz, 1H), 8.19 (d, *J*=7.8 Hz, 1H), 8.67 (s, 1H)

HRMS (ESI, *m/z*) calced for C$_{15}$H$_{15}$N$_2$ (M+H)$^+$ 223.1230, found 223.1226

Synthesis of compound 11

Step 1: 1-Cyclopropyl-3-fluoro-2-nitrobenzene (**66**)

A mixture of 1-bromo-3-fluoro-2-nitrobenzene (**65**, 49.8 g, 226 mmol), cyclopropylboronic acid (21.4 g, 249 mmol), and K$_3$PO$_4$ (106 g, 499 mmol) in DME (300 mL) and H_2O (150 mL) was treated with PdCl$_2$(dppf)-CH$_2$Cl$_2$ (9.25 g, 11.3 mmol) and stirred under reflux for 1 h. After cooling to room temperature, the mixture was diluted with AcOEt and H_2O, filtered through Celite®, and the organic layer was separated and washed with H_2O and brine. After filtration and concentration, the residue was purified by flash

chromatography (solvent : EtOAc/Hexane = 5/95 to 25/75) to give the title compound **66** (36.8 g, 89% yield).

^{1}H-NMR (400 MHz, DMSO-d$_6$) δ: 0.77-0.81 (m, 2H), 0.99-1.04 (m, 2H), 1.85-1.92 (m, 1H), 7.03-7.05 (m, 1H), 7.35-7.40 (m, 1H), 7.52-7.58 (m, 1H)

Step 2: Ethyl 2-cyano-2-(3-cyclopropyl-2-nitrophenyl) acetate (**67**)

Compound **66** (3.00 g, 16.6 mmol) and ethyl 2-cyanoacetate (3.50 mL, 33.1 mmol) was dissolved in DMF (15 mL). To the solution, K$_2$CO$_3$ (6.90 g, 49.7 mmol) was added, and the mixture was stirred at 90 °C for 3 h. After cooling to 0 °C, the reactant mixture was diluted with H$_2$O (50 mL), acidified by adding 6.0 M aqueous HCl solution (25 mL), and extracted with 1:1 n-hexane-AcOEt. The organic layer was washed with H$_2$O and brine, and dried over Na$_2$SO$_4$. After filtration, the solvent was evaporated *in vacuo* to give the title compound **67** (5.0 g, overweight) as a crude product which was used for the next step without further purification.

^{1}H-NMR (400 MHz, CDCl$_3$) δ: 0.64-0.77 (m, 2H), 0.96-1.06 (m, 2H), 1.30 (t, J =7.3 Hz, 3H), 1.98-2.06 (m, 1H), 4.26 (q, J =7.3 Hz, 2H), 5.00 (s, 1H), 7.21 (dd, J =7.7, 0.8 Hz, 1H), 7.04 (t, J =7.7 Hz, 1H), 7.54 (dd, J =7.7,1.6 Hz, 1H)

Step 3: Ethyl 2-amino-7-cyclopropyl-1*H*-indole-3-carboxylate (**68**)

To the solution of **67** (5.0 g, approx. 16 mmol) in AcOH (30 mL) was added iron powder (5.09 g, 91.1 mmol), and the mixture was stirred at 90 °C for 2 h. After cooling to room temperature, the reactant mixture was diluted with 1:1 toluene-H$_2$O (60 mL), filtered through Celite®, and the organic layer was separated and washed with H$_2$O, saturated NaHCO$_3$ solution and brine sequentially, and dried over Na$_2$SO$_4$. After filtration and concentration, the residue was purified by flash chromatography (Yamazen-Universal Premium L, eluted with *n*-hexane/EtOAc = 85/15 to 40/60 (v/v)) to give the title compound **68** (2.10 g, 52% two-step yield from **66**).

^{1}H-NMR (400 MHz, CDCl$_3$) δ: 0.71 (br s, 2H), 0.93 (br s, 2H), 1.42 (t, J =7.1 Hz, 3H), 1.91 (br s, 1H), 4.36 (q, J =7.1 Hz, 2H), 5.68 (br s, 1H), 6.79 (d, J =7.4 Hz, 1H), 7.04 (t, J =7.4 Hz, 1H), 7.64 (d, J =7.4 Hz, 1H), 7.92 (br s, 2H)

Step 4: 8-Cyclopropyl-2-methyl-9*H*-pyrimido[4,5-b]indol-4-ol (**69**)

Compound **68** (5.00 g, 20.5 mmol) was dissolved in 4 M aqueous HCl solution (25 mL). To the solution, MeCN (7.5 mL) was added, and the mixture was stirred at room temperature for 20 h. The resultant suspension was diluted with 1:1 *n*-hexane-dioxane (50 mL), and the precipitated solid was collected by filtration, washed with *n*-hexane, and dried *in vacuo*. The obtained solid was redissolved in MeOH (65 mL) and H_2O (20 mL). Saturated $NaHCO_3$ solution (40 mL) was added to the solution, and the reactant mixture was stirred at 75 °C for 2.5 h. After cooling to 0 °C, the reactant mixture was acidified by adding 6 M aqueous HCl solution (7.5 mL), diluted with H_2O (100 mL), and then stirred at 0 °C for 15 min. The precipitated solid was collected by filtration, washed with H_2O, and dried to give the title compound **69** (4.88 mg, 99% yield).

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.77-0.73 (m, 2H), 0.99-1.04 (m, 2H), 2.30-2.37 (m, 1H), 2.42 (s, 3H), 6.80 (d, *J* =7.5 Hz, 1H), 7.10 (t, *J* =7.5 Hz, 1H), 7.73 (dd, *J* =7.5, 0.9 Hz, 1H), 12.07 (s, 1H), 12.12 (s, 1H)

Step 5: 4-Chloro-8-cyclopropyl-2-methyl-9*H*-pyrimido [4,5-b] indole (**70**)

To a suspension of **69** (4.88 g, 20.4 mmol) in $CHCl_3$ (66 mL) was added thionyl chloride (7.42 mL, 102 mmol) and DMF (33 mL), and the mixture was stirred at 60 °C for 2 h. The resultant solution was cooled to 0 °C and quenched with H_2O. The mixture was extracted with AcOEt, and the organic layer was washed twice with H_2O and brine, and dried over Na_2SO_4. After filtration and concentration, the residue was purified by flash chromatography (Yamazen-Universal Premium 3L, eluted with *n*-hexane/EtOAc = 90/10 to 20/80 (v/v)) to give the title compound **70** (2.44 mg, 46% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.05-2.09 (m, 2H), 2.27-2.31 (m, 2H), 3.08-3.14 (m, 1H), 3.68 (s, 3H), 7.26-7.31 (m, 2H), 7.93-7.98 (m, 1H), 8.84 (s, 1H)

Step 6: 8-Cyclopropyl-2-methyl-9*H*-pyrimido[4,5-b]indole (**11**)

A solution of **70** (40.0 mg, 0.155 mmol) in THF (0.6 mL) and MeOH (0.6 mL) was treated with 10% palladium hydroxide on activated carbon (15.0 mg) and K_2CO_3 (32.0 mg, 0.233 mmol), and the mixture was stirred under 1.0 atm of hydrogen at room temperature for 7 h. After removal of the palladium catalyst by Celite® filtration, the filtrate was concentrated. The residue was suspended in 1:1 *n*-hexane-AcOEt and

slurried for a while. The precipitated solid was collected by filtration, washed with 2:1 *n*-hexane-AcOEt, and dried to give the title compound **11** (30.0 mg, 86% yield).

^{1}H-NMR (400 MHz, DMSO-d$_6$) δ: 0.77-0.81 (m, 2H), 1.04-1.09 (m, 2H), 2.33-2.40 (m, 1H), 2.71 (s, 3H), 7.02 (d, *J* =7.6 Hz, 1H), 7.20 (t, *J* =7.6 Hz, 1H), 7.97 (d, *J* =7.6 Hz, 1H), 9.30 (s, 1H), 12.27 (s, 1H).

HRMS (ESI, *m/z*) calced for C$_{14}$H$_{14}$N$_3$ (M+H)$^+$ 224.1182, found 224.1183

Synthesis of compound 12

Step 1: 4-Chloro-2-methyl-7-((2-(trimethylsilyl)ethoxy)methyl)-7*H*-pyrrolo[2,3-d]pyrimidine (**72**)

4-Chloro-2-methyl-7*H*-pyrrolo[2,3-d]pyrimidine (**71**, 5.00 g, 29.8 mmol) was added to a suspension of sodium hydride 60% dispersion in mineral oil (1.43 g, 35.8 mmol) and DMF (30 mL) at 0 °C. After stirring for 30 min at 0 °C, 2-(chloromethoxy)ethyltrimethylsilane (6.79 mL, 38.7 mmol) was added to the solution at 0 °C, and the mixture was stirred at room temperature for 1.5 h. The mixture was diluted with H$_2$O and extracted with EtOAc, and the organic layer was washed with H$_2$O and brine, and dried over MgSO$_4$. After filtration and concentration, the residue was purified by silica gel column chromatography (eluted with *n*-hexane/EtOAc = 100/0 to 90/10 (v/v)) to give the title compound **72** (7.80 g, 89% yield).

^{1}H-NMR (400 MHz, DMSO-d$_6$) δ: -0.10 (s, 9H), 0.81-0.86 (m, 2H), 2.65 (s, 3H), 3.50-3.55 (m, 2H), 5.59 (s, 2H), 6.63 (d, *J* =3.7 Hz, 1H), 7.75 (d, *J* =3.7 Hz, 1H)

Step 2: 4-Chloro-2-methyl-6-propyl-7-((2-(trimethylsilyl)ethoxy)methyl)-7*H*-pyrrolo[2,3-d]pyrimidine (**73**)

To a solution of **72** (2.00 g, 6.71 mmol) in THF (20 mL), 1.63 M *n*-butyllithium in hexane (5.34 mL, 8.70 mmol) was added at -78 °C. After stirring for 3 h at -78 °C, 1-iodopropane (847 μL, 8.72 mmol) was added to the reactant mixture, and then the mixture was stirred at room temperature for 1.5 h. The mixture was diluted with H$_2$O and extracted with AcOEt, and the organic layer was washed with H$_2$O and brine, and dried over MgSO$_4$. After filtration and concentration, the residue was loaded on silica gel column chromatography (eluted with *n*-hexane/EtOAc = 100/0 to 95/5 (v/v)) for elimination of the polar component. The title compound **73** (1.10 g) was obtained as a mixture with unknown compounds, which was applied to the next step without further purification.

Step 3: 2-Methyl-6-propyl-7-((2-(trimethylsilyl)ethoxy)methyl)-7*H*-pyrrolo[2,3-d]pyrimidine (**74**)

A solution of **73** (1.10 g, ~3.2 mmol) and potassium carbonate (450 mg, 3.26 mmol) in MeOH (11 mL) was treated with 10% palladium on activated carbon (110 mg) and stirred under 1.0 atm of hydrogen overnight at room temperature. After removal of the palladium catalyst by Celite® filtration, the filtrate was diluted with H_2O and extracted with AcOEt. The organic layer was washed with H_2O and brine, and dried over $MgSO_4$. After filtration and concentration, the title compound **74** (1.00 g) was obtained, which was applied to the next step without further purification.

Step 4: 5-Bromo-2-methyl-6-propyl-7-((2-(trimethylsilyl)ethoxy)methyl)-7*H*-pyrrolo[2,3-d]pyrimidine (**75**)

To a solution of **74** (1.00 g, ~3.2 mmol) in DMF (20 mL) was added *N*-bromosuccinimide (582 mg, 3.27 mmol). After stirring at room temperature for 1 h, the reactant mixture was quenched with saturated $NaHCO_3$ solution. The mixture was extracted with AcOEt, and the organic layer was washed with H_2O and brine, and dried over $MgSO_4$. After filtration and concentration, the residue was purified by silica gel column chromatography (eluted with *n*-hexane/EtOAc = 95/5 to 90/10 (v/v)) to give the title compound **75** (770 mg, 30% yield, 3 steps from **72**).

^{1}H-NMR (400 MHz, DMSO-d_6) δ: -0.10 (s, 9H), 0.82-0.87 (m, 2H), 0.95 (t, *J* =7.6 Hz, 3H), 1.69 (tq, *J* =7.6, 7.6 Hz, 2H), 2.67 (s, 3H), 2.84 (t, *J* =7.6 Hz, 2H), 3.48-3.54 (m, 2H), 5.64 (s, 2H), 8.72 (d, 1H)

Step 5: 2-Methyl-6-propyl-7-((2-(trimethylsilyl)ethoxy)methyl)-7*H*-pyrrolo[2,3-d]pyrimidine-5-carbonitrile (**76**)

To a solution of **75** (200 mg, 0.520 mmol) in THF (2.0 mL), 1.63 M *n*-butyllithium in hexane (414 μL, 0.676 mmol) was added at -78 °C. After stirring for 1 h at -78 °C, *p*-toluenesulfonyl cyanide (122 mg, 0.673 mmol) was added to the reactant mixture, and then the mixture was stirred at room temperature for 3 h. The mixture was quenched with saturated NH_4Cl solution and extracted with AcOEt. The organic layer was washed with H_2O and brine, and dried over $MgSO_4$. After filtration and concentration, the residue was purified by preparative TLC (*n*-hexane/AcOEt = 4/1 (v/v)) to give the title compound **76** (110 mg) as a mixture with 2-methyl-6-propyl-7-((2-(trimethylsilyl)ethoxy)methyl)-7*H*-pyrrolo[2,3-d]pyrimidine, which was applied to the next step without further purification.

Step 6: 2-Methyl-6-propyl-7H-pyrrolo[2,3-d]pyrimidine-5-carbonitrile (12)

To a solution of 76 (50.0 mg, ~0.150 mmol) in CH_2Cl_2 (1.0 mL), boron trifluoride diethyl etherate (57.0 μL, 0.512 mmol) was added at room temperature. After stirring overnight at room temperature, the mixture was quenched with saturated $NaHCO_3$ solution and extracted with AcOEt. The organic layer was washed with H_2O and brine, and dried over $MgSO_4$. After filtration and concentration, for purification, the residue was slurried in n-hexane/AcOEt (1/1 (v/v)). The precipitated solid was collected by filtration, washed with n-hexane, and dried to give the title compound 12 (13.0 mg, 28% yield, 2 steps from 75).

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.92 (t, J =7.4 Hz, 3H), 1.77 (tq, J =7.4, 7.4 Hz, 2H), 2.65 (s, 3H), 2.87 (t, J =7.4 Hz, 2H), 8.93 (s, 1H), 12.89 (br s, 1H)

HRMS (ESI, m/z, MH+) Calcd for $C_{11}H_{13}N_4$: 201.1135, Found: 201.1138

Synthesis of compound 13

Step 1: 2-Methyl-5-(pent-1-yn-1-yl)pyrimidin-4-amine (78)

A solution of 5-iodo-2-methylpyrimidin-4-amine (77; 640 mg, 2.72 mmol), 1-penthyne (805 μL, 8.17 mmol), [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (220 mg, 0.269 mmol), copper(I) iodide (51.0 mg, 0.268 mmol) and triethylamine (569 μL, 4.08 mmol) in DMF (10 mL) was stirred at 70 °C for 2 h under argon atmosphere. The mixture was cooled to room temperature, diluted with H_2O and extracted with AcOEt. The organic layer was washed with H_2O and brine, and dried over $MgSO_4$. After filtration and concentration, the residue was loaded on silica gel column chromatography (eluted with n-hexane/EtOAc = 50/50 (v/v)) for elimination of the polar component. The title compound 78 (305 mg, 64% yield) was obtained, which was advanced to the next step without further purification.

Step 2: 2-Methyl-6-propyl-7H-pyrrolo[2,3-d]pyrimidine (79)

To a solution of 78 (300 mg, 1.71 mmol) in NMP (3.0 mL), potassium *tert*-butoxide (400 mg, 3.56 mmol) was added at room temperature and then stirred at 100 °C for 1 h. The mixture was cooled to room temperature, quenched with 1 N HCl solution and extracted with AcOEt. The organic layer was washed

with H_2O and brine, and dried over $MgSO_4$. After filtration and concentration, the residue was purified by silica gel column chromatography (eluted with EtOAc) to give the title compound **79** (155 mg, 52% yield).

^{1}H-NMR (400 MHz, DMSO-d$_6$) δ: 0.92 (t, J =7.4 Hz, 3H), 1.70 (qt, J =7.4, 7.4 Hz, 2H), 2.58 (s, 3H), 2.68 (t, J =7.4 Hz, 2H), 6.19 (br s, 1H), 8.69 (s, 1H), 11.67 (br s, 1H)

Step 3: 5-Chloro-2-methyl-6-propyl-7*H*-pyrrolo[2,3-d]pyrimidine (**13**)

To a solution of **79** (30.0 mg, 0.171 mmol) in DMF (1.0 mL) was added *N*-chlorosuccinimide (34.0 mg, 0.255 mmol). After stirring at room temperature for 5 h, the reactant mixture was quenched with saturated $NaHCO_3$ solution. The mixture was extracted with AcOEt, and the organic layer was washed with H_2O and brine, and dried over $MgSO_4$. After filtration and concentration, the residue was purified by preparative TLC (*n*-hexane/AcOEt = 1/1 (v/v)) to give the title compound **13** (18.0 mg, 50% yield).

^{1}H-NMR (400 MHz, DMSO-d$_6$) δ: 0.90 (t, J =7.4 Hz, 3H), 1.69 (qt, J =7.4, 7.4 Hz, 2H), 2.62 (s, 3H), 2.72 (t, J =7.4 Hz, 2H), 8.73 (s, 1H), 12.11 (br s, 1H)

HRMS (ESI, m/z, MH+) Calcd for $C_{10}H_{13}N_3Cl$: 210.0793, Found: 210.0788

Synthesis of compound 14

To a solution of **79** (45.0 mg, 0.256 mmol) in $CHCl_3$ (1.0 mL) was added *N*-bromosuccinimide (55.0 mg, 0.308 mmol). After stirring overnight at room temperature, the reactant mixture was quenched with saturated $NaHCO_3$ solution. The mixture was extracted with AcOEt, and the organic layer was washed with H_2O and brine, and dried over $MgSO_4$. After filtration and concentration, for purification, the residue was slurried in *n*-hexane/AcOEt (2/1 (v/v)). The precipitated solid was collected by filtration, washed with *n*-hexane, and dried to give the title compound **14** (41.0 mg, 63% yield).

^{1}H-NMR (400 MHz, DMSO-d$_6$) δ: 0.90 (t, J =7.4 Hz, 3H), 1.69 (qt, J =7.4, 7.4 Hz, 2H), 2.63 (s, 3H), 2.71 (t, J =7.4 Hz, 2H), 8.65 (s, 1H), 12.23 (br s, 1H)

HRMS (ESI, m/z, MH+) Calcd for $C_{10}H_{13}N_3Br$: 254.0284, Found: 254.0287

Synthesis of compound 16

Step 1: 5-Bromo-4-chloro-2-methyl-6-propyl-7-((2-(trimethylsilyl)ethoxy)methyl)-7*H*-pyrrolo[2,3-d]pyrimidine (**80**)

To a solution of **73** (450 mg, 1.32 mmol) in DMF (5.0 mL) was added N-bromosuccinimide (260 mg, 1.46 mmol). After stirring overnight at room temperature, the reactant mixture was quenched with saturated NaHCO$_3$ solution. The mixture was extracted with AcOEt, and the organic layer was washed with H$_2$O and brine, and dried over MgSO$_4$. After filtration and concentration, the residue was loaded on silica gel column chromatography (eluted with n-hexane/EtOAc = 90/10 (v/v)) for elimination of the polar component. The title compound **80** (500 mg, 90% yield) was obtained, which was proceeded to the next step without further purification.

Step 2: 4-Chloro-2,5-dimethyl-6-propyl-7-((2-(trimethylsilyl)ethoxy)methyl)-7*H*-pyrrolo[2,3-d]pyrimidine (**81**)

To a solution of **80** (500 mg, 1.19 mmol) in THF (5.0 mL), 1.63 M *n*-butyllithium in hexane (950 μL, 1.55 mmol) was added at -78 °C. After stirring for 1 h at -78 °C, methyl iodide (96.0 μL, 1.54 mmol) was added to the reactant mixture, and then the mixture was stirred at room temperature for 2 h. The mixture was diluted with H$_2$O and extracted with AcOEt. The organic layer was washed with H$_2$O and brine, and dried over MgSO$_4$. After filtration and concentration, the residue was purified by silica gel column chromatography (eluted with n-hexane/EtOAc = 90/10 (v/v)) to give the title compound **81** (337 mg, 80% yield).

