

HETEROCYCLES, Vol. 103, No. 1, 2021, pp. 331 - 347. © 2021 The Japan Institute of Heterocyclic Chemistry
Received, 4th June, 2020, Accepted, 10th July, 2020, Published online, 29th July, 2020
DOI: 10.3987/COM-20-S(K)17

IDENTIFICATION OF A NOVEL INDOLEAMINE 2,3-DIOXYGENASE INHIBITOR BEARING AN EIGHT-MEMBERED RING FUSED INDOLE SCAFFOLD AND ITS STRUCTURE-ACTIVITY RELATIONSHIP

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Abstract – Indoleamine 2,3-dioxygenase 1 (IDO1) is a promising target for cancer immunotherapy because it is overexpressed in a variety of tumor cells. IDO1 also plays an important role in the process of immune escape by tumors. In this study, we identified a novel IDO1 inhibitor KPYC12532 (**5a**) bearing an eight-membered ring-fused indole scaffold by screening our compound library. To develop novel IDO1 inhibitors, we conducted a structure-activity relationship study of **5a** and found some compounds displaying comparable activity. These results provide useful insight for the design of novel IDO1 inhibitors.

INTRODUCTION

Indoleamine 2,3-dioxygenase 1 (IDO1) is a heme-containing enzyme that catalyzes the oxidative cleavage of the C2–C3 double bond of the tryptophan indole to provide *N*-formylkynurenine, which is the rate-limiting step of the kynurenine pathway.¹ IDO1 is overexpressed in a variety of tumor cells and plays an important role in the process of immune escape by tumors.^{2,3} The upregulation of IDO1 induced by INF- γ or other inflammatory cytokines leads to the depletion of tryptophan in the tumor microenvironment and contributes to local immunosuppression by suppressing the activity of T cells and enhancing that of local regulatory T cells.⁴ Hence, IDO1 inhibitors that can promote a host immunological response are considered useful for cancer immunotherapy.⁵

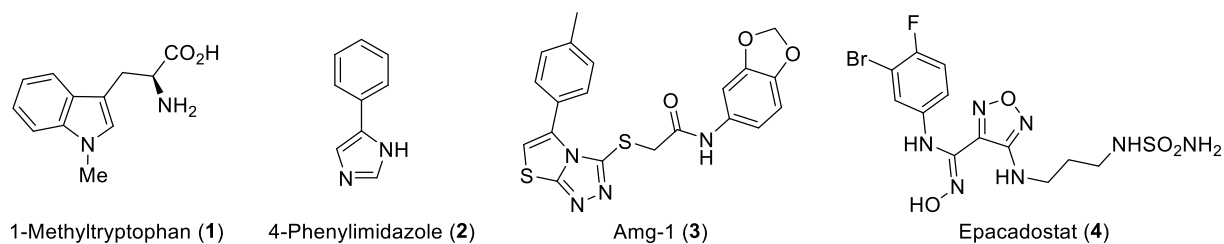


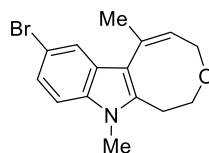
Figure 1. Representative IDO1 inhibitors

To date, a number of IDO1 inhibitors have been reported (Figure 1).^{5–9} The tryptophan analog 1-methyltryptophan (**1**) is widely used as an IDO1 inhibitor with moderate inhibitory activity.⁶ Although other tryptophan or indole analogs have been reported, most showed moderate or weak inhibitory activities (IDO1 enzyme assay: $IC_{50} > 5 \mu\text{M}$).^{10–13} The known heme binder, 4-phenylimidazole (**2**), was previously reported as an IDO1 inhibitor. More potent derivatives were designed on the basis of its structure-activity relationship (SAR) studies.^{14–16} Amg-1 (**3**, Figure 1) and epacadostat (**4**, Figure 1) are also known to bind to the heme iron of IDO1 and exhibit potent inhibitory activities.^{17,18} In particular, epacadostat (**4**) is currently being evaluated in clinical trials in combination with anti-PD-1 antibodies.^{19–21} Recently, Groves *et al.* identified novel inhibitors bound to IDO1 with a dissociated heme cofactor (apo-IDO1).²² Although several modes of inhibition have been reported, most potent inhibitors are heme binders (*e.g.*, imidazole, triazole, or *N*-hydroxyamidine moieties).

To find a novel structural class of IDO1 inhibitor with potent and selective inhibitory activity, we performed a screening of heterocyclic compounds and a SAR study based on the hit compound. From these studies, we identified a novel inhibitor bearing a unique eight-membered ring-fused indole scaffold.