^{1}H-NMR (400 MHz, DMSO-d$_6$) δ: -0.10 (s, 9H), 0.81-0.87 (m, 2H), 0.93 (t, *J* =7.4 Hz, 3H), 1.61 (qt, *J* =7.4, 7.4 Hz, 2H), 2.35 (s, 3H), 2.59 (s, 3H), 2.78 (t, *J* =7.4 Hz, 2H), 3.45-3.50 (m, 2H), 5.58 (s, 2H)

Step 3: 4-Chloro-2,5-dimethyl-6-propyl-7*H*-pyrrolo[2,3-d]pyrimidine (**16**)

Compound **81** (150 mg, 0.424 mmol) was dissolved in TFA (1.5 mL), and the mixture was stirred at room temperature for 1 h. After evaporation of TFA, the residue was diluted with THF and neutralized with 2 N NaOH solution, and then extracted with AcOEt. The organic layer was washed with H$_2$O, 1 N HCl solution and brine, and dried over MgSO$_4$. After filtration and concentration, for purification, the residue was slurried in *n*-hexane/AcOEt (10/1 (v/v)). The precipitated solid was collected by filtration, washed with *n*-hexane, and dried to give the title compound **16** (35.0 mg, 37% yield).

^{1}H-NMR (400 MHz, DMSO-d$_6$) δ: 0.88 (t, J =7.4 Hz, 3H), 1.63 (qt, J =7.4, 7.4 Hz, 2H), 2.31 (s, 3H), 2.55 (s, 3H), 2.65 (t, J =7.4 Hz, 2H), 11.88 (br s, 1H)

HRMS (ESI, m/z, MH+) Calcd for C$_{11}$H$_{15}$N$_3$Cl$_1$: 224.0949, Found: 224.0950

Synthesis of compound 15

A solution of **16** (25.0 mg, 0.112 mmol) and potassium carbonate (15.0 mg, 0.109 mmol) in MeOH (1.0 mL) was treated with 10% palladium on activated carbon (10 mg) and stirred under 1.0 atm of hydrogen at room temperature for 2 h. After removal of the palladium catalyst by Celite® filtration, the filtrate was diluted with H$_2$O and extracted with AcOEt. The organic layer was washed with H$_2$O and brine, and dried over MgSO$_4$. After filtration and concentration, for purification, the residue was slurried in n-hexane/AcOEt (1/1 (v/v)). The precipitated solid was collected by filtration, washed with n-hexane, and dried to give the title compound **15** (17.0 mg, 81% yield).

^{1}H-NMR (400 MHz, DMSO-d$_6$) δ: 0.88 (t, J =7.4 Hz, 3H), 1.64 (qt, J =7.4, 7.4 Hz, 2H), 2.18 (s, 3H), 2.57 (s, 3H), 2.64 (t, J =7.4 Hz, 2H), 8.67 (s, 1H), 11.40 (br s, 1H)

HRMS (ESI, m/z, MH+) Calcd for C$_{11}$H$_{16}$N$_3$: 190.1339, Found: 190.1342

Synthesis of compound 17

Step1: 5-Bromo-4-chloro-2-methyl-7H-pyrrolo[2,3-d]pyrimidine (**82**)

To a suspension of 4-chloro-2-methyl-7H-pyrrolo[2,3-d]pyrimidine (**71**, 2.00 g, 11.9 mmol) in CHCl$_3$ (20 mL) was added N-bromosuccinimide (2.55 mg, 14.3 mmol). After stirring overnight at room temperature, the reactant mixture was quenched with saturated NaHCO$_3$ solution. The mixture was extracted with CHCl$_3$ and AcOEt, and the organic layer was washed with H$_2$O and brine, and dried over MgSO$_4$. After filtration and concentration, for purification, the residue was slurried in n-hexane/AcOEt (1/1 (v/v)). The precipitated solid was collected by filtration, washed with n-hexane, and dried to give the title compound **82** (2.50 g, approx. 70% yield) as a mixture of 15% of 5,6-dibromo-4-chloro-2-methyl-7H-pyrrolo[2,3-d]pyrimidine.

^{1}H-NMR (400 MHz, DMSO-d$_6$) δ: 2.62 (s, 3H), 7.82 (d, J =2.5 Hz, 1H), 12.69 (br s, 1H)

Step 2: 4-Chloro-2,5-dimethyl-7*H*-pyrrolo[2,3-d]pyrimidine (**83**)

To a solution of **82** (85% purity, 2.50 g, 8.70 mmol) in THF (45 mL), 1.63 M n-butyllithium in hexane (15.0 mL, 23.3 mmol) was added at -78 °C. After stirring for 1 h at -78 °C, methyl iodide (662 μL, 10.6 mmol) was added to the reactant mixture, and then the mixture was stirred at 0 °C for 1 h. The mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with H_2O and brine, and dried over $MgSO_4$. After filtration and concentration, for purification, the residue was slurried in *n*-hexane/AcOEt (2/1 (v/v)). The precipitated solid was collected by filtration, washed with *n*-hexane, and dried to give the title compound **83** (660 mg, approx. 35% yield) as a mixture of 10 % of 4-chloro-2-methyl-7*H*-pyrrolo[2,3-d]pyrimidine.

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 2.38 (br s, 3H), 2.58 (s, 3H), 7.30 (br s, 1H), 11.94 (br s, 1H)

Step 3: 4-Chloro-2,5-dimethyl-7-tosyl-7*H*-pyrrolo[2,3-d]pyrimidine (**84**)

Compound **83** (90% purity, 660 mg, 3.27 mmol) was added to a suspension of sodium hydride 60% dispersion in mineral oil (160 mg, 4.00 mmol) was dissolved in DMF (6.0 mL) at 0 °C. After stirring for 30 min at 0 °C, *p*-toluenesulfonyl chloride (762 mg, 3.99 mmol) was added to the solution at 0 °C, and the mixture was stirred at room temperature for 30 min. The mixture was diluted with H_2O and extracted with AcOEt, and the organic layer was washed with H_2O and brine, and dried over $MgSO_4$. After filtration and concentration, for purification, the residue was slurried in *n*-hexane. The precipitated solid was collected by filtration, washed with *n*-hexane, and dried to give the title compound **84** (1.00 g, 91% yield) which was applied to the next step without further purification.

Step 4: Ethyl 4-chloro-2,5-dimethyl-7-tosyl-7*H*-pyrrolo[2,3-d]pyrimidine-6-carboxylate (**85**)

To a solution of **84** (1.00 g, 2.98 mmol) in THF (10 mL), 1.55 M *n*-butyllithium in hexane (2.88 mL, 4.46 mmol) was added at -78 °C. After stirring for 3 h at -78 °C, ethyl chloroformate (426 μL, 4.46 mmol) was added to the reactant mixture, and then the mixture was stirred overnight at room temperature. The mixture was diluted with H_2O and 1N HCl solution, and extracted with AcOEt. The organic layer was washed with H_2O and brine, and dried over $MgSO_4$. After filtration and concentration, the residue was purified by silica gel column chromatography (eluted with *n*-hexane/EtOAc = 90/10 to 80/20 (v/v)) to give

the title compound **85** (560 mg, 46% yield) as a mixture containing a small amount of impurities. This was advanced to the next step without further purification.

Step 5: 4-Chloro-2,5-dimethyl-7*H*-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid (**86**)

To a solution of **85** (560 mg, 1.37 mmol) in THF (5.0 mL), 1.0 M lithium hydroxide solution (6.00 mL, 6.00 mmol) was added. After stirring overnight at 50 °C, the mixture was diluted with H_2O. And then, the mixture was neutralized with 6.0 M aqueous HCl solution. Precipitated solid was collected by filtration, washed with H_2O, and dried to give the title compound **86** (300 mg, 97% yield) which was advanced to the next step without further purification.

Step 6: 4-Chloro-*N*,2,5-trimethyl-7*H*-pyrrolo[2,3-d]pyrimidine-6-carboxamide (**17**)

To a solution of **86** (100 mg, 0.443 mmol) in 1:1 $CHCl_3$-THF (2.0 mL) was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (169 mg, 0.886 mmol), *N,N*-dimethyl-4-aminopyridine (5.00 mg, 0.0409 mmol) and 40% methylamine in methanol (34.0 μg, 0.493 mmol), and the mixture was stirred overnight at room temperature. The mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with H_2O, saturated aqueous $NaHCO_3$ solution and brine, and dried over $MgSO_4$. After filtration and concentration, for purification, the residue was slurried in AcOEt. The precipitated solid was collected by filtration, washed with a small portion of AcOEt, and dried to give the title compound **17** (73.0 mg, 69% yield).

^{1}H-NMR (400 MHz, DMSO-d$_6$) δ: 2.60 (s, 3H), 2.61 (s, 3H), 2.81 (d, *J* =4.6 Hz, 3H), 8.11 (br d, *J* =4.6 Hz, 1H), 12.32 (br s, 1H)

HRMS (ESI, m/z, MH+) Calcd for $C_{10}H_{12}N_4Cl_1$: 239.0694, Found: 239.0688

Synthesis of compound 19

Step1: 4-Chloro-2-methyl-7-((2-(trimethylsilyl)ethoxy)methyl)-7*H*-pyrrolo[2,3-d]pyrimidine-6-carbaldehyde (**87**)

To a solution of **72** (1.50 g, 5.03 mmol) in THF (15 mL), 1.63 M *n*-butyllithium in hexane (4.01 mL, 6.54 mmol) was added at -78 °C. After stirring for 3 h at -78 °C, *N,N*-dimethylformamide (503 μL, 6.54

mmol) was added to the reactant mixture, and then the mixture was stirred at room temperature for 1 h. The mixture was diluted with H_2O and saturated aqueous NH_4Cl solution, and extracted with AcOEt. The organic layer was washed with H_2O and brine, and dried over $MgSO_4$. After filtration and concentration, the residue was loaded on silica gel column chromatography (eluted with *n*-hexane/EtOAc = 90/10 (v/v)) for elimination of the polar component. The title compound **87** (1.08 g, 66% yield) was obtained, which was applied to the next step without further purification.

Step 2: (E)-4-Chloro-2-methyl-6-(prop-1-en-1-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7*H*-pyrrolo[2,3-d]pyrimidine (**88**)

Phenyllithium (2.1 M) in dibutyl ether (613 μL, 1.28 mmol) was added at 0 °C to a solution of ethyltriphenylphosphonium bromide (250 mg, 0.673 mmol) in THF (2.5 mL) and stirred for 15 min at 0 °C. The mixture was cooled to -78 °C and a solution **87** (200 mg, 0.613 mmol) in THF (2.5 mL) was added. After stirring at -78 °C for 30 min, additional 2.1 M phenyllithium in dibutyl ether (292 μL, 0.613 mmol) was added to the mixture, and then stirred for another 1.5 h at -78 °C. The reactant mixture was diluted with H_2O and saturated aqueous NH_4Cl solution, and extracted with AcOEt. The organic layer was washed with H_2O and brine, and dried over $MgSO_4$. After filtration and concentration, the residue was purified by preparative TLC (*n*-hexane/AcOEt = 4/1 (v/v)) to give the title compound **88** (50.0 mg, 24% yield).

^{1}H-NMR (400 MHz, DMSO-d6) δ: -0.11 (s, 9H), 0.80-0.88 (m. 2H), 1.93 (d, *J* =4.6 Hz, 3H), 2.63 (s, 3H), 3.46-3.52 (m, 2H), 5.65 (s, 2H), 6.64-6.66 (m, 2H), 6.77 (s, 1H)

Step 3: (E)-4-Chloro-2-methyl-6-(prop-1-en-1-yl)-7*H*-pyrrolo[2,3-d]pyrimidine (**89**)

Compound **88** (50 mg, 0.153 mmol) was dissolved in 1:1 $CHCl_3$-TFA (2.0 mL), and the mixture was stirred at room temperature for 1 h. After evaporation of $CHCl_3$ and TFA, the residue was diluted with THF and neutralized with 2 N NaOH solution, and then extracted with AcOEt. The organic layer was washed with H_2O, 1 N HCl solution and brine, and dried over $MgSO_4$. After filtration and concentration, for purification, the residue was slurried in *n*-hexane/AcOEt (10/1 (v/v)). The precipitated solid was collected by filtration, washed with *n*-hexane, and dried to give the title compound **89** (20.0 mg, 65% yield).

^{1}H-NMR (400 MHz, DMSO-d6) δ: 1.89 (d, *J* =5.5 Hz, 3H), 2.59 (s, 3H), 6.38-6.58 (m, 3H), 12.37 (br s, 1H)

Step 4: (E)-4,5-Dichloro-2-methyl-6-(prop-1-en-1-yl)-7*H*-pyrrolo[2,3-d]pyrimidine (**19**)

To a solution of **89** (10.0 mg, 4.82 x 10-2 mmol) in DMF (1.0 mL) was added *N*-chlorosuccinimide (10.0 mg, 7.49 x 10-2 mmol). After stirring overnight at room temperature, the reactant mixture was quenched with saturated NaHCO$_3$ solution. The mixture was extracted with AcOEt, and the organic layer was washed with H$_2$O and brine, and dried over MgSO$_4$. After filtration and concentration, the residue was purified by preparative TLC (n-hexane/AcOEt = 1/1 (v/v)) to give the title compound **19** (5.0 mg, 43% yield).

^{1}H-NMR (400 MHz, CDCl$_3$) δ: 2.00 (dd, *J* =6.7, 1.7 Hz, 3H), 2.73 (s, 3H), 6.23 (dq, *J* =16.1, 6.7 Hz, 1H), 6.58 (dq, *J* =16.1, 1.7 Hz, 1H), 9.57 (br s, 1H)

HRMS (ESI, m/z, MH+) Calcd for C$_{10}$H$_{10}$N$_3$Cl$_2$: 242.0246, Found: 242.0527

Synthesis of compound 18

Step 1: 4-Chloro-2-methyl-7-((2-(trimethylsilyl)ethoxy)methyl)-7*H*-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid (**90**)

Sodium dihydrogen phosphate dihydrate (114 mg, 0.734 mmol), 2-methylbut-2-ene (650 μg, 6.14 mmol) and sodium chlorite (68.0 mg, 0.736 mmol) were sequentially added to a solution of **87** (200 mg, 0.613 mmol) in 2:2:1 *t*-BuOH-H$_2$O-THF (2.5 mL) and stirred overnight at room temperature. The reactant mixture was diluted with H$_2$O and extracted with AcOEt. The organic layer was washed with H$_2$O and brine, and dried over MgSO$_4$. After filtration and concentration, for purification, the residue was slurried in *n*-hexane. The precipitated solid was collected by filtration, washed with *n*-hexane, and dried to give the title compound **90** (120 mg, 57% yield) as a mixture with a small amount of impurities. This was used for the next step without further purification.

Step 2: 4-Chloro-*N*,2-dimethyl-7-((2-(trimethylsilyl)ethoxy)methyl)-7*H*-pyrrolo[2,3-d]pyrimidine-6-carboxamide (**91**)

To a solution of **90** (120 mg, 0.350 mmol) in CHCl$_3$ (2.0 mL) was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (74.0 mg, 0.386 mmol), N,N-dimethyl-4-aminopyridine (2.00 mg, 0.0164 mmol) and 40% methylamine in methanol (39.0 μL, 0.386 mmol), and the mixture was stirred overnight at

room temperature. The mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with H_2O, saturated aqueous $NaHCO_3$ solution and brine, and dried over $MgSO_4$. After filtration and concentration, the residue was loaded on silica gel column chromatography (eluted with *n*-hexane/EtOAc = 66/34 (v/v)) for elimination of the polar component. The title compound **91** (90.0 mg, 72% yield) was obtained, which was used for the next step without further purification.

Step 3: 5-Bromo-4-chloro-*N*-2-dimethyl-7-((2-(trimethylsilyl)ethoxy)methyl)-7*H*-pyrrolo[2,3-d]pyrimidine-6-carboxamide (**92**)

To a suspension of **91** (90.0 mg, 0.253 mmol) in $CHCl_3$ (2.0 mL) was added *N*-bromosuccinimide (54.0 mg, 0.304 mmol). After stirring at room temperature for 2.5 h, the reactant mixture was quenched with saturated $NaHCO_3$ solution. The mixture was extracted with AcOEt, and the organic layer was washed with H_2O and brine, and dried over $MgSO_4$. After filtration and concentration, the residue was purified by preparative TLC (*n*-hexane/AcOEt = 2/1 (v/v)) to give the title compound **92** (92.0 mg, 84% yield) as a mixture with a small amount of impurities. This mixture was used for the next step without further purification.

Step 4: 5-Bromo-4-chloro-*N*-2-dimethyl-7*H*-pyrrolo[2,3-d]pyrimidine-6-carboxamide (**18**)

To a solution of **92** (90.0 mg, 0.207 mmol) in CH_2Cl_2 (1.0 mL), boron trifluoride diethyl etherate (77.0 µL, 0.624 mmol) was added at room temperature. After stirring overnight at room temperature, the mixture was quenched with 2 N NaOH solution and extracted with AcOEt. The organic layer was washed with H_2O and brine, and dried over $MgSO_4$. After filtration and concentration, for purification, the residue was slurried in AcOEt. The precipitated solid was collected by filtration, washed with *n*-hexane, and dried to give the title compound **18** (49.0 mg, 77% yield).

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 2.63 (s, 3H), 2.84 (d, *J* =4.4 Hz, 3H), 8.29 (q, *J* =4.4 Hz, 1H), 13.17 (br s, 1H)

HRMS (ESI, m/z, MH+) Calcd for $C_9H_9O_1N_4Br_1Cl_2$: 302.9643, Found: 302.9636

Synthesis of compound 22

Step1 : 6-Amino-2-methylnicotinic acid (**94**)

6-amino-2-methylnicotinonitrile (**93**, 50.0 g, 3.76×10^2 mmol) was added to a solution of KOH (120 g, 2.14 mol) and H_2O (500 mL), and the mixture was stirred at 100 °C for 8 h. After cooling to room temperature, the reactant mixture was neutralized with 12 M aqueous HCl solution (178 mL, 2.14 mmol). The precipitated solid was collected by filtration, washed with H_2O, and dried to give the title compound **94** (40.2 g, 70% yield).

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 2.52 (s, 3H), 6.27 (d, J = 8.9 Hz, 1H), 6.56 (s, 2H), 7.81 (d, J = 8.9 Hz, 1H), 12.10 (s, 1H)

Step2 : Methyl 6-amino-2-methylnicotinate (**95**)

To a solution of **94** (40.0 g, 2.63×10^2 mmol) in MeOH (400 mL) was added thionyl chloride (52.0 mL, 7.21×10^2 mmol) at room temperature, and then the mixture was stirred at 80 °C for 15 h. Under cooling to 0 °C, the reactant solution was quenched with saturated aqueous $NaHCO_3$ solution (approx. 600 mL), dropwise. The precipitated solid was collected by filtration, washed with H_2O, and dried to give the title compound **95** (39.7 g, 91% yield).

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 2.52 (s, 3H), 3.73 (s, 3H), 6.29 (d, J = 8.5 Hz, 1H), 6.66 (s, 2H), 7.82 (d, J = 8.5 Hz, 1H)

Step3 : Methyl 6-amino-5-bromo-2-methylnicotinate (**96**)

To a solution of **95** (75.7 g, 4.55×10^2 mmol) in THF (500 mL) was added N-bromosuccinimide (89.2 mL, 5.01×10^2 mmol), portionwise, with stirring and the mixture was cooled externally by using a water bath. After stirring at room temperature for 2 h, the reactant solution was quenched with H_2O (500 mL). The precipitated solid was collected by filtration, washed with H_2O, and dried to give the title compound **96** (92.0 g, 82% yield).