RESULTS AND DISCUSSION

Compound Screening



KPYC12532 (**5a**)

- 1) 68% inhibition of kynurenine production in A431 cell at 10 μM
- 2) 47% inhibition of kynurenine production in enzymatic assay at 10 μM

Figure 2. Structure of KPYC12532 (**5a**)

To find novel scaffolds capable of inhibiting IDO1, we screened in-house chemical libraries. Among them, 226 compounds bearing a heterocyclic core (*e.g.*, indole, pyrrole and pyrazole) were selected for *in*

vitro assays, referring to the scaffolds of known IDO1 inhibitors shown in Figure 1. The initial screening was conducted on the basis of kynurenine production, which is enhanced by the IFN- γ mediated upregulation of IDO1. We measured the kynurenine production from A431 cells in the presence of the test compounds. This led to 15 hit compounds after the exclusion of cytotoxic compounds (Table S1; see Supporting Information). Next, these 15 compounds were assessed based on the inhibition of kynurenine production using recombinant human IDO1 (rhIDO1). From the enzymatic assay, we identified a candidate compound KPYC12532 (**5a**, Figure 2), which displayed 68% inhibition of kynurenine production in A431 cells. In addition, it inhibited kynurenine production by 47% in the enzymatic assay using rhIDO1 at 10 μ M. KPYC12532 (**5a**) consists of a unique eight-membered ring (dihydrooxocine)-fused indole scaffold, inspiring us to perform a SAR study to develop novel IDO1 inhibitors.

Design and Synthesis of KPYC12532 Derivatives

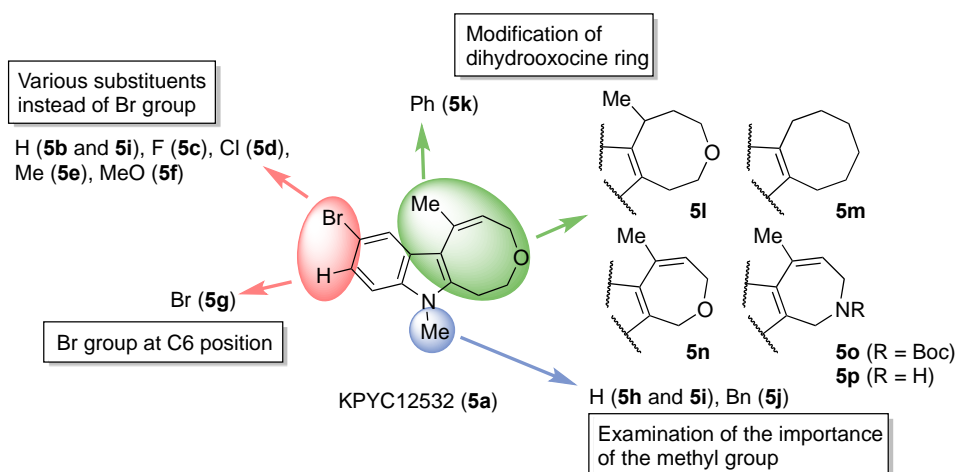
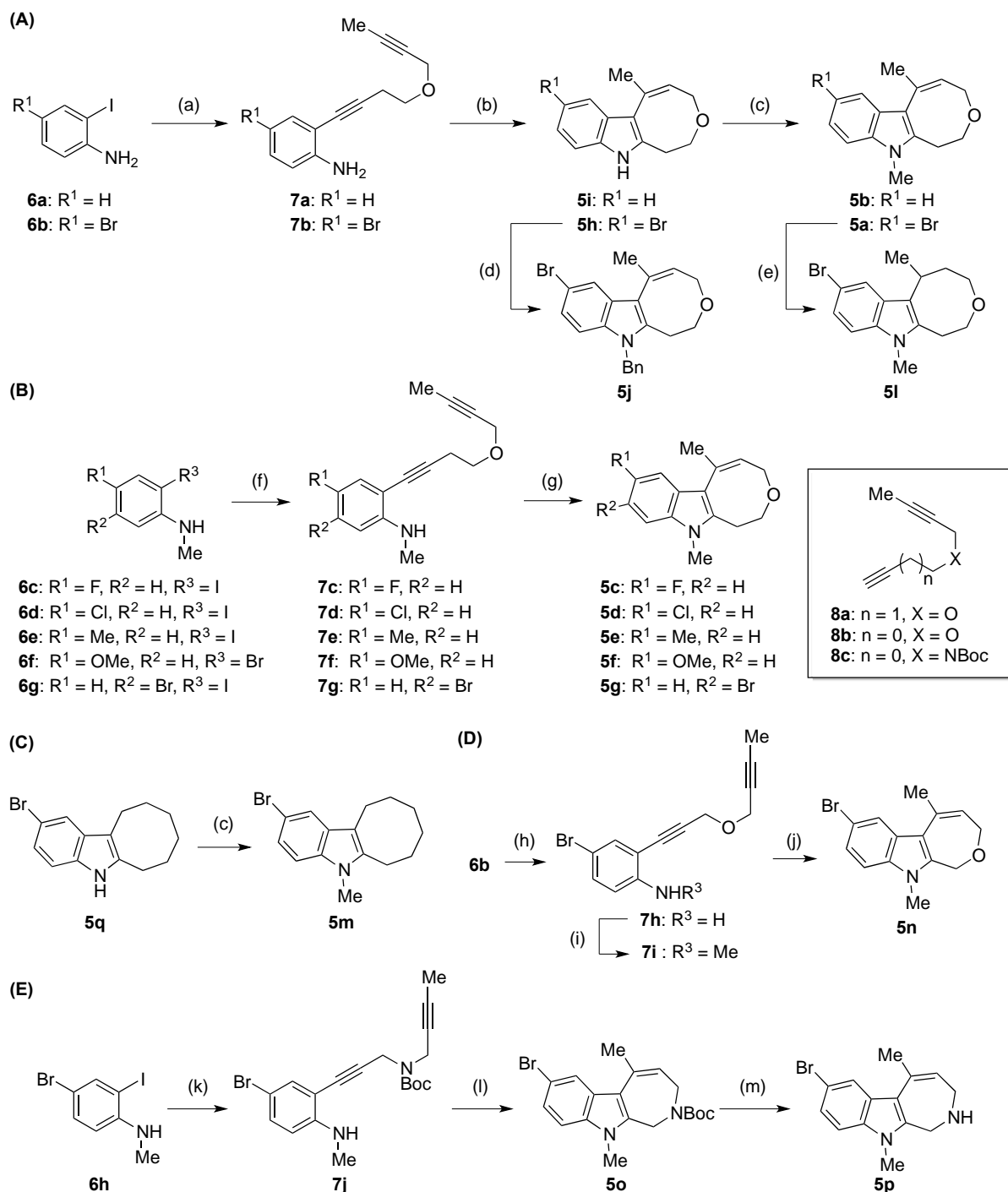