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 2.51 (s, 3H), 3.73 (s, 3H), 6.97 (br s, 2H), 8.05 (s, 1H)

Step 4 : Methyl 5-bromo-6-((2-cyclopropylphenyl)amino)-2-methylnicotinate (**97**)

A mixture of **96** (15.1 g, 6.16×10 mmol), **60** (16.5 g, 6.76×10 mmol), and sodium *tert*-pentoxide (10.2 g, 9.26×10 mmol) in toluene (160 mL) was treated with Pd(OAc)$_2$ (1.40 g, 6.24 mmol) and Xantphos (3.60

g, 6.22 mmol) and stirred at 140 °C for 4 h under argon atmosphere. After cooling to room temperature, the mixture was diluted with EtOAc and H_2O. The insoluble solid was filtered through Celite®. The organic layer was separated from the filtrate and concentrated. The residue was purified by flash chromatography (Biotage-SNAP Ultra 100g, eluted with n-hexane/EtOAc = 98/2 to 80/20 (v/v)) to give the title compound **97** (9.33 g, 38% yield).

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.62-0.66 (m, 2H), 0.92-0.97 (m, 2H), 1.82-1.89 (m, 1H), 2.57 (s, 3H), 3.79 (s, 3H), 7.06-7.14 (m, 2H), 7.23 (t, J = 7.8 Hz, 1H), 8.02 (d, J = 7.8 Hz, 1H), 8.25 (s, 1H), 8.39 (s, 1H)

Step 5 : Methyl 8-cyclopropyl-2-methyl-9H-pyrido[2,3-b]indole-3-carboxylate (**22**)

A solution of **97** (8.00 g, 2.21 x 10 mmol) and DBU (6.70 mL, 4.48 x 10 mmol) in DMA (50 mL) was treated with Pd(OAc)$_2$ (500 mg, 2.23 mmol) and CyJonphos (780 mg, 6.22 mmol) under argon atmosphere and stirred at 130 °C for 4 h. After cooling to room temperature, the mixture was diluted with H_2O and extracted with EtOAc. The organic layer was washed twice with H_2O and brine, and dried over Na_2SO_4. After filtration and concentration, the residue was purified by flash chromatography (Biotage-SNAP Ultra 100g, eluted with n-hexane/EtOAc = 94/6 to 50/50 (v/v)) to give the title compound **22** (4.79 g, 66% yield).

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.73-0.84 (m, 2H), 1.00-1.11 (m, 2H), 2.32-2.44 (m, 1H), 2.87 (s, 3H), 3.89 (s, 3H), 7.00 (d, J = 7.6 Hz, 1H), 7.16 (t, J = 7.6 Hz, 1H), 8.00 (d, J = 7.6 Hz, 1H), 8.95 (s, 1H), 12.25 (s, 1H)

^{13}C-NMR (400 MHz, DMSO-d_6) δ: 8.0 (2C), 10.4, 25.2, 51.7, 113.2, 115.9, 118.3, 120.0, 120.5, 121.4, 127.0, 138.9, 152.8, 156.9, 167.0, 170.2

HRMS (ESI, m/z, MH+) Calcd for $C_{17}H_{17}O_2N_2$: 281.1285, Found: 281.1284

Synthesis of compound 21

Step1 : 8-Cyclopropyl-2-methyl-9H-pyrido[2,3-b]indole-3-carboxylic acid (**98**)

Compound **22** (4.79 g, 1.71 x 10 mmol) was dissolved in 1:1 THF-MeOH (34 mL). To the solution, 4 M aqueous NaOH solution (17.1 mL, 6.84 x 10 mmol) was added, and the mixture was stirred at 60 °C for 4.5 h. After cooling to room temperature, the reactant mixture was acidified by adding 2 M aqueous HCl solution (42.8 mL, 8.56 x 10 mmol), and was stirred at room temperature for 1 h. The precipitated solid

was collected by filtration, washed with H_2O, and dried to give the title compound **98** (5.07 g, quant.), which was proceeded to the next step without further purification.

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.76-0.81 (m, 2H), 1.03-1.08 (m, 2H), 2.34-2.41 (m, 1H), 2.88 (s, 3H), 6.99 (d, J = 7.6 Hz, 1H), 7.15 (t, J = 7.6 Hz, 1H), 8.00 (d, J = 7.6 Hz, 1H), 8.96 (s, 1H), 12.21 (s, 1H), 12.75 (s, 1H)

Step2 : 8-Cyclopropyl-N,2-dimethyl-9H-pyrido[2,3-b]indole-3-carboxamide (**21**)

Methylamine (45.6 mg, 6.76 x 10^{-1} mmol), HATU (206 mg, 5.41 x 10^{-1} mmol) and Et$_3$N (137 mg, 1.35 mmol) were added to a solution of **98** (120 mg, 4.51 x 10^{-1} mmol) in DMF (1.2 mL), and the reactant mixture was stirred at room temperature for 2h. The mixture was quenched with saturated aqueous NaHCO$_3$ solution and extracted with CHCl$_3$. The organic layer was dried over MgSO$_4$. After filtration and concentration, the residue was purified by preparative TLC (CHCl$_3$/MeOH = 20/1 (v/v)) to give the title compound **21** (57.5 mg, 46% yield).

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.76-0.80 (m, 2H), 1.03-1.08 (m, 2H), 2.34-2.41 (m, 1H), 2.67 (s, 3H), 2.81 (d, J = 4.8 Hz, 3H), 6.96 (d, J = 7.5 Hz, 1H), 7.13 (t, J = 7.5 Hz, 1H), 7.92 (t, J = 7.5 Hz, 1H), 8.33 (q, J = 4.8 Hz, 1H), 8.47 (s, 1H), 12.00 (s, 1H)

^{13}C-NMR (400 MHz, DMSO-d_6) δ: 8.1 (2C), 10.4, 23.3, 26.1, 79.1, 112.4, 117.9, 119.9, 120.7, 123.8, 126.7, 127.8, 138.7, 151.6, 152.9, 169.3

HRMS (ESI, m/z, MH+) Calcd for C$_{17}$H$_{18}$ON$_3$: 280.1444, Found: 281.1441

Synthesis of compound 23

Step1 : 3,5-Dibromo-N-(2-cyclopropylphenyl)-6-methylpyridin-2-amine (**100**)

A mixture of **60** (5.00 g, 2.05 x 10 mmol), 3,5-dibromo-6-methylpyridin-2-amine (**99**; 5.45 g, 2.05 x 10 mmol) and sodium *tert*-pentoxide (3.39 g, 3.08 x 10 mmol) in toluene (50 mL) was treated with Pd(OAc)$_2$ (0.46 g, 2.05 mmol) and Xantphos (1.18 g, 2.05 mmol) and stirred at 140 °C for 4 h under argon atmosphere. After cooling to room temperature, the mixture was diluted with EtOAc and H_2O. The insoluble solid was filtered through Celite®. The organic layer was separated from the filtrate and concentrated. The residue

was purified by flash chromatography (Biotage-SNAP Ultra 100g, eluted with n-hexane/EtOAc = 98/2 to 80/20 (v/v)) to give the title compound **100** (2.89 g, 37% yield).

^{1}H-NMR (400 MHz, CDCl$_3$) δ: 0.65-0.79 (m, 2H), 0.98-1.12 (m, 2H), 1.77-1.77 (m, 1H), 2.55 (s, 3H), 7.20 (dt, J = 7.8, 1.4 Hz, 1H), 7.25 (td, J = 7.8, 1.4 Hz, 1H), 7.80 (br s, 2H), 7.83 (s, 1H), 8.02 (dd, J = 7.8, 1.4 Hz, 1H)

Step2 : 3-Bromo-8-cyclopropyl-2-methyl-9H-pyrido[2,3-b] indole (**101**)

A solution of **100** (2.89 g, 7.56 mmol) and DBU (2.28 mL, 1.51 x 10 mmol) in DMA (29 mL) was treated with Pd(OAc)$_2$ (693 mg, 7.56 x 10^{-1} mmol) and CyJonphos (1.06 g, 3.03 mmol) under argon atmosphere and stirred at 130 °C for 4 h. After cooling to room temperature, the mixture was diluted with H$_2$O and extracted with EtOAc. The organic layer was washed twice with H$_2$O and brine, and dried over Na$_2$SO$_4$. After filtration and concentration, the residue was purified by flash chromatography (Biotage-SNAP Ultra 100g, eluted with n-hexane/EtOAc = 94/6 to 50/50 (v/v)) to give the title compound **101** (590 mg, 26% yield).

^{1}H-NMR (400 MHz, CDCl$_3$) δ: 0.75-0.82 (m, 2H), 0.99-1.08 (m, 2H), 2.03-2.14 (m, 1H), 2.78 (s, 3H), 7.15-7.26 (m, 2H), 7.81 (d, J = 7.5 Hz, 1H), 8.41 (s, 1H), 8.69 (br s, 1H)

Step3 : 8-Cyclopropyl-2-methyl-3-phenyl-9H-pyrido[2,3-b]indole (**23**)

A mixture of the compound **101** (170 mg, 5.64 x 10^{-1} mmol), phenyl boronic acid (103 mg, 8.47 x 10^{-1} mmol), and K$_2$CO$_3$ (156 mg, 1.13 mmol) in 1,4-dioxane (2.5 mL) and H$_2$O (2.5 mL) was treated with PdCl$_2$(dppf)-CH$_2$Cl$_2$ (461 mg, 5.60 x 10^{-2} mmol) and stirred under 80 °C for 4 h. After cooling to room temperature, the mixture was diluted with EtOAc and H$_2$O, filtered through Celite®, and the organic layer was sepatated and washed with H$_2$O and brine. After filtration and concentration, the residue was purified by flashchromatography (Yamazen-Fuji Silysia Q-PACK SI50 SIZE400, eluted with n-hexane/EtOAc = 92/8 to 25/75 (v/v)) to give the title compound **23** (75.3 mg, 45% yield).

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.75-0.83 (m, 2H), 1.01-1.11 (m, 2H), 2.34-2.45 (m, 1H), 2.56 (s, 3H), 6.94 (d, J = 7.5 Hz, 1H), 7.09 (t, J = 7.5 Hz, 1H), 7.35-7.44 (m, 1H), 7.44-7.54 (m, 4H), 7.91 (d, J = 7.5 Hz, 1H), 8.29 (s, 1H), 11.87 (s, 1H)

^{13}C-NMR (400 MHz, DMSO-d_6) δ: 8.1 (2C), 10.4, 23.7, 113.5, 118.0, 119.5, 119.9, 120.5, 126.5, 126.7, 128.1, 128.2 (2C), 129.4, 129.5 (2C), 138.6, 140.8, 151.1, 151.8

HRMS (ESI, m/z, MH+) Calcd for $C_{21}H_{19}N_2$: 299.1543, Found: 299.1542

Synthesis of compound 24

Step1 : 8-Cyclopropyl-2-methyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-9H-pyrido[2,3-b]indole (**102**)

A mixture of 3-bromo-8-cyclopropyl-2-methyl-9H-pyrido[2,3-b]indole (**101**, 100 mg, 3.32 x 10^{-1} mmol), (Bpin)$_2$ (126 mg, 4.96 x 10^{-1} mmol), and KOAc (98.0 mg, 9.99 x 10^{-1} mmol) in DMSO (2.0 mL) was treated with PdCl$_2$(dppf)-CH$_2$Cl$_2$ (28.0 mg, 3.43 x 10^{-2} mmol) under argon atmosphere. The mixture was heated at 100 °C for 2 h. After cooling to room temperature, the reactant mixture was diluted with H$_2$O and extracted with AcOEt, and the organic layer was washed with H$_2$O and brine, and dried over MgSO$_4$. After filtration and concentration, the residue was purified by flashchromatography (Biotage-SNAP Ultra 10 g, eluted with n-hexane/EtOAc = 66/34 (v/v)) to give the title compound **102** (30.0 mg, 26% yield).

^{1}H-NMR (400 MHz, CDCl$_3$) δ: 0.77-0.81 (m, 2H), 1.02-1.07 (m, 2H), 1.40 (s, 12H), 2.07-2.14 (m, 1H), 2.88 (s, 3H), 7.16-7.21 (m, 2H), 7.85-7.89 (m, 1H), 8.71 (s, 1H), 9.36 (s, 1H)

Step2 : 8-Cyclopropyl-2-methyl-3-(pyridin-2-yl)-9H-pyrido[2,3-b]indole (**24**)

A mixture of **102** (57.8 mg, 1.66 x 10^{-1} mmol), 2-bromopyridine (52.2 mg, 3.32 x 10^{-1} mmol), and K$_2$CO$_3$ (69.0 mg, 4.98 x 10^{-1} mmol) in 1,4-dioxane (2.0 mL) was treated with PdCl$_2$(dppf)-CH$_2$Cl$_2$ (14.0 mg, 1.71 x 10^{-2} mmol) under argon atmosphere. The mixture was heated at 100 °C for 2 h. After cooling to room temperature, the reactant mixture was diluted with H$_2$O and extracted with AcOEt, and the organic layer was washed with H$_2$O and brine and dried over MgSO$_4$. After filtration and concentration, the residue was purified by preparative TLC (n-hexane/AcOEt = 1/1 (v/v)) to give the title compound **24** (11.0 mg, 22% yield).

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.77-0.81 (m, 2H), 1.04-1.09 (m, 2H), 2.37-2.44 (m, 1H), 2.67 (s, 3H), 6.96 (d, J = 7.5 Hz, 1H), 7.12 (t, J = 7.5 Hz, 1H), 7.40 (ddd, J = 7.5, 4.9, 0.9 Hz, 1H), 7.69 (dt, J = 7.8, 0.9 Hz, 1H), 7.93 (td, J = 7.8, 1.8 Hz, 1H), 7.95 (d, J = 7.5 Hz, 1H), 8.51 (s, 1H), 8.72 (ddd, J = 4.9, 1.8, 0.9 Hz, 1H), 11.96 (s, 1H)

^{13}C-NMR (400 MHz, DMSO-d_6) δ: 8.0 (2C), 10.4, 23.8, 113.4, 118.0, 119.7, 120.0, 120.6, 121.6, 124.3, 126.6, 127.3, 129.8, 136.5, 138.7, 149.0, 151.3, 152.5, 158.8

HRMS (ESI, m/z, MH+) Calcd for $C_{20}H_{18}N_3$: 300.1495, Found: 300.1496

Synthesis of compound 25

Step1 : *tert*-Butyl 2-(8-cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indole-3-carbonyl)hydrazine-1-carboxylate (**103**)

To a solution of **98** (5.00 g, 1.70 x 10 mmol) and *tert*-butyl carbazate (3.37 g, 2.55 x 10 mmol) in DMF (50 mL) was added WSC·HCl (4.89 g, 2.55 x 10 mmol) and HOBt·H$_2$O (3.91 g, 2.55 x 10   mmol), and the mixture was stirred at room temperature for 4 h. The reactant solution was dropped into saturated aqueous NaHCO$_3$ solution under stirring. The precipitated solid was collected by filtration, washed with H$_2$O, and dried to give the title compound **103** (6.61 g, overweight), which was proceeded to the next step without further purification.

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.76-0.80 (m, 2H), 1.03-1.08 (m, 2H), 1.45 (s, 9H), 2.34-2.41 (m, 1H), 2.71 (s, 3H), 6.97 (d, *J* = 7.5 Hz, 1H), 7.13 (t, *J* = 7.5 Hz, 1H), 7.93 (d, *J* = 7.5 Hz, 1H), 8.46 (s, 1H), 8.94 (br s, 1H), 9.99 (br s, 1H), 12.00 (br s, 1H)

Step2 : 8-Cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indole-3-carbohydrazide (**104**)

A suspension of **103** (6.60 g, approx. 1.7 x 10 mmol) in 1,4-dioxane (40 mL) was treated with 4 M HCl in 1,4-dioxane (40 mL) and stirred at 70 °C for 3 h. The resultant suspension was filtrated and washed with 1,4-dioxane, and dried to give the hydrochloride salt of the title compound **104** (5.43 g, 84% yield, 2steps), which was proceeded to the next step without further purification.

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.77-0.82 (m, 2H), 1.04-1.09 (m, 2H), 2.38-2.45 (m, 1H), 2.76 (s, 3H), 7.01 (d, *J* = 7.6 Hz, 1H), 7.17 (t, *J* = 7.6 Hz, 1H), 7.96 (d, *J* = 7.6 Hz, 1H), 8.69 (s, 1H), 10.79 (br s, 2H), 11.56 (s, 1H), 12.24 (s, 1H)

Step3 : 2-(8-Cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indol-3-yl)-5-methyl-1,3,4-oxadiazole   (**25**)

AcOH (45.5 mg, 7.58 x 10⁻¹ mmol), HATU (288 mg, 7.58 x 10⁻¹ mmol) and Et₃N (191 mg, 1.89 mmol) were added to a solution of **104** (200 mg, 6.31 x 10⁻¹ mmol) in DMF (1.2 mL), and the mixture was stirred at room temperature for 16 h. The mixture was quenched with saturated aqueous NaHCO₃ solution and extracted with CHCl₃. The organic layer was dried over MgSO₄. After concentration, the residue was suspended in 1:1 *n*-hexane-EtOAc and stirred. The precipitated solid was collected by filtration, washed with *n*-hexane, and dried to give the intermediate diacyl hydrazide compound. After adding THF (4.0 mL) and the Burgess reagent (300 mg, 1.26 mmol), the mixture was stirred at room temperature for 3 h. The mixture was diluted with H₂O and extracted with EtOAc. The organic layer was washed twice with H₂O and brine, and dried over MgSO₄. After filtration and concentration, the residue was purified by flash chromatography (Biotage-SNAP Ultra 100 g, eluted with *n*-hexane/EtOAc = 50/50 (v/v)) to give the title compound **25** (71.7 mg, 37% yield).

¹H-NMR (400 MHz, DMSO-*d₆*) δ: 0.78-0.82 (m, 2H), 1.04-1.09 (m, 2H), 2.36-2.43 (m, 1H), 2.63 (s, 3H), 2.95 (s, 3H), 7.02 (d, *J* = 7.5 Hz, 1H), 7.17 (t, *J* = 7.5 Hz, 1H), 8.05 (t, *J* = 7.5 Hz, 1H), 8.94 (s, 1H), 12.29 (s, 1H)

¹³C-NMR (400 MHz, DMSO-*d₆*) δ: 8.1 (2C), 10.4, 10.6, 25.2, 110.2, 113.6, 118.4, 119.7, 120.5, 121.5, 127.0, 129.0, 139.0, 152.4, 154.1, 163.2, 164.3

HRMS (ESI, m/z, MH+) Calcd for C₁₈H₁₇ON₄: 305.1397, Found: 305.1397

Synthesis of compound 27 – 30

2-(8-Cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indol-3-yl)-5-ethyl-1,3,4-oxadiazole   (**27**)

Compound **27** was synthesized in the same procedure described above for compound **25**, except using the propionic acid instead of AcOH to obtain the corresponding diacyl hydrazide intermediate (8-cyclopropyl-2-methyl-*N*′-propionyl-9*H*-pyrido[2,3-*b*]indole-3-carbohydrazide).   Starting   from   the compound **104** (200 mg, 6.31   x 10⁻¹ mmol), the title compound **27** (87.4 mg, 46% yield) was obtained.

¹H-NMR (400 MHz, DMSO-*d₆*) δ: 0.78-0.82 (m, 2H), 1.04-1.09 (m, 2H), 1.38 (t, *J* = 7.5 Hz, 3H), 2.36-2.43 (m, 1H), 2.95 (s, 3H), 2.99 (q, *J* = 7.5 H z, 2H), 7.02 (d, *J* = 7.6 Hz, 1H), 7.18 (t, *J* = 7.6 Hz, 1H), 8.06 (t, *J* = 7.6 Hz, 1H), 8.96 (s, 1H), 12.29 (s, 1H)

¹³C-NMR (400 MHz, DMSO-*d₆*) δ: 8.1 (2C), 10.4, 10.5, 18.4, 25.2, 110.3, 113.6, 118.5, 119.7, 120.5, 121.5, 127.0, 129.1, 139.0, 152.4, 154.1, 164.2, 167.0

HRMS (ESI, m/z, MH+) Calcd for $C_{19}H_{19}ON_4$: 319.1553, Found: 319.1556

2-(8-Cyclopropyl-2-methyl-9H-pyrido[2,3-b]indol-3-yl)-5-isopropyl-1,3,4-oxadiazole (**28**)

Compound **28** was synthesized in the same procedure described above for compound **25**, except using the isobutyric acid instead of AcOH. Starting from the compound **104** (200 mg, 6.31 x 10^{-1} mmol), the title compound **28** (97.0 mg, 46% yield) was obtained.