Figure 3. Design of KPYC12532 derivatives

We first designed analogs **5b–5f** with various substituents replacing the Br group at the C5 position of the indole and a compound **5g** with a Br group at the C6 position (Figure 3). Derivatives **5h** and **5i** lacking the *N*-Me group and a compound **5j** containing a Bn group were designed to examine the importance of the methyl group at the indole N1 position. To determine whether the dihydrooxocine ring could be modified, we prepared several derivatives such as a Ph group-containing **5k**, a reduced eight-membered ring-containing **5l**, and its carbocycle congener **5m**. We also synthesized a seven-membered ring derivative (**5n**) and its nitrogen analogs (**5o** and **5p**).

KPYC12532 (**5a**) and its derivatives were synthesized on the basis of our previous procedure involving the gold(I)-catalyzed cascade cyclization to allow for the direct construction of medium-sized ring-fused

indoles (Scheme 1).²³ The Sonogashira coupling of commercially available anilines **6a** and **6b** with the alkyne **8a** provided the diyne derivatives **7a** and **7b**. Subsequently, the gold(I)-catalyzed cascade



Scheme 1. Synthesis of KPYC12532 (**5a**) and its derivatives **5b–5p**. Reagents and conditions: (a) **8a**, PdCl₂(PPh₃)₂, CuI, Et₃N, 50 °C; (b) IPrAuNTf₂, AcOH, MS 3Å, *i*-PrOH, 80 °C; (c) MeI, NaH, DMF, 0 °C to rt; (d) BnBr, NaH, DMF, 0 °C to rt; (e) H₂ (balloon), PtO₂, EtOH, rt; (f) **8a**, PdCl₂(PPh₃)₂, CuI, Et₃N, DMF, rt or 80 °C; (g) IPrAuNTf₂, MS 3Å, *i*-PrOH, 80 °C; (h) **8b**, PdCl₂(PPh₃)₂, CuI, Et₃N, DMF, rt; (i) MeLi, MeI, THF, –78 °C to rt; (j) IPrAuNTf₂, *i*-PrOH, 80 °C (k) **8c**, PdCl₂(PPh₃)₂, CuI, Et₃N, DMF, rt; (l) JohnPhosAuNTf₂, *i*-PrOH, 80 °C; (m) TFA, CH₂Cl₂, rt

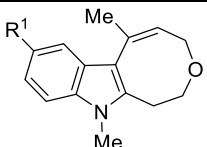
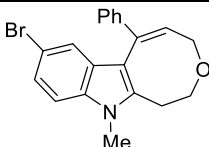
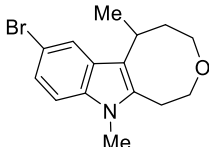
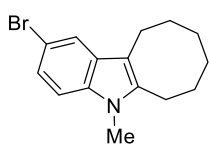
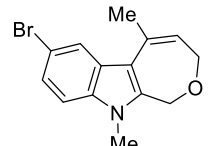
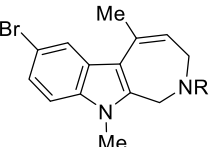
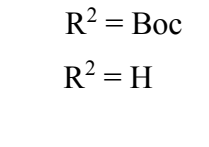
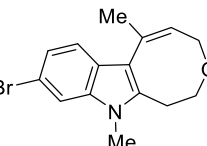
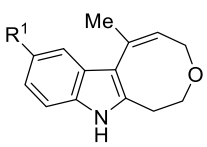
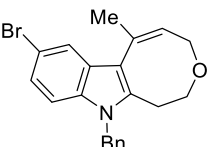
cyclization of **7a** and **7b** with IPrAuNTf₂ gave the corresponding oxocines **5i** and **5h**, which were converted to the compounds **5b** and **5a** through the methylation of the indole NH. The introduction of the Bn group to the indole NH of **5h** was conducted with BnBr and NaH. The compound **5l** was obtained from **5a** by reducing the olefin using Adams' catalyst. The preparation of compounds **5c–g** is shown in Scheme 1B. The Sonogashira coupling of *N*-methylanilines **6c–g** with the alkyne **8a** afforded the diyne derivatives **7c–g**, which were subjected to the gold(I)-catalyzed cascade cyclization to provide the compounds **5c–g** bearing the F, Cl, Me and MeO groups at the C5 position of the indole and Br group at the C6 position. The *N*-methylation of the commercially available compound **5q** provided the compound **5m** (Scheme 1C). The seven-membered ring derivative **5n** was also obtained following a similar procedure to the synthesis of **5a** (Scheme 1D). Thus, diyne **7i** was prepared from the commercially available aniline **6b**, and its gold(I)-catalyzed cascade cyclization with IPrAuNTf₂ resulted in the formation of the seven-membered ring derivative **5n**. Compounds **5o** and **5p** have a nitrogen atom in the seven-membered ring, and were synthesized as shown in Scheme 1E. The Sonogashira coupling of the *N*-methylaniline derivative **6h** with the alkyne **8c** bearing a Boc protected nitrogen atom furnished the cyclization precursor **7j**. The gold(I)-catalyzed cascade cyclization of **7j** with JohnPhosAuNTf₂ led to the formation of compound **5o**, which was converted to the compound **5p** by treatment with trifluoroacetic acid (TFA).

Structure-Activity Relationship Study of KPYC12532 Derivatives

With a series of KPYC12532 derivatives in hand, we performed enzymatic assays to determine the inhibitory activity of these synthesized compounds toward IDO1. We measured the production level of kynurenine mediated by rhIDO in the presence of the synthesized compounds at 30 μM to evaluate their inhibitory activities toward rhIDO1 (Table 1). Initially, we investigated how functional groups on the indole core affect the inhibitory activity. The removal of the Br group (**5b**) or the replacement of Br with a F, Cl, Me and MeO group (**5c–f**) at the indole C5 position resulted in less inhibitory activity than the parent compound **5a** (6–30% inhibition). The activity of compound **5g** bearing a Br group at the C6 position was also slightly weaker than that of **5a** (36%). Removing a methyl group of the indole NH led to slightly decreased activity (**5h**: 30%). Without the Br and methyl groups of the indole, compound **5i** abolished the inhibitory activity (4%). The substitution of a methyl group with a benzyl group also led to a reduction of the activity (**5j**: 25%). These results suggested that the Br group at the C5 position and the methyl group at the N1 position would contribute to the potency of KPYC12532 (**5a**). Next, we conducted SAR studies on the dihydrooxocine ring of KPYC12532 (**5a**). Changing a methyl group to a phenyl group on the eight-membered ring was tolerated (**5k**²³: 44%). The tetrahydrooxocine derivative **5l**

was found to be less potent than **5a** (27%). The replacement of an oxygen atom with a carbon atom (**5m**) showed comparable activity to **5a** (40%), despite lacking the methyl group on the eight-membered ring. The seven-membered ring derivative **5n** exhibited equal inhibitory potency to the parent **5a** molecule (58%). In contrast, compounds **5o** and **5p** with a nitrogen atom on the seven-membered ring did not provide any improvement in activity (**5o**: 21%, **5p**: 25%). These results revealed that modification of the oxocine moiety using oxa- or carbocycles could be promising for the development of more potent inhibitors.