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.78-0.82 (m, 2H), 1.05-1.09 (m, 2H), 1.43 (d, J = 7.2 Hz, 6H), 2.36-2.43 (m, 1H), 2.94 (s, 3H), 3.28-3.38 (m, 1H), 7.02 (d, J = 7.6 Hz, 1H), 7.18 (t, J = 7.6 Hz, 1H), 8.07 (t, J = 7.6 Hz, 1H), 8.98 (s, 1H), 12.29 (s, 1H)

^{13}C-NMR (400 MHz, DMSO-d_6) δ: 8.1 (2C), 10.4, 19.8 (2C), 25.2, 25.7, 110.3, 113.6, 118.5, 119.8, 120.5, 121.5, 127.0, 129.1, 139.0, 152.4, 154.1, 164.2, 170.0

HRMS (ESI, m/z, MH+) Calcd for $C_{20}H_{21}ON_4$: 333.1710, Found: 333.1711

2-(8-Cyclopropyl-2-methyl-9H-pyrido[2,3-b]indol-3-yl)-5-propyl-1,3,4-oxadiazole (**29**)

Compound **29** was synthesized in the same procedure described above for compound **28**, except using the butyric acid instead of AcOH. Starting from the compound **104** (200 mg, 6.31 x 10^{-1} mmol), the title compound **29** (97.6 mg, 47% yield) was obtained.

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.78-0.82 (m, 2H), 1.03 (t, J = 7.5 Hz, 3H), 1.05-1.11 (m, 2H), 1.80-1.90 (m, 2H), 2.36-2.43 (m, 1H), 2.95 (s, 3H), 2.95 (t, J = 7.5 Hz, 3H), 7.02 (d, J = 7.6 Hz, 1H), 7.18 (t, J = 7.6 Hz, 1H), 8.06 (t, J = 7.6 Hz, 1H), 8.96 (s, 1H), 12.29 (s, 1H)

^{13}C-NMR (400 MHz, DMSO-d_6) δ: 8.1 (2C), 10.4, 13.4, 19.5, 25.2, 26.4, 110.3, 113.6, 118.5, 119.7, 120.5, 121.5, 127.0, 129.1, 139.0, 152.4, 154.1, 164.2, 166.0

HRMS (ESI, m/z, MH+) Calcd for $C_{20}H_{21}ON_4$: 333.1710, Found: 333.1711

2-(8-Cyclopropyl-2-methyl-9H-pyrido[2,3-b]indol-3-yl)-5-isobutyl-1,3,4-oxadiazole (**30**)

Compound **30** was synthesized in the same procedure described above for compound **25**, except using the isovaleric acid instead of AcOH. Starting from the compound **104** (200 mg, 6.31 x 10^{-1} mmol), the title compound **30** (73.6 mg, 34% yield) was obtained.

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.78-0.82 (m, 2H), 1.03 (d, J = 6.6 Hz, 6H), 1.04-1.09 (m, 2H), 2.17-2.27 (m, 1H), 2.36-2.43 (m, 1H), 2.87 (d, J = 6.9 Hz, 2H), 2.94 (s, 3H), 7.02 (d, J = 7.6 Hz, 1H), 7.18 (t, J = 7.6 Hz, 1H), 8.06 (t, J = 7.6 Hz, 1H), 8.95 (s, 1H), 12.29 (s, 1H)

^{13}C-NMR (400 MHz, DMSO-d_6) δ: 8.1 (2C), 10.4, 22.0 (2C), 25.2, 26.6, 33.2, 110.3, 113.6, 118.5, 119.7, 120.5, 121.5, 127.0, 129.1, 139.0, 152.4, 154.1, 164.3, 165.4

HRMS (ESI, m/z, MH+) Calcd for $C_{21}H_{23}ON_4$: 347.1866, Found: 347.1870

Synthesis of compound 26

2-(8-Cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indol-3-yl)-5-ethyl-1,3,4-thiadiazole (**26**)

8-Cyclopropyl-2-methyl-*N*′-propionyl-9*H*-pyrido[2,3-*b*]indole-3-carbohydrazide (15.0 mg, 4.46 x 10^{-2} mmol) was prepared in the same method listed above for compound **27**. THF (2.0 mL) and Lawesson reagent (38.0 mg, 9.41 x 10^{-2} mmol) was added, and the reactant mixture was stirred at 80 °C for 19 h. After cooling to room temperature, the mixture was diluted with H_2O and extracted with EtOAc. After filtration and concentration, the residue was purified by preparative TLC (*n*-hexane/AcOEt = 2/1 (v/v)) to give the title compound **26** (6.0 mg, 40% yield).

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.78-0.82 (m, 2H), 1.04-1.09 (m, 2H), 1.41 (t, J = 7.5 Hz, 3H), 2.36-2.43 (m, 1H), 2.84 (s, 3H), 3.19 (q, J = 7.5 Hz, 2H), 7.00 (d, J = 7.6 Hz, 1H), 7.16 (t, J = 7.6 Hz, 1H), 8.02 (d, J = 7.6 Hz, 1H), 8.80 (s, 1H), 12.21 (s, 1H)

^{13}C-NMR (400 MHz, DMSO-d_6) δ: 8.1 (2C), 10.4, 14.0, 22.9, 24.7, 113.6, 116.4, 118.4, 119.7, 120.2, 121.3, 126.8, 130.7, 138.9, 152.0, 153.0, 166.9, 171.6

HRMS (ESI, m/z, MH+) Calcd for $C_{19}H_{19}N_4S$: 335.1325, Found: 335.1330

Synthesis of compound 32

Step1 : *tert*-Butyl (1-(2-(8-cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indole-3-carbonyl)hydrazineyl)-3-methyl-1-oxobutan-2-yl)carbamate (**106**)

To a solution of the hydrochloride salt of **104** (200 mg, 6.31 x 10^{-1} mmol) and *N*-(*tert*-butoxycarbonyl)-DL-valine (**105**; 164 mg, 7.57 x 10^{-1} mmol) in DMF (2.0 mL) was added WSC·HCl (145 mg, 7.56 x 10^{-1} mmol), HOBt·H_2O (116 mg, 7.58 x 10^{-1} mmol) and Et$_3$N (105 μL, 7.53 x 10^{-1} mmol), and the mixture was stirred at room temperature for 3 h. The mixture was quenched with saturated aqueous NaHCO$_3$ solution and extracted with EtOAc. The organic layer was washed twice with H_2O and brine, and dried over MgSO$_4$. After filtration, the solvent was evaporated *in vacuo* to give the title compound **106** (310 mg, overweight) as a crude product. This was used to the next step without further purification.

Step2 : *tert*-Butyl (1-(5-(8-cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indol-3-yl)-1,3,4-oxadiazol-2-yl)-2-methylpropyl)carbamate (**107**)

To a solution of **106** (310 mg, approx. 6.3 x 10^{-1} mmol) in THF (5.0 mL) was added Burgess reagent (300 mg, 1.26 mmol), and the mixture was stirred at room temperature for 3 h. The mixture was diluted with H_2O and extracted with EtOAc. The organic layer was washed twice with H_2O and brine, and dried over MgSO$_4$. After filtration and concentration, the residue was suspended in 1:2 *n*-hexane-EtOAc and slurried for a while. The precipitated solid was collected by filtration, washed with *n*-hexane, and dried to give the title compound **107** (200 mg), which was used to the next step without further purification.

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.77-0.83 (m, 2H), 0.93 (d, *J* = 6.7 Hz, 3H), 1.02 (d, *J* = 6.7 Hz, 3H), 1.04-1.10 (m, 2H), 1.42 (s, 9H), 2.22-2.31 (m, 1H), 2.36-2.44 (m, 1H), 2.93 (s, 3H), 4.69-4.76 (m, 1H), 7.03 (d, *J* = 7.5 Hz, 1H), 7.19 (t, *J* = 7.5 Hz, 1H), 7.71 (d, *J* = 8.3 Hz, 1H) 8.00 (d, *J* = 7.5 Hz, 1H), 8.94 (s, 1H), 12.31 (s, 1H)

Step3 : 1-(5-(8-Cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indol-3-yl)-1,3,4-oxadiazol-2-yl)-2-methylpropan-1-amine (**108**)

Compound **107** (200 mg, approx. 4.3 x 10^{-1} mmol) was treated with 4 M HCl in 1,4-dioxane (2.0 mL) and stirred at room temperature for 1 h. The solvent was removed by *in vacuo*, and then the residue was suspended in EtOAc and slurried for a while. The precipitated solid was collected by filtration, washed with EtOAc, and dried to give the title compound **108** (170 mg, 68%, 3 steps).

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.78-0.83 (m, 2H), 1.02 (d, J = 6.8 Hz, 3H), 1.04-1.11 (m, 2H), 1.13 (d, J = 6.8 Hz, 3H), 2.35-2.48 (m, 2H), 2.98 (s, 3H), 4.71-4.83 (m, 1H), 7.04 (d, J = 7.5 Hz, 1H), 7.20 (t, J = 7.5 Hz, 1H), 8.02 (d, J = 7.5 Hz, 1H), 9.04 (s, 1H), 8.94-9.08 (br m, 3H), 12.37 (s, 1H)

Step4 : *N*-(1-(5-(8-Cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indol-3-yl)-1,3,4-oxadiazol-2-yl)-2-methylpropyl)-3-phenylpropanamide (**32**)

To a solution of **108** (20.0 mg, 5.03 x 10^{-2} mmol) and 3-phenylpropanoyl chloride (**109**; 9.00 μL, 6.36 x 10^{-2} mmol) in CHCl$_3$ (1.0 mL) was added Et$_3$N (18.0 μL, 1.28 x 10^{-1} mmol), and the reactant mixture was stirred at room temperature for 1 h. The mixture was quenched with saturated aqueous NaHCO$_3$ solution, and extracted with EtOAc. The organic layer was washed with H$_2$O and brine, and dried over MgSO$_4$. After filtration and concentration, the residue was suspended in *n*-hexane and slurried for a while. The precipitated solid was collected by filtration, washed with *n*-hexane and dried to give the title compound **32** (24.0 mg, 96%).

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.77-0.83 (m, 2H), 0.90 (d, J = 6.7 Hz, 3H), 0.96 (d, J = 6.7 Hz, 3H), 1.04-1.10 (m, 2H), 2.21-2.31 (m, 1H), 2.35-2.44 (m, 1H), 2.48-2.61 (m, 2H), 2.86 (t, J = 7.6 Hz, 1H), 2.92 (s, 3H), 5.07 (dd, J = 8.3, 7.9 Hz, 1H), 7.03 (d, J = 7.6 Hz, 1H), 7.06-7.13 (m, 1H), 7.15-7.31 (m, 5H), 8.02 (d, J = 7.6 Hz, 1H), 8.58 (d, J = 8.6 Hz, 1H), 8.92 (s, 1H), 12.32 (s, 1H)

^{13}C-NMR (400 MHz, DMSO-d_6) δ: 8.1 (2C), 10.4, 18.4, 18.9, 25.1, 30.8, 30.9, 36.5, 50.4, 109.9, 113.5, 118.3, 119.6, 120.5, 125.7, 127.1, 128.06 (2C), 128.10 (2C), 128.18, 128.23, 129.1, 141.0, 152.4, 154.1, 164.2, 165.4, 171.6

HRMS (ESI, m/z, MH+) Calcd for C$_{30}$H$_{32}$O$_2$N$_5$: 494.2551, Found: 494.2550

Synthesis of compound 33

Step1 : Diethyl 2-(2-(benzyloxy)ethyl)-2-isopropylmalonate (**112**)

To a suspension of sodium hydride (60% dispersion in mineral oil, 480 mg, 1.20 x 10 mmol) in DMSO (10 mL), diethyl 2-isopropylmalonate (**110**, 2.00 mL, 1.00 x 10 mmol) and ((2-bromoethoxy)methyl)benzene (**111**, 1.60 mL, 1.00 x 10 mmol) were added at room temperature. Then the mixture was stirred at 60 °C for 3 h. The mixture was quenched with aqueous NH$_4$Cl solution and extracted with AcOEt, and the organic layer was washed with brine, and dried over MgSO$_4$. After filtration and

concentration, the residue was purified by silica gel column chromatography (eluted with *n*-hexane/EtOAc = 90/10 (v/v)) to give the title compound **112** (2.56 g, 76% yield).

^{1}H-NMR (400 MHz, DMSO-*d*[6]) δ: 0.91 (d, *J* = 7.0 Hz, 6H), 1.14 (d, *J* = 7.0 Hz, 6H), 2.10 (t, *J* = 6.9 Hz, 2H), 2.20-2.30 (m, 1H), 3.44 (t, *J* = 6.9 Hz, 2H), 4.40 (s, 2H), 7.25-7.36 (m, 5H)

Step2 : Ethyl 4-(benzyloxy)-2-isopropylbutanoate (**113**)

To a solution of **112** (2.56 g, 7.60 mmol) in EtOH (26 mL), EtOH (4.0 mL) solution of KOH (2.55 g, 4.56 x 10 mmol) was added at 0 °C, and the reactant mixture was stirred at reflux temperature for 17 h. The reactant solution was acidified by adding 2 M aqueous HCl solution (30 mL), extracted with EtOAc, and the organic layer was washed with brine. The solvent was evaporated *in vacuo* to give the title compound **113** (2.50 g, overweight) as a crude product. That was used in the next step without further purification.

Step3 : 4-(Benzyloxy)-2-isopropylbutanoic acid (**114**)

To a solution of **113** (2.50 g, approx. 7.6 mmol) in EtOH (25 mL), 8 M aqueous KOH solution (4.80 mL, 3.84 x 10 mmol) and H_2O (10 mL) were added, and the reactant mixture was stirred at reflux temperature for 8 h. The reactant solution was quenched with 1 M aqueous HCl solution (40 mL) and extracted with EtOAc. The organic layer was washed with brine, and dried over $MgSO_4$. After filtration and concentration, the residue was purified by flash chromatography (Biotage-SNAP Ultra 50g, eluted with *n*-hexane/EtOAc = 66/34 (v/v)) to give the title compound **114** (850 mg, 47% yield, 2 steps).

^{1}H-NMR (400 MHz, DMSO-*d*[6]) δ: 0.89 (d, *J* = 6.7 Hz, 6H), 1.64-1.86 (m, 3H), 2.13-2.20 (m, 1H), 3.32-3.47 (m, 2H), 4.43 (s, 2H), 7.24-7.37 (m, 5H), 12.08 (br s, 1H)

Step4 : *tert*-Butyl 2-(4-(benzyloxy)-2-isopropylbutanoyl)hydrazine-1-carboxylate (**115**)

To a solution of **114** (536 mg, 2.27 mmol) and *tert*-butyl carbazate (300 mg, 2.27 mmol) in DMF (3.0 mL) were added WSC・HCl (522 mg, 2.27 mmol) and HOBt・H_2O (263 mg, 2.27 mmol), and the mixture was stirred overnight at room temperature. The reactant solution was diluted with aqueous $NaHCO_3$ solution and extracted with *n*-hexane/EtOAc (3/1 (v/v)). The organic layer was washed with brine, and

dried over MgSO$_4$. After filtration, the solvent was evaporated *in vacuo* to give the title compound **115** (801 mg, quant.) as a crude product.

Step5 : 4-(Benzyloxy)-2-isopropylbutanehydrazide hydrochloride (**116**)

The compound **115** (800 mg, approx. 2.2 mmol) was dissolved in 4 N HCl dioxane solution (8.0 mL), and the mixture was stirred at room temperature for 3.5 h. The reaction solution was concentrated *in vacuo* to give the title compound **116** (700 mg, quant.), which was used for the next step without further purification.

Step6 : *N'*-(4-(Benzyloxy)-2-isopropylbutanoyl)-8-cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indole-3-carbohydrazide (**117**)

To the solution of **98** (160 mg, 6.01 x 10^{-1} mmol) and **116** (350 mg, approx. 1.1 mmol) in DMF (2.0 mL) was added WSC·HCl (176 mg, 9.18 x 10^{-1} mmol), HOBt·H$_2$O (140 mg, 9.14 x 10^{-1} mmol) and *i*-Pr$_2$EtN (260 μL, 1.53 mmol), and the mixture was stirred at room temperature for 1 h. The reactant solution was dropped into saturated aqueous NaHCO$_3$ solution under stirring. The precipitated solid was collected by filtration, washed with H$_2$O, and dried to give the title compound **117** (232 mg, 76%) as a crude product.

Step7 : 2-(1-(Benzyloxy)-4-methylpentan-3-yl)-5-(8-cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indol-3-yl)-1,3,4-oxadiazole (**118**)

A solution of **117** (460 mg, 9.23 x 10^{-1} mmol) in POCl$_3$ (3.0 mL) stirred at 110 °C for 2 h. The reactant solution was poured into H$_2$O, and then the mixture was extracted with CHCl$_3$. The organic layer was washed with saturated aqueous NaHCO$_3$ solution, and dried over MgSO$_4$. After filtration and concentration, the residue was purified by silica gel column chromatography (eluted with *n*-hexane/EtOAc = 75/25 to 67/33 (v/v)) to give the title compound **118** (250 mg, 57% yield).

^{1}H-NMR (400 MHz, DMSO-*d$_6$*) δ: 0.78-0.83 (m, 2H), 0.91 (d, *J* = 6.7 Hz, 3H), 1.03 (d, *J* = 6.7 Hz, 3H), 1.04-1.10 (m, 2H), 2.15-2.25 (m, 1H), 2.35-2.44 (m, 1H), 2.91 (s, 3H), 3.84-3.95 (m, 2H), 4.52 (dd, *J* = 27.5, 12.3 Hz, 2H), 7.03 (t, *J* = 7.5 Hz, 1H), 7.16-7.32 (m, 6H), 8.03 (d, *J* = 7.5 Hz, 1H), 8.91 (s, 1H), 12.28 (br s, 1H)

Step8 : 3-(5-(8-Cyclopropyl-2-methyl-9H-pyrido[2,3-b]indol-3-yl)-1,3,4-oxadiazol-2-yl)-4-methylpentan-1-ol (**119**)

A solution of **118** (230 mg, 4.79 x 10^{-1} mmol) in THF (3.0 mL) was treated with ASCA-2 (120 mg) and stirred under 1.0 atm of hydrogen at room temperature for 2 days. After removal of the catalyst by Celite$^®$ filtration and concentration, the residue was purified by silica gel column chromatography (eluted with n-hexane/EtOAc = 67/33 (v/v)) to give the title compound **119** (97.0 mg, 52% yield).

1H-NMR (400 MHz, CDCl$_3$) δ: 0.79-0.84 (m, 2H), 1.02 (d, J = 6.7 Hz, 3H), 1.08 (d, J = 6.7 Hz, 3H), 1.04-1.11 (m, 2H), 2.06-2.24 (m, 4H), 3.02 (s, 3H), 3.16-3.24 (m, 1H), 3.65-3.81 (m, 2H), 7.21-7.26 (m, 2H), 7.87-7.93 (m, 1H), 8.79 (s, 1H), 9.45 (br s, 1H)

Step9 : 3-(5-(8-Cyclopropyl-2-methyl-9H-pyrido[2,3-b]indol-3-yl)-1,3,4-oxadiazol-2-yl)-4-methylpentanal (**120**)

To a solution of **119** (97.0 mg, 2.48 x 10^{-1} mmol) in CHCl$_3$ (2.0 mL) was added Dess-Martin Periodinane (158 mg, 3.73 x 10^{-1} mmol), and the mixture was stirred at room temperature for 4 h. The reaction was quenched with saturated aqueous NaHCO$_3$ solution. The mixture was extracted with CHCl$_3$, and the organic layer was washed with aqueous Na$_2$CO$_3$ and brine. After dried over Na$_2$SO$_4$ and filtration, the solvent was evaporated *in vacuo* to give the title compound **120** (108 mg, overweight) as a crude product. That was used to the next step without further purification.

Step10 : 3-(5-(8-Cyclopropyl-2-methyl-9H-pyrido[2,3-b]indol-3-yl)-1,3,4-oxadiazol-2-yl)-4-methylpentanoic acid (**121**)

To the solution of **120** (108 mg, approx. 2.5 x 10^{-1} mmol) in 8:2:1 THF-*t*-BuOH-H$_2$O (5.5 mL) was added 2-methyl-2-butene (300 μg, 2.78 mmol), NaH$_2$PO$_4$ (130 mg, 8.33 x 10^{-1} mmol) and NaClO$_2$ (75.0 mg, 8.33 x 10^{-1} mmol), and the mixture was stirred at room temperature for 1.5 h. The reaction was quenched with aqueous Na$_2$S$_2$O$_4$ solution, and then acidified by adding 6 M aqueous HCl solution under stirring. The mixture was extracted with EtOAc, and the organic layer was washed with brine. After dried over MgSO$_4$ and filtration, the solvent was evaporated *in vacuo*. The residue was resolved into CHCl$_3$, and

the insoluble residue was removed by filtration. The organic layer was concentrated to give the title compound **121** (132 mg, overweight).

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.76-0.84 (m, 2H), 0.94 (d, J = 6.7 Hz, 3H), 0.99 (d, J = 6.7 Hz, 3H), 1.04-1.12 (m, 2H), 2.05-2.23 (m, 1H), 2.30-2.47 (m, 1H), 2.93 (s, 3H), 2.74-2.97 (m, 2H), 3.32-3.45 (m, 1H), 7.03 (d, J = 7.7 Hz, 1H), 7.18 (t, J = 7.7 Hz, 1H), 8.07 (d, J = 7.7 Hz, 1H), 8.96 (s, 1H), 12.29 (s, 1H), 12.34 (br s, 1H)

Step11 : *N*-Benzyl-3-(5-(8-cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indol-3-yl)-1,3,4-oxadiazol-2-yl)-4-methylpentanamide (**33**)

To a solution of **121** (25.0 mg, approx. 4.7 x 10^{-2} mmol) and benzylamine (15.0 μL, 1.37 x 10^{-2} mmol) in CHCl$_3$ (2.0 mL) was added WSC·HCl (20.0 mg, 1.04 x 10^{-1} mmol) and HOBt·H$_2$O (16.0 mg, 1.04 x 10^{-1} mmol), and the mixture was stirred at room temperature for 1 h. The mixture was quenched with saturated aqueous NaHCO$_3$ solution and extracted with CHCl$_3$. The organic layer was dried over MgSO$_4$. After filtration and concentration, the residue was purified by preparative TLC (*n*-hexane/EtOAc = 1/2 (v/v)) twice to give the title compound **33** (11.0 mg, 51%, 3 steps).

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.76-0.84 (m, 2H), 0.94 (d, J = 6.9 Hz, 3H), 1.01 (d, J = 6.9 Hz, 3H), 1.03-1.12 (m, 2H), 2.06-2.19 (m, 1H), 2.35-2.45 (m, 1H), 2.68-2.90 (m, 2H), 2.93 (s, 3H), 3.45-3.53 (m, 1H), 4.25 (ddd, J = 35.3, 15.4, 5.8 Hz, 2H), 7.03 (d, J = 7.7 Hz, 1H), 7.11-7.23 (m, 6H), 8.05 (d, J = 7.7 Hz, 1H), 8.56 (t, J = 7.7 Hz, 1H), 8.93 (s, 1H), 12.28 (br s, 1H)

^{13}C-NMR (400 MHz, DMSO-d_6) δ: 8.1 (2C), 10.4, 19.1 (2C), 20.0, 25.1, 30.6, 35.3, 41.9, 110.2, 113.5, 118.4, 119.6, 120.4, 121.5, 126.5, 126.9 (2C), 127.0, 128.0 (2C), 129.0, 138.9, 139.2, 152.3, 154.0, 164.0, 167.2, 169.9

HRMS (ESI, m/z, MH+) Calcd for C$_{30}$H$_{32}$O$_2$N$_5$: 494.2551, Found: 494.2549

Synthesis of compound 34

Step1 : (*S*)-4-Benzyl-3-(3-methylbutanoyl)oxazolidin-2-one (**124**)

To a solution of isovaleric acid (**122**, 10.0 g, 5.65 x 10 mmol) and (*S*)-4-benzyl-2-oxazolidinone (**123**, 7.40 mL, 6.78 x 10 mmol) in MeCN (100 mL) were added DMAP (3.40 g, 2.79 x 10 mmol) and WSC·

HCl (12.0 g, 6.28 x 10 mmol), and the mixture was stirred at room temperature for 19 h. The reaction was quenched with saturated aqueous $NaHCO_3$ solution. The mixture was extracted with toluene, and the organic layer was washed with H_2O, 1 M aqueous HCl solution, H_2O and brine. After drying over $MgSO_4$ and filtration, the solvent was evaporated *in vacuo* to give the title compound **124** (15.0 g, quant.), which was applied to the next step without further purification.

^{1}H-NMR (400 MHz, CDCl$_3$) δ: 1.01 (d, J = 6.6 Hz, 3H), 1.03 (t, J = 6.6 Hz, 3H), 2.17-2.28 (m, 1H), 2.75 (dd, J = 13.2, 9.6 Hz, 1H), 2.84 (ddd, J = 44.9, 16.2, 6.9 Hz, 2H), 3.32 (dd, J = 13.2, 3.4 Hz, 1H), 4.13-4.22 (m, 2H), 4.65-4.72 (m, 1H), 7.12-7.38 (m, 5H)

Step2 : *tert*-Butyl (*S*)-3-((*S*)-4-benzyl-2-oxooxazolidine-3-carbonyl)-4-methylpentanoate (**125**)

The compound **124** (2.00 g, approx. 7.7 mmol) was dissolved in THF (20 mL). To the solution, 1.0 M LiHMDS in THF (8.43 mL, 8.43 mmol) was added at -78 °C. *tert*-Butyl bromoacetate (1.66 mL, 1.15 x 10 mmol) was added to the reactant mixture, and then the mixture was stirred at room temperature for 3.5 h. The mixture was quenched with aqueous NH_4Cl solution and extracted with AcOEt, and the organic layer was washed with aqueous $NaHCO_3$ solution and brine, and dried over $MgSO_4$. After filtration and concentration, the residue was crystallize in CHCl$_3$-*n*-hexane. The precipitated solid was collected by filtration, washed with *n*-hexane, and dried to give the title compound **125** (1.52 g, 53% yield, 2 steps).

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.80 (d, J = 6.8 Hz, 3H), 0.92 (d, J = 6.8 Hz, 3H), 1.39 (s, 9H), 1.89-2.00 (m, 1H), 2.45 (dd, J = 16.5, 3.7 Hz, 1H), 2.64 (dd, J = 16.5, 11.6 Hz, 1H), 2.85 (dt, J = 13.4, 8.0 Hz, 1H), 2.99 (dd, J = 13.4, 3.0 Hz, 1H), 4.00 (ddd, J = 11.6, 3.7, 3.0 Hz, 1H), 4.14 (dd, J = 8.8, 2.5 Hz, 1H), 4.32 (t, J = 8.0 Hz, 1H), 4.65 (tt, J = 8.0, 2.5 Hz, 1H), 7.23-7.33 (m, 5H)

Step3 : (*S*)-4-(*tert*-Butoxy)-2-isopropyl-4-oxobutanoic acid (**126**)

The compound **125** (1.52 g, 4.05 mmol) was dissolved in THF (40 mL). To the solution, 30% aqueous H_2O_2 solution (1.65 mL, 1.62 x 10 mmol) and 0.4 M aqueous LiOH solution (2.03 x 10 mL, 8.10 mmol) was added at 0 °C, and then the mixture was stirred at room temperature for 1 h. After cooling to 0 °C, the reactant mixture was quenched with aqueous $Na_2S_2O_3$ solution. After concentration of the organic layer, the aqueous layer was washed with CHCl$_3$ three times, and then acidified by adding 6 N HCl solution. The mixture was extracted with EtOAc, and the organic layer was washed with H_2O and brine. After drying

over MgSO$_4$ and filtration, the solvent was evaporated *in vacuo* to give the title compound **126** (840 mg, 96%).

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.85 (d, *J* = 6.8 Hz, 3H), 0.88 (d, *J* = 6.8 Hz, 3H), 1.37 (s, 9H), 1.82-1.94 (m, 1H), 2.30 (dd, *J* = 16.0, 3.9 Hz, 1H), 2.36-2.49 (m, 2H), 12.16 (br s, 1H)

Step4 ： *tert*-Butyl (*S*)-3-(2-(8-cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indole-3-carbonyl)hydrazine-1-carbonyl)-4-methylpentanoate (**127**)

To a solution of the hydrochloride salt of **104** (500 mg, 1.58 mmol) and (*S*)-4-(*tert*-butoxy)-2-isopropyl-4-oxobutanoic acid (**126**, 512 mg, 2.37 mmol) in DMF (5.0 mL) was added WSC·HCl (290 mg, 1.89 mmol), HOBt·H$_2$O (363 mg, 1.90 mmol) and *i*-Pr$_2$EtN (670 μL, 3.94 mmol), and the mixture was stirred at room temperature for 2.5 h. The reactant solution was dropped into saturated aqueous NaHCO$_3$ solution under stirring. The precipitated solid was collected by filtration, washed with H$_2$O, and dried to give the title compound **127** (716 mg, 95% yield).

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.77-0.83 (m, 2H), 0.94 (d, *J* = 6.7 Hz, 3H), 0.99 (d, *J* = 6.7 Hz, 3H), 1.03-1.10 (m, 2H), 1.32 (s, 9H), 2.07-2.18 (m, 1H), 2.35-2.44 (m, 1H), 2.78 (dd, *J* = 16.3, 5.2 Hz, 1H), 2.86 (dd, *J* = 16.3, 10.0 Hz, 1H), 2.93 (s, 3H), 3.33-3.40 (m, 1H), 7.03 (d, *J* = 7.6 Hz, 1H), 7.18 (t, *J* = 7.6 Hz, 1H), 8.06 (d, *J* = 7.6 Hz, 1H), 8.96 (s, 1H), 12.30 (s, 1H)

Step5 ： *tert*-Butyl (*S*)-3-(5-(8-cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indol-3-yl)-1,3,4-oxadiazol-2-yl)-4-methylpentanoate (**128**)

To a solution of **127** (716 mg, 1.50 mmol) in THF (14 mL) was added Burgess reagent (1.07 g, 4.49 mmol), and the mixture was stirred at room temperature for 30 min. The mixture was diluted with H$_2$O and extracted with EtOAc. The organic layer was washed twice with H$_2$O and brine, and dried over Na$_2$SO$_4$. After filtration and concentration, the residue was purified by silica gel column chromatography (eluted with *n*-hexane/EtOAc = 85/15 to 67/33 (v/v)) to give the title compound **128** (432 mg, 63% yield).

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.77-0.83 (m, 2H), 0.94 (d, *J* = 6.8 Hz, 3H), 0.99 (d, *J* = 6.8 Hz, 3H), 1.04-1.10 (m, 2H), 1.32 (s, 9H), 2.08-2.18 (m, 1H), 2.35-2.44 (m, 1H), 2.78 (dd, *J* = 16.2, 5.3 Hz, 1H), 2.86 (dd, *J* = 16.2, 10.2 Hz, 1H), 2.93 (s, 3H), 3.33-3.40 (m, 1H), 7.02 (d, *J* = 7.4 Hz, 1H), 7.18 (t, *J* = 7.4 Hz, 1H), 8.06 (d, *J* = 7.4 Hz, 1H), 8.96 (s, 1H), 12.30 (s, 1H), 12.38 (br s, 1H)

Step6 : (*S*)-3-(5-(8-Cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indol-3-yl)-1,3,4-oxadiazol-2-yl)-4-methylpentanoic acid (**129**)

The compound **128** (432 mg, 9.38 x 10^{-1} mmol) was dissolved in TFA (2.0 mL), and the mixture was stirred at room temperature for 1 h. The reaction solution was concentrated *in vacuo* and azeotroped twice with toluene. The residue was purified by twice precipitation in CHCl$_3$/*n*-hexane/EtOAc (1/1/1 (v/v)) and MeOH/H$_2$O (1/2 (v/v). The precipitated solid was collected by filtration and dried to give the title compound **129** (311 mg, 82% yield).

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.77-0.84 (m, 2H), 0.94 (d, *J* = 6.9 Hz, 3H), 0.99 (d, *J* = 6.9 Hz, 3H), 1.03-1.11 (m, 2H), 2.09-2.20 (m, 1H), 2.35-2.44 (m, 1H), 2.79 (dd, *J* = 16.7, 4.8 Hz, 1H), 2.93 (s, 3H), 2.86-2.94 (m, 1H), 3.34-3.45 (m, 1H), 7.02 (d, *J* = 7.6 Hz, 1H), 7.18 (t, *J* = 7.6 Hz, 1H), 8.07 (d, *J* = 7.6 Hz, 1H), 8.97 (s, 1H), 12.29 (s, 1H), 12.38 (br s, 1H)

Step7：(*S*)-3-(5-(8-Cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indol-3-yl)-1,3,4-oxadiazol-2-yl)-4-methyl-*N*-((*R*)-1-phenylethyl)pentanamide (**34**)

To the solution of **129** (20.0 mg, 4.94 x 10^{-2} mmol) and (*R*)-1-phenylethan-1-amine (9.50 µL, 7.45 x 10^{-2} mmol) in DMF (1.0 mL) was added WSC·HCl (14.0 mg, 7.30 x 10^{-2} mmol) and HOBt·H$_2$O (12.0 mg, 7.84 x 10^{-2} mmol), and the reactant mixture was stirred at room temperature for 1.5 h. The reactant solution was dropped into saturated aqueous NaHCO$_3$ solution under stirring. The precipitated solid was collected by filtration, washed with H$_2$O, and dried to give the title compound **34** (25.0 mg, 99% yield).

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.77-0.83 (m, 2H), 0.94 (d, *J* = 6.9 Hz, 3H), 1.00 (d, *J* = 6.9 Hz, 3H), 1.03-1.11 (m, 2H), 1.32 (d, *J* = 7.2 Hz, 3H), 2.05-2.16 (m, 1H), 2.35-2.44 (m, 1H), 2.66-2.74 (m, 2H), 2.84 (dd, *J* = 15.1, 10.1 Hz, 1H), 2.90 (s, 3H), 3.40-3.48 (m, 1H), 4.81-4.91 (m, 1H), 7.00-7.12 (m, 4H), 7.14-7.21 (m, 3H), 8.04 (dd, *J* = 7.8, 1.0 Hz, 1H), 8.92 (s, 1H), 12.28 (s, 1H)

^{13}C-NMR (400 MHz, DMSO-d_6) δ: 8.1 (2C), 10.5, 19.2 (2C), 20.1, 22.5, 25.2, 30.8, 35.5, 47.7, 110.3, 113.5, 118.4, 119.7, 120.4, 121.5, 125.6 (2C), 126.3, 127.1, 127.9 (2C), 129.1, 139.0, 144.4, 152.4, 154.1, 164.0, 167.2, 169.1

$[α]^{25}_D$ = +44.0 (*c* = 0.10, DMSO)

HRMS (ESI, m/z, MH+) Calcd for C$_{31}$H$_{34}$O$_2$N$_5$: 508.2707, Found: 508.2709

Synthesis of compound 35

A microwave vial was charged with **70** (150 mg, 5.82 x 10^{-1} mmol), pyrrolidine (49.7 mg, 6.98 x 10^{-1} mmol), *i*-Pr$_2$NEt (301 mg, 2.328 mmol), and NMP (3.00 mL). The vial was heated to 140 °C and then irradiated with the microwave apparatus for 2 h. The mixture was diluted with H$_2$O and extracted with AcOEt, and the organic layer was washed with H$_2$O and brine, and dried over Na$_2$SO$_4$. After filtration and concentration, the residue was purified by flash chromatography (Biotage-SNAP Ultra 100 g, eluted with *n*-hexane/EtOAc = 50/50 (v/v)) to give the title compound **35** (117 mg, 69% yield).

^{1}H-NMR (400 MHz, DMSO-*d*$_6$) δ: 0.70-0.73 (m, 2H), 0.97-1.02 (m, 2H), 1.90-1.94 (m, 4H), 2.28-2.35 (m, 1H), 2.44 (s, 3H), 3.82-3.85 (m, 4H), 6.82 (d, *J* = 7.7 Hz, 1H), 7.03 (t, *J* = 7.7 Hz, 1H), 7.81 (d, *J* = 7.7 Hz, 1H), 11.88 (s, 1H)

^{13}C-NMR (400 MHz, DMSO-*d*$_6$) δ: 7.8 (2C), 10.4, 25.1 (2C), 25.8, 49.4 (2C), 94.6, 118.5, 119.2, 119.5, 120.1, 126.2, 136.1, 156.9, 157.5, 161.8

HRMS (ESI, m/z, MH+) Calcd for C$_{18}$H$_{21}$N$_4$: 293.1761, Found: 293.1761

Synthesis of compound 36 – 42

8-Cyclopropyl-4-(2-ethylpyrrolidin-1-yl)-2-methyl-9*H*-pyrimido[4,5-*b*]indole   (**36**)

Compound **36** was synthesized in the same procedure described above for compound **35**, except using the 2-ethyl pyrrolidine instead of the pyrrolidine. Starting from the compound **70** (150 mg, 5.82 x 10 mmol), the title compound **36** (175 mg, 94% yield) was obtained.

^{1}H-NMR (400 MHz, DMSO-*d*$_6$) δ: 0.70-0.74 (m, 2H), 0.87 (t, *J* = 7.4 Hz, 3H), 0.98-1.03 (m, 2H), 1.49 (dq, *J* = 22.0, 6.7 Hz, 1H), 1.58-1.71 (m, 2H), 1.85-1.95 (m, 2H), 2.10-2.18 (m, 1H), 2.29-2.36 (m, 1H), 2.46 (s, 3H), 3.75-3.79 (m, 1H), 3.96-4.02 (m, 1H), 4.50-4.57 (m, 1H), 6.83 (d, *J* = 7.7 Hz, 1H), 7.06 (t, *J* = 7.7 Hz, 1H), 7.70 (d, *J* = 7.7 Hz, 1H), 11.89 (s, 1H)

^{13}C-NMR (400 MHz, DMSO-*d*$_6$) δ: 7.8, 7.9, 9.8, 10.4, 25.2, 25.9, 26.3, 29.6, 51.9, 58.7, 95.4, 118.5, 119.1, 119.5, 120.1, 126.2, 136.2, 157.2, 157.7, 161.7

HRMS (ESI, m/z, MH+) Calcd for C$_{20}$H$_{25}$N$_4$: 321.2074, Found: 321.2076

(*S*)-(1-(8-Cyclopropyl-2-methyl-9*H*-pyrimido[4,5-*b*]indol-4-yl)pyrrolidin-2-yl)methanol   (**37**)

Compound **37** was synthesized in the same procedure described above for compound **35**, except using the (*S*)-2-pyrrolidine methanol instead of the pyrrolidine. Starting from the compound **70** (150 mg, 5.82 x 10 mmol), the title compound **37** (111 mg, 59% yield) was obtained.

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.70-0.77 (m, 2H), 0.99-1.04 (m, 2H), 1.24-1.25 (m, 2H) 1.87-2.21 (m, 3H), 2.30-2.37 (m, 1H), 2.47 (s, 3H), 3.53-3.59 (m, 1H), 3.68-3.73 (m, 1H), 3.85 (t, *J* = 7.6 Hz, 1H), 4.65-4.71 (m, 1H), 4.96 (t, *J* = 5.1 Hz, 1H), 6.84 (d, *J* = 7.8 Hz, 1H), 7.06 (t, *J* = 7.8 Hz, 1H), 7.76 (d, *J* = 7.8 Hz, 1H), 11.93 (s, 1H)

^{13}C-NMR (400 MHz, DMSO-d_6) δ: 7.8, 7.9, 10.4, 24.9, 25.7, 27.6, 51.9, 59.4, 62.6, 95.1, 118.6, 119.4, 119.5, 120.1, 126.2, 136.2, 157.3, 157.7, 161.6

$[α]^{25}_D$ = -272 (*c* = 1.00, DMSO)

HRMS (ESI, m/z, MH+) Calcd for $C_{19}H_{23}ON_4$: 323.1866, Found: 323.1866

(*R*)-(1-(8-Cyclopropyl-2-methyl-9*H*-pyrimido[4,5-*b*]indol-4-yl)pyrrolidin-2-yl)methanol   (**38**)

Compound **38** was synthesized in the same procedure described above for compound **35**, except using the (*R*)-2-pyrrolidine methanol instead of the pyrrolidine. Starting from the compound **70** (150 mg, 5.82 x 10 mmol), the title compound **38** (88.6 mg, 47% yield) was obtained.