Table 1. Inhibitory activities toward the rhIDO of KPYP12532 derivatives

Compd	rhIDO % inhibition at 30 μ M	Compd	rhIDO % inhibition at 30 μ M
	5a $R^1 = \text{Br}$ 50%		5k 44%
5b $R^1 = \text{H}$ 27%		5l 27%	
5c $R^1 = \text{F}$ 24%		5m 40%	
5d $R^1 = \text{Cl}$ 30%		5n 58%	
5e $R^1 = \text{Me}$ 28%		5o $R^2 = \text{Boc}$ 21%	
5f $R^1 = \text{OMe}$ 6%		5p $R^2 = \text{H}$ 25%	
	5g 36%	4 Epacadostat (1 μ M) 98%	
	5h $R^1 = \text{Br}$ 30%		
5i $R^1 = \text{H}$ 4%			
	5j 25%		

Finally, we investigated the dose-dependent inhibition of KPYC12532 (**5a**) in a cell-based assay. KPYC12532 (**5a**) exhibited dose-dependent inhibition in kynurenine production in A431 cells ($IC_{50} = 4.7 \mu\text{M}$, Figure 4a). No cytotoxicity was observed in the concentration range of 0.3–30 μM of the compound (Figure 4b).

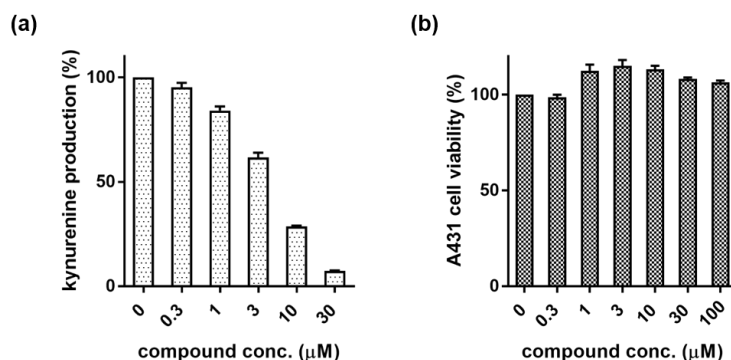


Figure 4. (a) Investigation of the dose-dependent inhibition of KPYC12532 (**5a**) in cell-based assay (A431 cell); (b) Investigation of the A431 cell viability of KPYC12532 (**5a**).

CONCLUSION

In summary, we identified a novel IDO1 inhibitor KPYC12532 (**5a**) having a unique eight-membered ring-fused indole scaffold by screening our compound library. We performed a SAR study of the hit compound **5a** via enzymatic assay and found that some compounds displayed comparable activity to the parent **5a**, providing information about the design of novel inhibitors. Compound **5a** exhibited an IC_{50} value of 4.7 μM in the kynurenine production assay in A431 cells. Among the known inhibitors against IDO1 except for heme binder, the compound **5a** that displays the inhibitory activity at μM level, appears to be a promising lead for the development of novel IDO1 inhibitors.

EXPERIMENTAL

Chemistry

General methods

^1H NMR spectra were recorded using a JEOL ECA-500 spectrometer at 500 MHz frequency. Chemical shifts are reported in δ (ppm) relative to Me_4Si [in CDCl_3 or $\text{DMSO}-d_6$] as the internal standard. ^{13}C NMR spectra were recorded using a JEOL ECA-500 or a JEOL ECZ600R, and referenced to the residual solvent signal. IR spectra were obtained on a JASCO FT/IR-4100 spectrometer. Exact mass (HRMS) spectra were recorded on a Shimadzu LC-ESI-IT-TOF-MS equipment (ESI). Column chromatography was performed using a forced flow (flash chromatography) of the indicated solvent system on Wakogel

C-300E (Wako) or Biotage Isolera flash purification system on Presep[®] Silica Gel Type M (Wako), Presep[®] Silica Gel Type L (Wako), Biotage[®] Sfär Silica D. All the heating experiments were performed in an oil bath. Compounds **5q**, **6a**, **6b**, are commercially available. The known compounds **6c**,²³ **6d**,²³ **6e**,²⁴ **6f**,²⁴ **6g**,²³ **6h**,²⁴ **8a**,²⁵ **8b**,²⁶ **8c**²⁷ were synthesized according to the literatures. The ¹H NMR spectra of **5a**,²⁴ **5b**²³ and **5m**²⁸ were in good accordance with those reported in literatures.

Synthesis

2-[4-(But-2-yn-1-yloxy)but-1-yn-1-yl]aniline (**7a**)