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.70-0.77 (m, 2H), 0.99-1.04 (m, 2H), 1.69 (td, *J* = 17.6, 9.2 Hz, 1H), 1.87-2.21 (m, 3H), 2.30-2.37 (m, 1H), 2.47 (s, 3H), 3.53-3.59 (m, 1H), 3.68-3.73 (m, 1H), 3.85 (t, *J* = 7.6 Hz, 1H), 4.03 (td, *J* = 9.2, 6.4 Hz, 1H), 4.65-4.71 (m, 1H), 4.96 (t, *J* = 5.2 Hz, 1H), 6.84 (d, *J* = 7.8 Hz, 1H), 7.06 (t, *J* = 7.8 Hz, 1H), 7.76 (d, *J* = 7.8 Hz, 1H), 11.93 (s, 1H)

^{13}C-NMR (400 MHz, DMSO-d_6) δ: 7.8, 7.9, 10.4, 24.9, 25.7, 27.6, 51.9, 59.4, 62.6, 95.1, 118.6, 119.4, 119.5, 120.1, 126.2, 136.2, 157.3, 157.7, 161.6

$[α]^{25}_D$ = +284 (*c* = 1.00, DMSO)

HRMS (ESI, m/z, MH+) Calcd for $C_{19}H_{23}ON_4$: 323.1866, Found: 323.1867

((2*S*,4*S*)-1-(8-Ccyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indol-4-yl)-4-phenylpyrrolidin-2-yl)methanol (**39**)

Compound **39** was synthesized in the same procedure described above for compound **35**, except using pyrrolidine **130** instead of the pyrrolidine. Starting from the compound **70** (20.0 mg, 7.76 x 10^{-2} mmol), the title compound **39** (13.0 mg, 42% yield) was obtained.

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.67-0.77 (m, 2H), 0.96-1.05 (m, 2H), 2.13-2.22 (m, 1H), 2.28-2.37 (m, 1H), 2.40-2.48 (m, 1H), 2.48 (s, 3H), 3.84 (dd, 1H, J = 9.9, 5.3 Hz, 1H), 4.57 (dd, J = 10.1, 7.1 Hz, 1H), 4.87-4.94 (m, 1H), 4.98 (t, J = 5.3 Hz, 1H), 6.84 (d, J = 7.8 Hz, 1H), 7.05 (t, J = 7.8 Hz, 1H), 7.11-7.27 (m, 6H), 7.79 (d, J = 7.8 Hz, 1H), 11.96 (s, 1H)

^{13}C-NMR (400 MHz, DMSO-d_6) δ: 7.7, 7.8, 10.3, 25.7, 34.5, 41.7, 57.3, 59.0, 62.1, 94.8, 118.6, 119.2, 119.3, 120.1, 126.2 (2C), 126.6 (2C), 128.4 (2C), 136.1, 143.3, 156.8, 157.7, 161.7

[α]^{25}D = -321 (c = 0.40, DMSO)

HRMS (ESI, m/z, MH+) Calcd for C$_{25}$H$_{27}$N$_4$O: 399.2179, Found: 399.2181

((2*S*,4*R*)-4-Benzyl-1-(8-cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indol-4-yl)pyrrolidin-2-yl)methanol (**40**)

Compound **40** was synthesized in the same procedure described above for compound **35**, except using pyrrolidine **131** instead of the pyrrolidine. Starting from the compound **70** (20.0 mg, 7.76 x 10^{-2} mmol), the title compound **40** (26.0 mg, 81% yield) was obtained.

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.75 (td, J = 5.4, 3.2 Hz, 2H), 1.03 (ddd, J = 9.1, 5.4, 3.2 Hz, 2H), 1.81-1.90 (m, 1H), 2.03-2.11 (m, 1H), 2.31-2.40 (m, 1H), 2.54 (s, 3H), 2.53-2.57 (m, 1H), 2.65-2.76 (m, 1H), 3.58 (dd, J = 10.7, 6.0 Hz, 1H), 3.66 (dd, J = 10.7, 4.5 Hz, 1H), 3.73-3.65 (m, 1H), 4.21 (dd, J = 9.8, 6.4 Hz, 1H), 4.77-4.85 (m, 1H), 6.88 (d, J = 7.4 Hz, 1H), 7.18-7.25 (m, 2H), 7.70 (d, J = 8.1 Hz, 1H), 12.28 (br s, 1H)

^{13}C-NMR (400 MHz, DMSO-d_6) δ: 8.0, 8.1, 10.4, 24.5, 32.2, 38.2, 38.7, 56.5, 59.4, 62.3, 94.9, 119.1, 119.3, 119.5, 120.8, 126.0, 126.8, 128.2 (2C), 128.7 (2C), 136.2, 140.2, 155.7 (br, 2C), 159.9 (br)

[α]^{25}$_D$ = -295 (*c* = 0.20, DMSO)

HRMS (ESI, m/z, MH+) Calcd for C$_{26}$H$_{29}$N$_4$O: 413.2336, Found: 413.2338

((2*S*,4*S*)-4-Benzyl-1-(8-cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indol-4-yl)pyrrolidine-2,4-diyl)dimethanol (**41**)

Compound **41** was synthesized in the same procedure described above for compound **35**, except using pyrrolidine **132** instead of the pyrrolidine. Starting from the compound **70** (20.0 mg, 7.76 x 10^{-2} mmol), the title compound **41** (19.0 mg, 55% yield) was obtained.

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.71-0.78 (m, 2H), 0.98-1.07 (m, 2H), 1.20-1.32 (m, 1H), 1.20-1.32 (m, 1H), 1.71-1.85 (m, 2H), 2.28-2.40 (m, 2H), 2.48 (s, 3H), 3.32-3.43 (m, 2H), 3.53-3.60 (m, 1H), 3.62-3.68 (m, 1H), 3.68 (d, J = 10.5 Hz, 1H), 3.94 (d, J = 10.5 Hz, 1H), 4.76-4.85 (m, 1H), 4.87 (t, J = 5.4 Hz, 1H), 4.95 (t, J = 5.0 Hz, 1H), 6.84 (d, J = 7.6 Hz, 1H), 6.89-6.95 (m, 2H), 7.02-7.10 (m, 4H), 7.68 (d, J = 7.6 Hz, 1H), 11.96 (s, 1H)

^{13}C-NMR (400 MHz, DMSO-d_6) δ: 7.8 (2C), 10.4, 25.7, 33.4, 39.1, 48.2, 57.9, 59.8, 62.8, 64.2,106.0, 118.4, 119.1, 119.5, 120.2, 125.8, 126.5, 127.6 (2C), 129.9 (2C), 130.2, 137.0, 138.2, 157.6 (2C), 161.8

$[\alpha]^{25}_D$ = -344 (c = 0.04, DMSO)

HRMS (ESI, m/z, MH+) Calcd for $C_{27}H_{31}N_4O_2$: 443.2442, Found: 443.2438

(3S,5S)-3-Benzyl-1-(8-cyclopropyl-2-methyl-9H-pyrido[2,3-b]indol-4-yl)-5-(hydroxymethyl)pyrrolidine-3-carboxamide (**42**)

Compound **42** was synthesized in the same procedure described above for compound **35**, except using pyrrolidine **133** instead of the pyrrolidine. Starting from the compound **70** (103 mg, 4.00 x 10^{-1} mmol), the title compound **42** (34.0 mg, 19% yield) was obtained.

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.71-0.79 (m, 2H), 0.99-1.08 (m, 2H), 2.03-2.27 (m, 2H), 2.30-2.41 (m, 1H), 2.67 (s, 3H), 2.83-3.06 (m, 1H), 3.52-3.70 (m, 2H), 3.85 (d, J = 10.5 Hz, 1H), 4.13 (d, J = 10.5 Hz, 1H), 4.81-4.94 (br m, 2H), 6.80-6.87 (m, 3H), 7.00-7.10 (m, 4H), 7.16 (br s, 1H), 7.19-7.38 (m, 2H), 7.47 (br s, 1H), 7.65 (d, J = 8.1 Hz, 1H), 11.98 (s, 1H)

^{13}C-NMR (400 MHz, DMSO-d_6) δ: 7.8, 7.9, 10.4, 25.8, 34.5, 40.0, 53.8, 57.5, 58.1, 62.2, 95.4, 118.6, 119.1, 119.3, 120.1, 126.1 (2C), 127.7 (2C), 129.2 (2C), 136.2, 137.2, 157.6, 157.7, 161.8, 174.9

$[\alpha]^{25}_D$ = -359 (c = 0.30, DMSO)

HRMS (ESI, m/z, MH+) Calcd for $C_{27}H_{30}N_2O_5$: 456.2394, Found: 456.2397

Synthesis of pyrrolidine 130

Step1 : *tert*-Butyl (2*S*,4*S*)-2-(hydroxymethyl)-4-phenylpyrrolidine-1-carboxylate (**135**)

To a solution of (2*S*,4*S*)-1-(*tert*-butoxycarbonyl)-4-phenylpyrrolidine-2-carboxylic acid (**134**, 100 mg, 3.43 x 10^{-1} mmol) in THF (1.0 mL) was added 1.0 M BH$_3$-THF in THF (1.00 mL, 1.00 mmol) at room temperature, and then the mixture was stirred at 80 °C for 20 minutes. The reactant mixture was quenched with saturated aqueous NaHCO$_3$ solution, and the mixture was extracted with EtOAc. The extract was washed with H$_2$O and brine, and dried over Na$_2$SO$_4$. The solvent was evaporated *in vacuo* to give the title compound **135** (105 mg, overweight), which was advanced to the next step without further purification.

^{1}H-NMR (400 MHz, CDCl$_3$) δ: 1.48 (s, 9H), 1.96-2.07 (br m, 1H), 2.18 (dd, *J* = 20.3, 10.6 Hz, 1H), 3.34-3.52 (br m, 2H), 3.66-3.85 (m, 3H), 4.15-4.27 (br m, 2H), 7.19-7.25 (m, 3H), 7.29-7.36 (m, 2H)

Step2 : ((2*S*,4*S*)-4-Phenylpyrrolidin-2-yl)methanol (**130**)

Compound **135** (105 mg, approx. 3.4 x 10^{-1} mmol) was treated with 4 M HCl in 1,4-dioxane (500 μL) and stirred at room temperature for 1.5 h. The reaction mixture was evaporated and dried *in vacuo* to give the hydrochloride salt of the title compound **130** (75.0 mg, 94% yield (2 steps)).

^{1}H-NMR (400 MHz, CDCl$_3$) δ: 1.48 (s, 9H), 1.96-2.07 (br m, 1H), 2.18 (dd, *J* = 20.3, 10.6 Hz, 1H), 3.34-3.52 (br m, 2H), 3.66-3.85 (m, 3H), 4.15-4.27 (br m, 2H), 7.19-7.25 (m, 3H), 7.29-7.36 (m, 2H)

Synthesis of pyrrolidine 131

Step1 : *tert*-Butyl (*S*)-2-oxo-5-((trityloxy)methyl)pyrrolidine-1-carboxylate (**137**)

To a solution of (*S*)-5-((trityloxy)methyl)pyrrolidin-2-one (**136**, 9.00 g, 2.52 x 10 mmol) in DMF (50 mL) were added Boc$_2$O (6.60 mL, 3.02 x 10 mmol) and DMAP (3.70 g, 3.03 x 10 mmol) at room temperature. The reaction mixture was stirred at 70 °C for 4 h. After cooling to room temperature, the reaction was quenched by an addition of TMEDA (1.63 mL, 1.25 x 10 mmol). After stirring for 30 minutes at room temperature, the mixture was diluted with 1 M HCl solution and extracted with EtOAc. The organic layer was washed with H$_2$O and brine, and dried over Na$_2$SO$_4$. Evaporation of the solvent *in vacuo* gave the title compound **137** (11.9 g, overweight), which was applied to the next step without further purification.

^{1}H-NMR (400 MHz, CDCl$_3$) δ: 1.44 (s, 9H), 1.90-1.99 (m, 1H), 2.05-2.17 (m, 1H), 2.42 (ddd, J = 17.7, 9.8, 1.8 Hz, 1H), 2.80 (ddd, J = 19.3, 9.8, 8.2 Hz, 1H), 3.11 (dd, J = 9.5, 2.8 Hz, 1H), 3.48 (dd, J = 9.5, 4.6 Hz, 1H), 4.22-4.30 (m, 1H), 7.18-7.41 (m, 15H)

Step2 : *tert*-Butyl (3*R*,5*S*)-3-benzyl-2-oxo-5-((trityloxy)methyl)pyrrolidine-1-carboxylate (**138**)

To a solution of **137** (250 mg, approx. 5.4 x 10^{-1} mmol) in THF (7.0 mL) was added 1 M LiHMDS in THF (655 μL, 6.55 x 10^{-1} mmol) at -78 °C. The reaction mixture was stirred at -30 °C for 15 minutes before adding benzyl bromide (84.0 μL, 7.07 x 10^{-1} mmol) at -78 °C and stirred 1 h at room temperature. The mixture was diluted with saturated NH$_4$Cl solution and extracted with EtOAc. The organic layer was washed with H$_2$O and brine, and dried over Na$_2$SO$_4$. After filtration and concentration, the residue was purified by flash chromatography (Biotage-SNAP Ultra 25g, eluted with *n*-hexane/EtOAc = 90/10 to 60/40 (v/v)) to give the title compound **138** (232 mg, 77% yield (2 steps)).

^{1}H-NMR (400 MHz, CDCl$_3$) δ: 1.43 (s, 9H), 1.78 (td, J = 12.4, 8.8 Hz, 1H), 1.78 (dd, J = 12.4, 8.8 Hz, 1H), 1.94 (dd, J = 12.4, 8.8 Hz, 1H), 2.57 (dd, J = 14.0, 9.9 Hz, 1H), 3.09 (dd, J = 19.5, 3.0 Hz, 1H), 3.11-3.21 (m, 1H), 3.33 (dd, J = 14.0, 4.0 Hz, 1H), 3.41 (dd, J = 9.5, 4.6 Hz, 1H), 4.11-4.16 (m, 1H), 7.14-7.35 (m, 20H)

Step3 : *tert*-Butyl (2*S*,4*R*)-4-benzyl-2-((trityloxy)methyl)pyrrolidine-1-carboxylate (**139**)

To a solution of **138** (232 mg, 4.23 x 10^{-1} mmol) in THF (2.0 mL) was added 1.0 M BH$_3$-THF in THF (2.10 mL, 2.10 mmol) at room temperature, and then the mixture was stirred at 80 °C for 30 minutes. The reactant mixture was quenched with saturated aqueous NaHCO$_3$ solution and H$_2$O at 0 °C, and the mixture was extracted with EtOAc. The extract was washed with H$_2$O and brine, and dried over Na$_2$SO$_4$. After filtration and concentration, the residue was purified by flash chromatography (Biotage-SNAP Ultra 25g, eluted with *n*-hexane/EtOAc = 100/0 to 70/30 (v/v)) to give the title compound **139** (175 mg, 78%).

^{1}H-NMR (400 MHz, CDCl$_3$) δ: 1.24-1.51 (m, 9H), 1.58-1.77 (m, 1H), 1.87-2.13 (m, 1H), 2.49-2.90 (m, 4H), 2.96-3.15 (m, 2H), 3.24-3.52 (m, 1H), 3.91-4.09 (m, 1H), 7.08-7.47 (m, 9H)

Step4 : ((2*S*,4*R*)-4-Benzylpyrrolidin-2-yl)methanol (**131**)

Compound **139** (175 mg, 3.28 x 10^{-1} mmol) was treated with 4 M HCl in 1,4-dioxane (800 μL) and stirred at room temperature for 2 h. The reaction mixture was evaporated and dried *in vacuo*, and the residue was decanted three times with *n*-hexane to give the hydrochloride salt of the title compound **131** (75.0 mg, overweight).

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.67-1.80 (m, 2H), 2.49-2.60 (m, 1H), 2.62-2.91 (m, 3H), 3.14-3.24 (m, 3H), 3.49 (dd, J =11.6, 7.3 Hz, 1H), 3.60 (t, J = 11.6, 4.2 Hz, 1H), 3.65-3.76 (m, 1H), 5.33 (br s, 1H), 7.19-7.26 (m, 3H), 7.28-7.34 (m, 2H), 8.69 (br s, 1H), 9.54 (br s, 1H)

Synthesis of pyrrolidine 132

Step1 : 1-(*tert*-Butyl) 3-methyl (3*R*,5*S*)-2-oxo-5-((trityloxy)methyl)pyrrolidine-1,3-dicarboxylate (**140**)

To a solution of **137** (3.60 g, approx. 6.6 mmol) in THF (50 mL) was added 0.93 M LiHMDS in THF (8.50 mL, 7.87 mmol) at -78 °C. The reaction mixture was stirred at -30 °C for 20 minutes before adding methyl chloroformate (755 μL, 9.83 mmol) at -78 °C followed by stirring 1 h at -30 °C. The mixture was diluted with H$_2$O and extracted with EtOAc. The organic layer was washed with H$_2$O and brine, and dried over Na$_2$SO$_4$. After filtration and concentration, the residue was purified by flash chromatography (Biotage-SNAP Ultra 50g, eluted with *n*-hexane/acetone = 90/10 to 65/35 (v/v)) to give the title compound **140** (2.70 g, 80% yield (2 steps)) as a mixture of diastereomers.

^{1}H-NMR (400 MHz, CDCl$_3$) δ: 1.42 (s, 4.5H), 1.47 (s, 4.5H), 2.11 (ddd, J = 13.0, 9.0, 1.0 Hz, 0.5H), 2.49-2.59 (m, 0.5H), 2.83 (dd, J = 14.1, 3.3 Hz, 0.5H), 2.92 (dd, J = 14.1, 8.4 Hz, 0.5H), 3.03-3.11 (m, 1H), 3.35 (dd, J = 9.0, 3.9 Hz, 0.5H), 3.54-3.60 (m, 0.5H), 3,79 (s, 1.5H), 3.81 (s, 1.5H), 3.79-3.84 (m, 1H), 3.92 (dd, J = 11.2, 9.1 Hz, 0.5H), 4.38-4.26 (m, 1H), 7.14-7.43 (m, 15H)

Step2 : *tert*-Butyl (2*S*,4*R*)-4-(hydroxymethyl)-2-((trityloxy)methyl)pyrrolidine-1-carboxylate (**141**)

To a solution of **140** (2.70 g, 5.24 mmol) in THF (10 mL) was added 1.0 M BH$_3$-THF in THF (30.0 mL, 2.62 x 10 mmol) at room temperature, and then the mixture was stirred at 80 °C for 2.5 h. The reactant was cooled at 0 °C and quenched with H$_2$O, and the mixture was extracted with EtOAc. The extract was washed with 1M HCl solution, saturated aqueous NaHCO$_3$ solution and brine, and dried over Na$_2$SO$_4$. After filtration and concentration, the residue was purified by flash chromatography (Biotage-SNAP Ultra 50g,

eluted with *n*-hexane/acetone = 90/10 to 65/35 (v/v)) to give the title compound **141** (1.15 g, 46% yield) as a mixture of diastereomers.

^{1}H-NMR (400 MHz, CDCl$_3$) δ: 1.32 (br s, 4.5H), 1.47 (br s, 4.5H), 1.59-1.99 (br m, 2H), 2.07-2.63 (br m, 2H), 2.88-3.74 (br m, 5H), 3.96-4.17 (br m, 1H), 7.17-7.46 (m, 15H)

Step3 : (3*R*,5*S*)-1-(*tert*-Butoxycarbonyl)-5-((trityloxy)methyl)pyrrolidine-3-carboxylic acid (**142**)

To a solution of **141** (1.15 g, 2.43 mmol) in 3:1 *t*-BuOH-H$_2$O (8.0 mL) were added 5% NaClO solution (362 μL, 2.42 x 10^{-1} mmol), 1-Me-AZADO (41.0 mg, 2.47 x 10^{-1} mmol) and 80% NaClO$_2$ (549 mg, 4.86 mmol) at 0 °C, and then the mixture was stirred for 1.5 h at room temperature. The reactant was quenched with Na$_2$SO$_3$ (918 mg, 7.28 mmol) and H$_2$O, and then the mixture was extracted with EtOAc. The extract was washed with brine, and dried over Na$_2$SO$_4$. Evaporation of the solvent *in vacuo* gave the title compound **142** (1.20 g, overweight), which was advanced to the next step without further purification.