A mixture of 2-iodoaniline (**6a**) (232 mg, 1.06 mmol), **8a** (143 mg, 1.17 mmol), PdCl₂(PPh₃)₂ (74.4 mg, 0.106 mmol), and CuI (10.1 mg, 0.0530 mmol) in Et₃N (5.3 mL) was stirred at 50 °C for 10.5 h under Ar. The mixture was diluted with saturated aqueous NH₄Cl and 28% NH₄OH. The resulting mixture was extracted with EtOAc twice. The combined organic layer was washed with brine, dried over Na₂SO₄, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (hexane/acetone = 10/1) to give **7a** (121 mg, 53%) as yellow oil; IR (neat cm⁻¹): 3465 (NH); ¹H NMR (500 MHz, CDCl₃): δ 1.86 (t, *J* = 2.3 Hz, 3H), 2.77 (t, *J* = 6.9 Hz, 2H), 3.72 (t, *J* = 6.9 Hz, 2H), 4.17 (q, *J* = 2.3 Hz, 2H), 4.22 (s, 2H), 6.62–6.69 (m, 2H), 7.08 (dd, *J* = 7.7, 7.7 Hz, 1H), 7.23 (d, *J* = 7.7 Hz, 1H); ¹³C{¹H} NMR (125 MHz, CDCl₃): δ 3.6, 20.8, 58.7, 68.0, 74.8, 78.1, 82.7, 91.9, 108.3, 114.0, 117.6, 129.0, 131.9, 147.9; HRMS (ESI-TOF) *m/z*: [M + H]⁺ calcd for C₁₄H₁₆NO, 214.1226; found, 214.1217.

4-Bromo-2-[4-(but-2-yn-1-yloxy)but-1-yn-1-yl]aniline (**7b**)

A mixture of 4-bromo-2-iodoaniline (**6b**) (269 mg, 0.902 mmol), **8a** (121 mg, 0.992 mmol), PdCl₂(PPh₃)₂ (31.6 mg, 0.0451 mmol), and CuI (8.59 mg, 0.0451 mmol) in Et₃N (4.5 mL) was stirred at 50 °C for 3.5 h under Ar. The mixture was diluted with saturated aqueous NH₄Cl. The resulting mixture was extracted with EtOAc twice. The combined organic layer was washed with brine, dried over Na₂SO₄, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (hexane/acetone = 10/1) to give **7b** (229 mg, 87%) as yellow oil; IR (neat cm⁻¹): 3456 (NH); ¹H NMR (500 MHz, CDCl₃): δ 1.86 (t, *J* = 2.3 Hz, 3H), 2.75 (t, *J* = 6.9 Hz, 2H), 3.70 (t, *J* = 6.9 Hz, 2H), 4.17 (q, *J* = 2.3 Hz, 2H), 4.24 (s, 2H), 6.54 (d, *J* = 8.6 Hz, 1H), 7.15 (dd, *J* = 8.6, 2.3 Hz, 1H), 7.33 (d, *J* = 2.3 Hz, 1H); ¹³C{¹H} NMR (125 MHz, CDCl₃): δ 3.6, 20.8, 58.7, 67.8, 74.8, 77.0, 82.8, 93.3, 108.6, 110.2, 115.5, 131.8, 134.0, 147.0; HRMS (ESI-TOF) *m/z*: [M + H]⁺ calcd for C₁₄H₁₅BrNO, 292.0332; found, 292.0330.

(Z)-6-Methyl-1,2,4,11-tetrahydrooxocino[4,5-*b*]indole (**5i**)

To a stirred mixture of **7a** (23.5 mg, 0.110 mmol) in *i*-PrOH (1.1 mL) were added AcOH (6.3 μL) and MS 3 Å at room temperature. After the mixture was stirred at this temperature for 30 min, IPrAuNTf₂ (4.78 mg, 0.00551 mmol) was added to the mixture. After being stirred at 80 °C for 3 h, the reaction

mixture was filtered through a short pad of celite and diluted with saturated aqueous NaHCO₃. The resulting mixture was extracted with EtOAc twice. The combined organic layer was washed with brine, dried over Na₂SO₄, filtered, and concentrated in vacuo. The residue was purified by PTLC twice (hexane/acetone = 5/1; toluene/EtOAc = 5/1) to give **5i** (2.85 mg, 12%) as white solid; mp 128–131 °C; ¹H NMR (500 MHz, DMSO-*d*₆, 100 °C): δ 2.25 (s, 3H), 2.81–2.84 (m, 2H), 3.52–3.56 (m, 2H), 3.70–3.74 (m, 2H), 5.72 (t, *J* = 7.9 Hz, 1H), 6.98 (dd, *J* = 8.0, 8.0 Hz, 1H), 7.05 (dd, *J* = 8.0, 8.0 Hz, 1H), 7.32 (d, *J* = 8.0 Hz, 1H), 7.51 (d, *J* = 8.0 Hz, 1H), 10.79 (s, 1H); ¹³C{¹H} NMR (125 MHz, DMSO-*d*₆, 100 °C): δ 22.3, 29.9, 63.4, 66.5, 110.5, 111.7, 118.4, 118.5, 120.1, 121.3, 126.4, 135.1, 135.7, 135.8; HRMS (ESI-TOF) *m/z*: [M + H]⁺ calcd for C₁₄H₁₆NO, 214.1226; found 214.1225.

(Z)-8-Bromo-6-methyl-1,2,4,11-tetrahydrooxocino[4,5-*b*]indole (5h)

According to the procedure described for the preparation of **5i**, compound **7b** (143 mg, 0.489 mmol) was converted to the title compound **5h** (6.9 mg, 4.8%) by the reaction with IPrAuNTf₂ (21.2 mg, 0.0245 mmol) and AcOH (28.0 μL, 0.489 mmol) in *i*-PrOH (4.9 mL) at 80 °C for 4 h: orange solid; mp 172–174 °C; ¹H NMR (500 MHz, DMSO-*d*₆, 100 °C): δ 2.20 (d, *J* = 1.1 Hz, 3H), 2.81 (t, *J* = 4.6 Hz, 2H), 3.52 (t, *J* = 4.6 Hz, 2H), 3.69 (d, *J* = 7.4 Hz, 2H), 5.73 (t, *J* = 7.4 Hz, 1H), 7.15 (dd, *J* = 8.6, 1.7 Hz, 1H), 7.27 (d, *J* = 8.6 Hz, 1H), 7.61 (d, *J* = 1.7 Hz, 1H), 11.03 (s, 1H); ¹³C{¹H} NMR (125 MHz, CDCl₃): δ 23.0, 31.4, 64.2, 67.7, 112.1, 113.1, 113.6, 122.3, 123.2, 124.5, 128.9, 133.8, 136.2, 136.4; HRMS (ESI-TOF) *m/z*: [M + H]⁺ calcd for C₁₄H₁₅BrNO, 292.0332; found, 292.0330.

(Z)-6,11-Dimethyl-1,2,4,11-tetrahydrooxocino[4,5-*b*]indole (5b)

To a suspension of NaH (3.69 mg, 0.154 mmol) in anhydrous THF (0.80 mL) at 0 °C was added dropwise the solution of **5i** (11.5 mg, 0.00539 mmol) in THF (0.40 mL). After the mixture was stirred at 0 °C for 2 h, a solution of MeI (9.6 μL 0.154 mmol) in THF (0.34 mL) was added to the reaction mixture at 0 °C. The mixture was stirred at room temperature for additional 18 h, and then quenched with water. The resulting mixture was extracted with EtOAc twice. The combined organic layer was washed with brine, dried over Na₂SO₄, filtered, and concentrated in vacuo. The residue was purified by PTLC (hexane/acetone = 5/1) to give **5b** (5.50 mg, 45%) as an orange solid. ¹H NMR (500 MHz, CDCl₃): δ 2.30 (s, 3H), 2.67–2.86 (br m, 1H), 3.08–3.28 (br m, 2H), 3.29–3.43 (br m, 1H), 3.72 (s, 3H), 4.02–4.16 (br m, 1H), 4.20–4.33 (br m, 1H), 5.85 (dd, *J* = 8.0, 8.0 Hz, 1H), 7.12 (dd, *J* = 8.0, 8.0 Hz, 1H), 7.22 (dd, *J* = 8.0, 8.0 Hz, 1H), 7.32 (d, *J* = 8.0 Hz, 1H), 7.62 (d, *J* = 8.0 Hz, 1H). The ¹H NMR spectrum was in good agreement with that reported.²³

(Z)-8-Bromo-6,11-dimethyl-1,2,4,11-tetrahydrooxocino[4,5-*b*]indole (5a)

According to the procedure described for the preparation of **5b**, compound **5h** (15.4 mg, 0.0527 mmol) was converted to the title compound **5a** (12.5 mg, 77%) by the reaction with MeI (65.7 μL) and NaH

(42.2 mg, 1.05 mmol) in DMF (2.6 mL) at room temperature for 1 h. ^1H NMR (500 MHz, CDCl_3): δ 2.26 (s, 3H), 2.74–2.76 (br m, 1H), 3.10–3.31 (br m, 3H), 3.69 (s, 3H), 4.08–4.25 (br m, 2H), 5.84 (dd, $J = 7.7$, 7.7 Hz, 1H), 7.17 (d, $J = 8.6$ Hz, 1H), 7.28 (dd, $J = 8.6$, 1.7 Hz, 1H), 7.72 (d, $J = 1.7$ Hz, 1H). The ^1H NMR spectrum was in good agreement with that reported.²⁴

(Z)-11-Benzyl-8-bromo-6-methyl-1,2,4,11-tetrahydrooxocino[4,5-*b*]indole (5j)