Step4 : 3-Benzyl 1-(*tert*-butyl) (3*R*,5*S*)-5-((trityloxy)methyl)pyrrolidine-1,3-dicarboxylate (**143**)

To a solution of **142** (500 mg, approx. 1.0 mmol) in DMF (5.0 mL) were added K$_2$CO$_3$ (212 mg, 1.53 mmol) and benzyl bromide (146 μL, 1.23 mmol) at room temperature and stirred 1.5 h. The mixture was diluted with H$_2$O and extracted with EtOAc. The organic layer was washed with H$_2$O and brine, and dried over Na$_2$SO$_4$. After filtration and concentration, the residue was purified by flash chromatography (Biotage-SNAP Ultra 25g, eluted with *n*-hexane/EtOAc = 95/5 to 60/40 (v/v)) to give the title compound **143** (410 mg, 69% yield (2 steps)) as a mixture of diastereomers.

^{1}H-NMR (400 MHz, CDCl$_3$) δ: 1.31 (br s, 4.5H), 1.47 (br s, 4.5H), 1.81-1.89 (br m, 1H), 2.08-2.33 (br m, 1H), 2.96-3.43 (br m, 2H), 3.59-4.78 (br m, 3H), 3.93-4.18 (br m, 1H), 5.07-5.16 (m, 2H), 7.18-7.43 (m, 20H)

Step5 : 3-Benzyl 1-(*tert*-butyl) (3*S*,5*S*)-3-benzyl-5-((trityloxy)methyl)pyrrolidine-1,3-dicarboxylate (**144**)

To a solution of **143** (410 mg, 7.10 x 10^{-1} mmol) in THF (8.0 mL) was added 1.0 M LiHMDS in THF (852 μL, 8.52 x 10^{-1} mmol) at -78 °C. The reaction mixture was stirred at -30 °C for 10 minutes before adding benzyl bromide (127 μL, 1.07 mmol) at -78 °C and stirred for 30 minutes at room temperature. The

mixture was diluted with H_2O and extracted with EtOAc. The organic layer was washed with H_2O and brine, and dried over Na_2SO_4. After filtration and concentration, the residue was purified by flash chromatography (Biotage-SNAP Ultra 25g, eluted with *n*-hexane/EtOAc = 95/5 to 60/40 (v/v)) to give the title compound **144** (325 mg, 69% yield) as a mixture of diastereomers.

^{1}H-NMR (400 MHz, CDCl$_3$) δ: 1.31 (br s, 4.5H), 1.31-1.43 (br m, 1H), 1.52 (br s, 4.5H), 2.00-2.30 (br m, 2H), 2.35-2.57 (br m, 1H), 2.76-2.92 (br m, 1H), 2.94-3.06 (m, 2H), 3.21-3.51 (br m, 1H), 3.54-3.90 (br m, 2H), 3.92-4.17 (br m, 1H), 4.91-5.17 (m, 2H), 6.97-7.07 (m, 2H), 7.16-7.42 (m, 18H)

Step6 : *tert*-Butyl (2*S*,4*S*)-4-Benzyl-4-(hydroxymethyl)-2-((trityloxy)methyl)pyrrolidine-1-carboxylate (**145**)

To a solution of **144** (325 mg, 4.87 x 10^{-1} mmol) in THF (8.0 mL) was added LAH (37.0 mg, 9.75 x 10^{-1} mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 30 minutes before quenching by H_2O (37 μL), 4 M NaOH solution (37 μL) and H_2O (74 μL). After filtration and concentration, the residue was purified by flash chromatography (Biotage-SNAP Ultra 25g, eluted with *n*-hexane/EtOAc = 90/10 to 60/40 (v/v)) to give the title compound **145** (244 mg, 89% yield) as a single diastereomer.

^{1}H-NMR (400 MHz, CDCl$_3$) δ: 1.24-1.59 (br m, 9H), 1.75-2.06 (br m, 3H), 2.63-2.76 (m, 2H), 2.94-3.78 (br m, 6H), 3.88-4.16 (br m, 1H), 7.17-7.33 (br m, 14H), 7.38-7.43 (m, 6H)

Step7 : ((2*S*,4*S*)-4-Benzylpyrrolidine-2,4-diyl)dimethanol (**132**)

Compound **145** (100 mg, 1.77 x 10^{-1} mmol) was treated with 4 M HCl in 1,4-dioxane (500 μL) and stirred at room temperature for 2 h. The reaction mixture was evaporated and dried *in vacuo*, and the residue was decanted three times by 1:1 *n*-hexane-AcOEt to give the hydrochloride salt of the title compound **132** (46.0 mg, quant.).

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.45 (dd, *J* = 13.4, 10.5 Hz, 1H), 1.81 (dd, *J* = 13.4, 7.0 Hz, 1H), 2.75 (dd, *J* = 29.1, 13.4 Hz, 1H), 3.01 (dd, *J* = 43.0, 11.7 Hz, 1H), 3.23 (s, 2H), 3.42-3.70 (m, 2H), 5.11 (br s, 1H), 5.34 (br s, 1H), 7.20-7.36 (m, 5H), 8.93 (br s, 2H)

Synthesis of pyrrolidine 133

Step1 : *tert*-Butyl (2*S*,4*R*)-4-methoxy-2-((trityloxy)methyl)pyrrolidine-1-carboxylate hydrate (**147**)

To a solution of *tert*-butyl (2*S*,4*R*)-4-hydroxy-2-(hydroxymethyl)pyrrolidine-1-carboxylate (**146**, 5.00 g, 2.30 x 10 mmol) in DMF (30 mL) were added TrCl (7.10 g, 2.55 x 10 mmol), Et$_3$N (9.60 mL, 6.90 x 10 mmol) and DMAP (845 mg, 6.92 mmol) under cooling in a water bath, and the mixture was stirred at 60 °C for 4 h. After cooling to room temperature, the reactant mixture was quenched with 2 M aqueous HCl solution, and the mixture was extracted with EtOAc. The extract was washed with H$_2$O and brine, and dried over Na$_2$SO$_4$. After filtration and concentration, the residue was purified by flash chromatography (Biotage-SNAP Ultra 200g, eluted with *n*-hexane/EtOAc = 30/70 to 0/100 (v/v)) to give the title compound **147** (10.1 g, 95% yield).

^{1}H-NMR (400 MHz, CDCl$_3$) δ: 1.29-1.48 (br m, 9H), 1.62 (br s, 1H), 1.90-2.11 (br m, 1H), 2.15-2.22 (m, 1H), 2.92-3.64 (br m, 4H), 4.10 (br s, 1H), 4.51 (br s, 1H), 7.18-7.29 (m, 9H), 7.29-7.36 (m, 6H)

Step2 : *tert*-Butyl (2*S*,4*R*)-4-((methylsulfonyl)oxy)-2-((trityloxy)methyl)pyrrolidine-1-carboxylate (**148**)

To a solution of **147** (2.00 g, 4.35 mmol) in CHCl$_3$ (10 mL) were added MsCl (510 µL, 7.55 mmol) and Et$_3$N (1.20 mL, 8.71 mmol) at 0 °C, and the mixture was stirred at 0 °C for 30 minutes. The reactant mixture was quenched with 1 M aqueous HCl solution, and the mixture was extracted with EtOAc. The extract was washed with saturated aqueous NaHCO$_3$ solution and brine, and dried over Na$_2$SO$_4$. The solvent was evaporated *in vacuo* to give the title compound **148** (2.40 mg, overweight), which was applied to the next step without further purification.

^{1}H-NMR (400 MHz, CDCl$_3$) δ: 1.30-1.53 (br m, 9H), 2.16-2.51 (br m, 2H), 3.01 (s, 1H), 2.89-3.34 (br m, 2H), 3.56-4.00 (br m, 2H), 4.14 (br d, *J* = 24.7 Hz, 1H), 5.38 (br d, *J* = 31.7 Hz, 1H), 7.20-7.33 (m, 9H), 7.35-7.40 (m, 6H)

Step3 : *tert*-Butyl (2*S*,4*S*)-4-cyano-2-((trityloxy)methyl)pyrrolidine-1-carboxylate (**149**)

A mixture of **148** (2.40 g, approx. 4.3 mmol) and tetraethylammonium cyanide (2.1 g, 1.34 x 10 mmol) in MeCN (10 mL) was stirred at 100 °C for 4 h. After cooling to room temperature, the mixture was diluted with H$_2$O, and the mixture was extracted with EtOAc. The extract was washed with saturated aqueous NaHCO$_3$ solution and brine, and dried over Na$_2$SO$_4$. The organic layer was separated and concentrated.

The residue was purified by flash chromatography (Biotage-SNAP Ultra 50g, eluted with *n*-hexane/EtOAc = 85/15 to 50/50 (v/v)) to give the title compound **149** (1.50 g, 74% yield (2 steps)).

^{1}H-NMR (400 MHz, CDCl$_3$) δ: 1.25-1.53 (br m, 9H), 2.26-2.61 (br m, 2H), 2.89-3.01 (br m, 1H), 3.07-3.60 (br m, 3H), 4.06 (br dd, *J* = 11.0, 7.5 Hz, 1H), 7.20-7.34 (m, 9H), 7.39-7.43 (m, 6H)

Step4 : *tert*-Butyl (2*S*,4*S*)-4-benzyl-4-cyano-2-((trityloxy)methyl)pyrrolidine-1-carboxylate (**150**)

To a solution of **149** (900 mg, 1.92 mmol) in THF (7.0 mL) was added 1 M LiHMDS in THF (2.30 mL, 2.30 mmol) at -78 °C. The reaction mixture was stirred at -30 °C for 10 minutes before adding benzyl bromide (342 mL, 2.88 mmol) at -78 °C and stirred 30 minutes at room temperature. The mixture was diluted with H$_2$O and extracted with EtOAc. The organic layer was washed with H$_2$O and brine, and dried over Na$_2$SO$_4$. After filtration and concentration, the residue was purified by flash chromatography (Biotage-SNAP Ultra 25g, eluted with *n*-hexane/EtOAc = 99/1 to 65/35 (v/v)) to give the title compound **150** (383 mg, 35% yield).

^{1}H-NMR (400 MHz, CDCl$_3$) δ: 1.25-1.53 (br m, 9H), 2.26-2.61 (br m, 2H), 2.89-3.01 (br m, 1H), 3.07-3.60 (br m, 3H), 4.06 (br dd, *J* = 11.0, 7.5 Hz, 1H), 7.20-7.34 (m, 9H), 7.39-7.43 (m, 6H)

Step5 : (3*S*,5*S*)-3-Benzyl-5-(hydroxymethyl)pyrrolidine-3-carbonitrile (**133**)

Compound **150** (383 mg, 6.85 x 10^{-1} mmol) was treated with 4 M HCl in 1,4-dioxane (2.0 mL) and stirred at room temperature for 2 h. The reaction mixture was evaporated and dried *in vacuo*, and the residue was decanted three times by *n*-hexane to give the hydrochloride salt of the title compound **133** (152 mg, 88% yield).

^{1}H-NMR (400 MHz, DMSO-*d*$_6$) δ: 1.94-2.18 (m, 2H), 2.95-3.19 (m, 3H), 3.41-3.56 (m, 2H), 3.57-3.68 (m, 1H), 3.69-3.88 (br m, 1H), 5.34-5.41 (br m, 1H), 7.09-7.44 (m, 5H), 9.52 (br s, 2H)

Synthesis of compound 43

Step1 : *tert*-Butyl (2*S*,4*R*)-4-(benzyloxy)-2-(hydroxymethyl)pyrrolidine-1-carboxylate (**152**)

To a solution of *tert*-butyl (2*S*,4*R*)-4-(benzyloxy)-2-(1-hydroxyvinyl)pyrrolidine-1-carboxylate (**151**, 25.0 g, 7.80 x 10 mmol) in THF (100 mL) was added 0.94 mol/L THF solution of BH$_3$-THF (99.0 mL, 9.30

x 10 mmol) at temperature, and the mixture was stirred at 50 °C for 2 h. The reactant mixture was quenched with saturated aqueous NaHCO₃ solution, and the mixture was extracted with EtOAc. The extract was washed with H₂O and brine, and dried over Na₂SO₄. After filtration, the solvent was evaporated *in vacuo* to give the title compound **152** (19.4 g, 81%).

^{1}H-NMR (400 MHz, CDCl₃) δ: 1.47 (s, 9H), 1.58-1.68 (m, 1H), 2.13-2.24 (m, 1H), 3.40 (dd, J = 12.1, 4.0 Hz, 1H), 3.56 (ddd, J = 11.3, 7.2, 2.9 Hz, 1H), 3.59-3.78 (m, 2H), 4.05 (br s, 1H), 4.08-4.19 (m, 1H), 4.50 (dd, J = 16.3, 11.3 Hz, 1H), 4.88 (d, J = 8.1 Hz, 1H), 7.28-7.38 (m, 5H)

Step2 : *tert*-Butyl (2S,4R)-4-(benzyloxy)-2-formylpyrrolidine-1-carboxylate (**153**)

To a solution of **152** (9.15 g, 2.98 x 10 mmol) in CH₂Cl₂ (92 mL) was added Dess-Martin Periodinane (4.20 g, 9.79 mmol) at temperature, and the mixture was stirred at room temperature for 1.5 h. The reaction was quenched with aqueous NaHCO₃ solution and aqueous Na₂S₂O₃ solution. The mixture was extracted with CHCl₃ using a Phase Separator. The solvent was evaporated *in vacuo* to give the title compound **153** (9.18 g, overweight), which was advanced to the next step without further purification.

Step3 : *tert*-Butyl (2S,4R)-4-(benzyloxy)-2-vinylpyrrolidine-1-carboxylate (**154**)

Ph₃PMeBr (31.6 g, approx. 8.8 x 10 mmol) was dissolved in THF (90 mL). To the solution, *t*-BuOK (9.92 g, 8.80 x 10 mmol) was added at 0 °C, and the mixture was stirred at 0 °C for 15 min. And then, the solution of **153** (9.00 g, 2.95 x 10 mmol) in THF (45 mL) was added to the reactant mixture at 0 °C followed by the stirring 5.5 h at room temperature. The mixture was diluted with H₂O and extracted with AcOEt, and the organic layer was washed with H₂O and brine, and dried over Na₂SO₄. After filtration and concentration, the residue was purified by flash chromatography (Biotage-SNAP Ultra 100 g, eluted with *n*-hexane/EtOAc = 97/3 to 70/30 (v/v)) to give the title compound **154** (7.18 g, 20% yield (2 steps)).

^{1}H-NMR (400 MHz, CDCl₃) δ: 1.44 (s, 9H), 1.88 (dt, J = 13.1, 5.8 Hz, 1H), 2.15-2.27 (m, 1H), 3.48 (dd, J = 11.5, 4.6 Hz, 1H), 3.70 (br s, 1H), 4.07-4.14 (m, 1H), 4.36 (br s, 1H), 4.50 (dd, J = 17.5, 12.1 Hz, 2H), 4.96-5.19 (m, 2H), 5.72 (br s, 1H), 7.26-7.38 (m, 5H)

Step4 : *tert*-Butyl (2R,4R)-2-ethyl-4-hydroxypyrrolidine-1-carboxylate (**155**)

A solution of **154** (7.18 g, 2.37 x 10 mmol) in THF (36 mL) and MeOH (36 mL) was treated with 5% palladium on activated carbon (N.E. Chemcat PE-type, 2.5 g) and stirred under 1.0 atm of hydrogen at room temperature for 24 h. After removal of the palladium catalyst by Celite® filtration, the filtrate was concentrated to give the title compound **155** (5.20 g, overweight), which was advanced to the next step without further purification.

^{1}H-NMR (400 MHz, CDCl$_3$) δ: 0.84 (t, J = 7.5 Hz, 3H), 1.46 (s, 9H), 1.64-1.97 (m, 3H), 1.99-2.10 (m, 1H), 3.39 (dd, J = 12.0, 4.2 Hz, 1H), 3.48 (s, 1H), 3.54 (br s, 1H), 3.87 (br s, 1H), 4.35-4.42 (m, 2H)

Step5 : *tert*-Butyl (*R*)-2-ethyl-4-oxopyrrolidine-1-carboxylate (**156**)

To a solution of **155** (5.20 g, approx. 2.4 x 10 mmol) in CH$_2$Cl$_2$ (104 mL) was added Dess-Martin Periodinane (15.4 g, 3.62 x 10 mmol) at temperature of a water bath, and the mixture was stirred at room temperature for 1 h. The reaction was quenched with aqueous NaHCO$_3$ solution and aqueous Na$_2$S$_2$O$_3$ solution. The mixture was extracted with CHCl$_3$ using a Phase Separator. The solvent was evaporated *in vacuo* to give the title compound **156** (6.02 g, overweight), which was advanced to the next step without further purification.

Step6 : *tert*-Butyl (*R*)-2-ethyl-4-((((trifluoromethyl)sulfonyl)oxy)-2,3-dihydro-1*H*-pyrrole-1-carboxylate (**157**)

Compound **156** (5.15 g, approx. 2.0 x 10 mmol) was dissolved in THF (155 mL). To the solution, 1.1 M LiHMDS in THF (26.3 mL, 2.90 x 10 mmol) was added at -78 °C, and the mixture was stirred at -30 °C for 15 min. After cooling to -78 °C, Tf$_2$NPh (10.8 g, 3.02 x 10 mmol) was added to the reactant mixture, and then the mixture was stirred at room temperature for 1 h. The mixture was diluted with aqueous NH$_4$Cl solution and extracted with AcOEt, and the organic layer was washed with H$_2$O and brine, and dried over Na$_2$SO$_4$. After filtration and concentration, the residue was purified by flash chromatography (Biotage-SNAP Ultra 50 g, eluted with *n*-hexane/EtOAc = 100/0 to 80/20 (v/v)) to give the title compound **157** (3.66 g, 50% yield (3 steps)).

^{1}H-NMR (400 MHz, CDCl$_3$) δ: 0.85 (t, J = 7.5 Hz, 3H), 1.49 (s, 9H), 1.76-1.90 (m, 2H), 4.14 (dd, J = 14.9, 5.1 Hz, 1H), 4.30 (dd, J = 29.7, 14.9 Hz, 1H), 4.53-4.70 (m, 1H), 5.66 (d, J = 19.7 Hz, 1H)

Step7 : 1'-(*tert*-Butyl) 5-methyl (*R*)-5'-ethyl-4',5'-dihydro-1*H*,1'*H*-[2,3'-bipyrrole]-1',5-dicarboxylate (**158**)

A mixture of **157** (1.80 g, 5.21 mmol), (5-(methoxycarbonyl)-1*H*-pyrrol-2-yl)boronic acid (1.32 g, 7.82 mmol) and Na_2CO_3 (600 μL, 1.20 mmol) in toluene (14 mL) and H_2O (7.0 mL) was treated with $PdCl_2$(dppf)-CH_2Cl_2 (426 mg, 5.21 x 10^{-1} mmol) and stirred at 90 °C for 2 h under argon atmosphere. After cooling to room temperature, the mixture was diluted with AcOEt and H_2O, and the organic layer was washed with H_2O and brine, and dried over Na_2SO_4. After filtration and concentration, the residue was purified by flash chromatography (Biotage-SNAP Ultra 25 g, eluted with *n*-hexane/EtOAc = 96/4 to 80/20 (v/v)) to give the title compound **158** (1.09 g, 65%) as a mixture of isomers on the olefine part.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.85 (t, *J* = 7.5 Hz, 3H), 1.50 (s, 4.5H), 1.51 (m, 4.5H), 1.71-1.94 (m, 3H), 3.86 (s, 1.5H), 3.87 (br s, 1H), 4.29-4.70 (m, 2H), 5.03 (d, *J* = 28.1 Hz, 0.5H), 6.21 (dd, *J* = 6.4, 3.7 Hz, 0.5H), 6.27 (dt, *J* = 4.6, 1.9 Hz, 0.5H), 6.86-6.89 (m, 0.5H), 6.90-6.98 (m, 1H), 9.24 (br s, 1H)

Step8 : Methyl 5-((3*S*,5*R*)-1-(*tert*-butoxycarbonyl)-5-ethylpyrrolidin-3-yl)-1*H*-pyrrole-2-carboxylate (**159**)

A solution of **158** (1.00 g, 3.12 mmol) in THF (5.0 mL) and MeOH (5.0 mL) was treated with 10% palladium on activated carbon (200 mg) and stirred under 1.0 atm of hydrogen at room temperature for 24 h. After removal of the palladium catalyst by Celite® filtration, the filtrate was concentrated and purified by flash chromatography (Biotage-SNAP Ultra 25 g, eluted with *n*-hexane/EtOAc = 96/4 to 80/20 (v/v)) to give the title compound **159** (762 mg, 75%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.86 (t, *J* = 7.5 Hz, 3H), 1.47 (s, 9H), 1.64-2.14 (m, 3H), 2.44-2.56 (m, 1H), 3.14-3.31 (m, 2H), 3.79 (br s, 1H), 3.84 (s, 3H), 3.93-4.20 (m, 1H), 6.04 (t, *J* = 3.5 Hz, 1H), 6.84 (dd, *J* = 3.5, 2.5 Hz, 1H), 9.27 (br s, 1H)

Step9 : Methyl 5-((3*S*,5*R*)-5-ethylpyrrolidin-3-yl)-1*H*-pyrrole-2-carboxylate (**160**)

Compound **159** (760 mg, 2.36 mmol) was dissolved in 4 M HCl in 1,4-dioxane solution (7.6 mL) and stirred at room temperature for 1.5 h. The reactant mixture was dried under vacuum to give the title compound **160** (615 mg, overweight) as a hydrochloride salt, which was applied to the next step without further purification.

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.96 (t, J = 7.5 Hz, 3H), 1.61-1.85 (m, 3H), 2.41-2.50 (m, 1H), 3.04-3.23 (m, 1H), 3.38 (br s, 1H), 3.42-3.59 (s, 3H), 3.74 (s, 3H), 6.11 (dd, J = 3.6, 2.6 Hz, 1H), 6.72 (dd, J = 3.6, 2.6 Hz, 1H), 9.02 (br s, 1H), 9.73 (br s, 1H), 11.86 (s, 1H)

Step10 ： Methyl 5-((3S,5R)-1-(8-cyclopropyl-2-methyl-9H-pyrimido[4,5-b]indol-4-yl)-5-ethylpyrrolidin-3-yl)-1H-pyrrole-2-carboxylate (**161**)

A microwave vial was charged with **160** (250 mg, 9.70 x 10^{-1} mmol), **70** (251 mg, 9.70 x 10^{-1} mmol), i-Pr$_2$NEt (800 μL, 4.40 mmol), and NMP (8.0 mL). The vial was heated to 140 °C, irradiated the microwave for 2 h. The mixture was diluted with H$_2$O and extracted with EtOAc, and the organic layer was washed with H$_2$O and brine, and dried over Na$_2$SO$_4$. After filtration and concentration, the residue was purified by preparative TLC (EtOAc/MeOH = 20/1 (v/v)) to give the title compound **161** (191 mg, 45% yield).

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.68-0.78 (m, 2H), 0.95 (t, J = 7.3 Hz, 3H), 1.00-1.04 (m, 2H), 1.57-1.69 (m, 1H), 1.92-2.03 (m, 2H), 2.31-2.38 (m, 1H), 2.51 (s, 3H), 2.46-2.55 (m, 1H), 3.12-3.21 (m, 1H), 3.73 (s, 3H), 3.92 (t, J = 10.3 Hz, 1H), 4.16 (t, J = 8.1 Hz, 1H), 4.67-4.75 (m, 1H), 6.15 (dd, J = 3.6, 2.4 Hz, 1H), 6.73 (dd, J = 3.6, 2.4 Hz, 1H), 6.85 (d, J = 7.7 Hz, 1H), 7.08 (t, J = 7.7 Hz, 1H), 7.63 (d, J = 7.7 Hz, 1H), 11.78 (br s, 1H), 11.97 (s, 1H)

Step11 ： 5-((3S,5R)-1-(8-Cyclopropyl-2-methyl-9H-pyrimido[4,5-b]indol-4-yl)-5-ethylpyrrolidin-3-yl)-1H-pyrrole-2-carboxylic acid (**43**)

Compound **161** (190 mg, 4.32 x 10^{-1} mmol) was dissolved in 1:1 THF-MeOH (6.0 mL). To the solution, 2M aqueous NaOH solution (1.74 mL, 3.60 mmol) was added, and the mixture was stirred at 80 °C for 4 h. After cooling to room temperature, the reactant mixture was neutralized with 2.0 M aqueous HCl solution and diluted with H$_2$O. The precipitated solid was collected by filtration, washed with H$_2$O, and dried to give the title compound **43** (165 mg, 90% yield).

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.68-0.78 (m, 2H), 0.95 (t, J = 7.3 Hz, 3H), 1.00-1.04 (m, 2H), 1.57-1.69 (m, 1H), 1.92-2.05 (m, 2H), 2.31-2.38 (m, 1H), 2.51 (s, 3H), 2.47-2.54 (m, 1H), 3.11-3.20 (m, 1H), 3.92 (t, J = 10.3 Hz, 1H), 4.16 (t, J = 8.1 Hz, 1H), 4.67-4.74 (m, 1H), 6.11 (dd, J = 3.5, 2.5 Hz, 1H), 6.67 (dd, J = 3.5, 2.4 Hz, 1H), 6.85 (d, J = 7.8 Hz, 1H), 7.08 (t, J = 7.8 Hz, 1H), 7.64 (d, J = 7.8 Hz, 1H), 11.64 (s, 1H), 11.98 (s, 1H), 12.09 (s, 1H)

^{13}C-NMR (400 MHz, DMSO-d_6) δ: 7.8, 8.0, 9.7, 10.5, 25.9, 26.5, 35.7, 37.4, 57.9, 58.5, 95.6, 106.6, 114.9, 118.7, 119.0, 119.4, 120.2, 122.3, 126.3, 136.2, 137.0, 157.2, 157.6, 161.8 (2C)

$[α]^{25}_D$ = -310 (c = 1.00, DMSO)

HRMS (ESI, m/z, MH+) Calcd for $C_{25}H_{28}O_2N_5$: 430.2238, Found: 430.2239

Synthesis of compound 44 and 45

Step1 : *tert*-Butyl (*S*)-4-oxo-2-((trityloxy)methyl)pyrrolidine-1-carboxylate (**162**)

To a solution of **147** (3.00 g, 6.52 mmol) in CHCl$_3$ (15 mL) was added Dess-Martin Periodinane (4.20 g, 9.79 mmol) at temperature of a water bath, and the mixture was stirred at room temperature for 1 h. The reaction was quenched with NaHCO$_3$ (2.50 g, 19.8 mmol) and H$_2$O (25 mL). The mixture was diluted with H$_2$O and extracted with AcOEt, and the organic layer was washed with aqueous Na$_2$CO$_3$ and brine. After drying over Na$_2$SO$_4$ and filtration, the solvent was evaporated *in vacuo* to give the title compound **162** (2.90 g, 97%).

^{1}H-NMR (400 MHz, CDCl$_3$) δ: 1.38-1.51 (br m, 9H), 2.51-2.42 (br m, 1H), 2.65-3.03 (br m, 2H), 3.46-3.69 (br m, 1H), 3.89-4.05 (br m, 2H), 4.35-4.51 (br m, 1H), 7.33-7.20 (m, 15H)

Step2 : *tert*-Butyl (*S*)-4-(((trifluoromethyl)sulfonyl)oxy)-2-((trityloxy)methyl)-2,3-dihydro-1*H*-pyrrole-1-carboxylate (**163**)

The compound **162** (1.40 g, 3.17 mmol) was dissolved in THF (10 mL). To the solution, 1.0 M LiHMDS in THF (3.80 mL, 3.80 mmol) was added at -78 °C, and the mixture was stirred at -30 °C for 15 min. After cooling to -78 °C, *N*-phenyltrifluoromethanesulfonimide (Tf$_2$NPf, 1.50 g, 4.00 mmol) was added to the reactant mixture, and then the mixture was stirred at room temperature for 1 h. The mixture was diluted with aqueous NH$_4$Cl solution and extracted with AcOEt, and the organic layer was washed with H$_2$O and brine, and dried over Na$_2$SO$_4$. After filtration and concentration, the residue was purified by flashchromatography (Yamazen-Universal Premium L, eluted with *n*-hexane/EtOAc = 95/5 to 65/35 (v/v)) to give the title compound **163** (1.66 mg, 89% yield).

^{1}H-NMR (400 MHz, CDCl$_3$) δ: 1.26-1.53 (br m, 9H), 3.18-3.53 (br m, 2H), 4.19-4.36 (br m, 2H), 4.63-4.70 (br m, 1H), 5.66-5.84 (br m, 1H), 7.21-7.30 (m, 9H), 7.36-7.41 (m, 6H)

Step3 : 1'-(*tert*-Butyl) 5-methyl (*S*)-5'-((trityloxy)methyl)-4',5'-dihydro-1*H*,1'*H*-[2,3'-bipyrrole]-1',5-dicarboxylate (**164**)

A mixture of **163** (300 mg, 5.09 x 10^{-1} mmol), (5-(methoxycarbonyl)-1*H*-pyrrol-2-yl)boronic acid (172 mg, 1.02 mmol) and 2.0 M aqueous Na$_2$CO$_3$ solution (600 μL, 1.20 mmol) in DMA (1.5 mL) was treated with PdCl$_2$(dppf)-CH$_2$Cl$_2$ (42.0 mg, 5.14 x 10^{-2} mmol) and stirred at 90 °C for 1 h under argon atmosphere. After cooling to room temperature, the mixture was diluted with AcOEt and H$_2$O, and the organic layer was washed with H$_2$O and brine, and dried over Na$_2$SO$_4$. After filtration and concentration, the residue was purified by preparative TLC (*n*-hexane/AcOEt = 2/1 (v/v)) to give the title compound **164** (167 mg, 58% yield).

^{1}H-NMR (400 MHz, CDCl$_3$) δ: 1.32-1.51 (br m, 9H), 3.13-3.50 (m, 2H), 3.88 (s, 3H), 4.34-4.58 (br m, 2H), 4.75-4.82 (br m, 1H), 5.82-6.02 (br m, 1H), 6.23 (br s, 1H), 6.88 (br s, 1H), 7.20-7.30 (m, 9H), 7.37-7.41 (m, 6H), 9.14-9.21 (br m, 1H)

Step4 : Methyl 5-((3*S*,5*S*)-1-(*tert*-butoxycarbonyl)-5-((trityloxy)methyl)pyrrolidin-3-yl)-1*H*-pyrrole-2-carboxylate (**165**)

A solution of **164** (167 mg, 2.96 x 10^{-1} mmol) in THF (8.0 mL) and MeOH (8.0 mL) was treated with 10% palladium on activated carbon (80.0 mg) and stirred under 1.0 atm of hydrogen at room temperature for 5 h. After removal of the palladium catalyst by Celite® filtration, the filtrate was concentrated and purified by preparative TLC (*n*-hexane/AcOEt = 2/1 (v/v)) to give the title compound **165** (129 mg, 77% yield).

^{1}H-NMR (400 MHz, CDCl$_3$) δ: 1.32-1.48 (br m, 9H), 2.11-2.21 (m, 1H), 2.38-2.56 (m, 1H), 3.09-3.52 (br m, 4H), 3.84 (s, 3H), 4.06-4.13 (br m, 2H), 6.04 (br s, 1H), 6.83 (br s, 1H), 7.20-7.30 (m, 9H), 7.39-7.42 (m, 6H), 9.01 (br s, 1H)

Step5 : Methyl 5-((3*S*,5*S*)-5-(hydroxymethyl)pyrrolidin-3-yl)-1*H*-pyrrole-2-carboxylate (**166**)

Compound **165** (129 mg, 2.28 x 10^{-1} mmol) was dissolved in 4 N HCl in 1,4-dioxane solution (2.0 mL) and stirred at room temperature for 2 h. The reactant mixture was diluted with H$_2$O (250 μL) and washed

with *n*-hexane (1.0 mL) three times. The aqueous layer was dried under vacuum to give the title compound **166** (56.0 mg, overweight), which was applied to the next step without further purification.

Step6 : Methyl 5-((3*S*,5*S*)-1-(8-cyclopropyl-2-methyl-9*H*-pyrimido[4,5-*b*]indol-4-yl)-5-(hydroxymethyl)pyrrolidin-3-yl)-1*H*-pyrrole-2-carboxylate (**167**)

A microwave vial was charged with **70** (58.0 mg, 2.25 x 10^{-1} mmol), **166** (56.0 mg, approx. 2.3 x 10^{-1} mmol), *i*-Pr$_2$NEt (200 μL, 1.1 mmol), and NMP (2.0 mL). The vial was heated to 140 °C, irradiated the microwave for 2 h. The mixture was diluted with H$_2$O and extracted with EtOAc, and the organic layer was washed with H$_2$O and brine, and dried over Na$_2$SO$_4$. After filtration and concentration, the residue was purified by preparative TLC (EtOAc/MeOH = 20/1 (v/v)) to give the title compound **167** (68.8 mg, 69% yield).

^{1}H-NMR (400 MHz, DMSO-*d*$_6$) δ: 0.67-0.79 (m, 2H), 1.00-1.04 (m, 2H), 2.13-2.22 (m, 1H), 2.31-2.38 (m, 1H), 2.43-2.49 (m, 1H), 2.51 (s, 3H), 3.13-3.22 (m, 1H), 3.64-3.70 (m, 1H), 3.72 (s, 3H), 3.75-3.80 (m, 1H), 3.89 (t, *J* = 10.3 Hz, 1H), 4.24 (t, *J* = 8.1 Hz, 1H), 4.81-4.88 (br m, 1H), 4.94 (t, *J* = 5.4 Hz, 1H), 6.13 (dd, *J* = 3.3, 2.5 Hz, 1H), 6.74 (dd, *J* = 3.7, 2.5 Hz, 1H), 6.85 (d, *J* = 7.8 Hz, 1H), 7.07 (t, *J* = 7.8 Hz, 1H), 7.64 (d, *J* = 7.8 Hz, 1H), 11.77 (s, 1H), 11.98 (s, 1H)

Step7 : 5-((3*S*,5*S*)-1-(8-Cyclopropyl-2-methyl-9*H*-pyrimido[4,5-*b*]indol-4-yl)-5-(hydroxymethyl)pyrrolidin-3-yl)-1*H*-pyrrole-2-carboxylic acid (**44**)

Compound **167** (57 mg, 1.3 x 10^{-1} mmol) was dissolved in 1:1 THF-MeOH (2.0 mL). To the solution, 2M aqueous NaOH solution (580 μL, 1.2 mmol) was added, and the mixture was stirred at 60 °C for 3 h. After cooling to room temperature, the reactant mixture was neutralized with 2.0 M aqueous HCl solution and diluted with H$_2$O. The precipitated solid was collected by filtration, washed with H$_2$O, and dried to give the title compound **44** (41 mg, 73% yield).

^{1}H-NMR (400 MHz, DMSO-*d*$_6$) δ: 0.67-0.79 (m, 2H), 0.98-1.06 (m, 2H), 2.13-2.22 (m, 1H), 2.31-2.38 (m, 1H), 2.42-2.48 (m, 1H), 2.50 (s, 3H), 3.12-3.21 (m, 1H), 3.67 (dd, *J* = 10.5, 5.7 Hz, 1H), 3.78 (dd, *J* = 10.5, 3.9 Hz, 1H), 3.89 (t, *J* = 10.3 Hz, 1H), 4.24 (t, *J* = 8.1 Hz, 1H), 4.81-4.88 (m, 1H), 4.95 (br s, 1H), 6.09 (dd, *J* = 3.4, 2.6 Hz, 1H), 6.68 (dd, *J* = 3.4, 2.4 Hz, 1H), 6.85 (d, *J* = 7.7 Hz, 1H), 7.08 (t, *J* = 7.7 Hz, 1H), 7.65 (d, *J* = 7.7 Hz, 1H), 11.62 (s, 1H), 11.98 (s, 1H), 12.07 (br s, 1H)

^{13}C-NMR (400 MHz, DMSO-d_6) δ: 7.7, 8.1, 10.5, 25.8, 33.6, 37.3, 58.0, 59.1, 62.8, 95.4, 106.3, 114.9, 118.7, 119.2, 119.4, 120.3, 122.3, 126.3, 136.3, 137.1, 157.4, 157.7, 161.8 (2C)

$[α]^{25}_D$ = -342 (c = 1.00, DMSO)

HRMS (ESI, m/z, MH+) Calcd for $C_{24}H_{26}O_3N_5$: 432.2030, Found: 432.2032

Step8 : 5-((3S,5S)-1-(8-Cyclopropyl-2-methyl-9H-pyrimido[4,5-b]indol-4-yl)-5-(hydroxymethyl) pyrrolidin-3-yl)-1H-pyrrole-2-carboxylic acid (**45**)

Ammonium chloride (7.2 mg, 1.4 x 10^{-1} mmol), HATU (39 mg, 1.0 x 10^{-1} mmol) and Et$_3$N (47 μL, 3.4 x 10^{-1} mmol) were added to a solution of **44** (29 mg, 6.7 x 10^{-2} mmol) in CHCl$_3$ (1.0 mL), and the reactant mixture was stirred at room temperature for 18 h. The mixture was diluted with H$_2$O and extracted with CHCl$_3$, using ISOLUTE® Phase Separator manufactured by Biotage AB. After concentration, the residue was suspended in 1:1 n-hexane-EtOAc and stirred. The precipitated solid was collected by filtration, washed with n-hexane, and dried to give the title compound **45** (7.2 mg, 25% yield).

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.66-0.77 (m, 2H), 0.98-1.02 (m, 2H), 2.14 (dd, J = 22.5, 12.1 Hz, 1H), 2.29-2.36 (m, 1H), 2.39-2.44 (m, 1H), 2.48 (s, 3H), 3.08-3.17 (m, 1H), 3.63-3.68 (m, 1H), 3.74-3.78 (m, 1H), 3.84 (t, J = 10.2 Hz, 1H), 4.22 (t, J = 8.2 Hz, 1H), 4.78-4.85 (m, 1H), 4.92 (t, J = 5.4 Hz, 1H), 5.99 (t, J = 2.7 Hz, 1H), 6.69 (dd, J = 3.5, 2.7 Hz, 1H), 6.79 (br s, 1H), 6.83 (d, J = 7.7 Hz, 1H), 7.06 (t, J = 7.7 Hz, 1H), 7.38 (br s, 1H), 7.62 (d, J = 7.7 Hz, 1H), 11.30 (s, 1H), 11.96 (s, 1H)

^{13}C-NMR (400 MHz, DMSO-d_6) δ: 7.7, 8.1, 10.5, 25.8, 33.6, 37.2, 58.2, 59.1, 62.8, 95.3, 105.4, 110.7, 118.7, 119.2, 119.5, 120.2, 125.7, 126.3, 135.0, 136.2, 157.4, 157.7, 161.8, 162.3

$[α]^{25}_D$ = -332 (c = 1.00, DMSO)

HRMS (ESI, m/z, MH+) Calcd for $C_{24}H_{27}O_2N_6$: 431.2190, Found: 431.2191

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主論文

本論文の内容は，以下の学術雑誌に公表した。

第 1 章

T. Akaki, Y. Bessho, T. Ito, S. Fujioka, M. Ubukata, G. Mori, K. Yamanaka, T. Orita, S. Doi, T. Iwanaga, K. Ikegashira, Y. Hantani, I. Nakanishi, T. Adachi

"Fragment-based lead discovery to identify novel inhibitors that target the ATP binding site of pyruvate dehydrogenase kinases"

Bioorganic & Medicinal Chemistry, vol. 44, 116283, (2021).

第 2 章

Y. Bessho, **T. Akaki** (Co-first author), Y. Hara, M. Yamakawa, S. Obika, G. Mori, M. Ubukata, K. Yasue, Y. Nakane, Y. Terasako, T. Orita, S. Doi, T. Iwanaga, A. Fujishima, T. Adachi, H. Ueno, T. Motomura

"Structure-based drug design of novel and highly potent pyruvate dehydrogenase kinase inhibitors"

Bioorganic & Medicinal Chemistry, vol. 52, 116514, (2021).

第 3 章 第 1 項

S. Nakamura, **T. Akaki** (Co-first author), K. Nishiwaki, M. Nakatani, Y. Kawase, Y. Takahashi, I. Nakanishi

"System truncation accelerates binding affinity calculations with the fragment molecular orbital method: A benchmark study"

Journal of Computational Chemistry, vol. 44. pp. 824-831, (2023).

第 3 章 第 2 項

T. Akaki, S. Nakamura, K. Nishiwaki, I. Nakanishi

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