To a suspension of NaH (11.2 mg, 0.281 mmol) in anhydrous DMF (0.70 mL) at 0 °C was added dropwise the solution of **5h** (8.20 mg, 0.0281 mmol) in DMF (0.70 mL). After the mixture was stirred at 0 °C for 30 min, a solution of BnBr (33.3 μL , 0.281 mmol) was added to the reaction mixture at 0 °C. The mixture was stirred at room temperature for additional 4 h, and then quenched with saturated aqueous NH_4Cl . The resulting mixture was extracted with EtOAc twice. The combined organic layer was washed with brine, dried over Na_2SO_4 , filtered, and concentrated in vacuo. The residue was purified by PTLC (hexane/EtOAc = 5/1) to give **5j** (5.8 mg, 54%) as pale yellow solid; mp 103–106 °C; ^1H NMR (500 MHz, CDCl_3): δ 2.30 (s, 3H), 2.63–2.89 (br m, 2H), 2.90–3.07 (br m, 1H), 3.20–3.33 (br m, 1H), 3.78–3.90 (br m, 1H), 4.17–4.29 (br m, 1H), 5.33 (s, 2H), 5.86 (dd, $J = 7.4$, 7.4 Hz, 1H), 6.95 (d, $J = 6.9$ Hz, 2H), 7.15 (d, $J = 8.6$ Hz, 1H), 7.24–7.30 (m, 4H), 7.77 (d, $J = 1.1$ Hz, 1H); $^{13}\text{C}\{^1\text{H}\}$ NMR (125 MHz, CDCl_3): δ 23.2, 28.5, 46.7, 63.5, 67.5, 111.0, 113.0, 113.8, 122.4, 123.3, 124.4, 125.9 (2C), 127.7, 127.8, 129.0 (2C), 135.6, 136.1, 137.2, 137.8; HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{21}\text{H}_{21}\text{BrNO}$, 382.0801; found, 382.0802.

8-Bromo-6,11-dimethyl-1,2,4,5,6,11-hexahydrooxocino[4,5-*b*]indole (5l)

A mixture of **5a** (9.30 mg, 0.0304 mmol) and PtO_2 (1.38 mg, 0.00607 mmol) in EtOH (0.61 mL) was stirred at room temperature for 5.5 h under H_2 . The reaction mixture was filtered through a short pad of celite and silica gel, and the filtrate was evaporated in vacuo to give a crude product, which was purified by PTLC (hexane/acetone = 5/1) to give **5l** (2.33 mg, 25%) as white solid; mp 138–140 °C; ^1H NMR (500 MHz, CDCl_3): δ 1.52–1.58 (m, 1H), 1.65 (d, $J = 7.4$ Hz, 3H), 2.05–2.15 (m, 1H), 2.84–2.91 (m, 2H), 3.11–3.17 (m, 1H), 3.36–3.44 (m, 2H), 3.66 (s, 3H), 3.80–3.85 (m, 1H), 4.10–4.14 (m, 1H), 7.15 (d, $J = 8.6$ Hz, 1H), 7.23 (dd, $J = 8.6$, 1.7 Hz, 1H), 7.83 (d, $J = 1.7$ Hz, 1H); $^{13}\text{C}\{^1\text{H}\}$ NMR (125 MHz, CDCl_3): δ 19.9, 27.4, 28.5, 29.6, 40.3, 70.7, 72.2, 110.6, 112.0, 114.8, 122.2, 123.1, 127.9, 135.8, 137.0; HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{15}\text{H}_{19}\text{BrNO}$, 308.0645; found, 308.0645.

2-[4-(But-2-yn-1-yloxy)but-1-yn-1-yl]-4-fluoro-*N*-methylaniline (7c)

A mixture of 4-fluoro-2-iodo-*N*-methylaniline (**6c**) (220 mg, 0.876 mmol), **8a** (118 mg, 0.963 mmol), $\text{PdCl}_2(\text{PPh}_3)_2$ (61.5 mg, 0.0876 mmol), CuI (8.34 mg, 0.0438 mmol) and Et_3N (0.38 mL) in DMF (2.9 mL) was stirred at room temperature for 10 h under Ar. The mixture was diluted with saturated aqueous NH_4Cl . The resulting mixture was extracted with EtOAc twice. The combined organic layer was washed with brine, dried over Na_2SO_4 , filtered, and concentrated in vacuo. The residue was purified by column

chromatography on silica gel (hexane/acetone = 10/1) to give **7c** (129 mg, <60%), including impurities, as red oil; IR (neat cm^{-1}): 3397 (NH); ^1H NMR (500 MHz, CDCl_3): δ 1.87 (t, $J = 2.3$ Hz, 3H), 2.77 (t, $J = 6.9$ Hz, 2H), 2.87 (s, 3H), 3.72 (t, $J = 6.9$ Hz, 2H), 4.18 (q, $J = 2.3$ Hz, 2H), 4.55 (s, 1H), 6.46 (dd, $J = 8.9$, 4.6 Hz, 1H), 6.91 (ddd, $J = 8.9$, 8.9, 3.2 Hz, 1H), 6.96 (dd, $J = 8.9$, 3.2 Hz, 1H); $^{13}\text{C}\{^1\text{H}\}$ NMR (125 MHz, CDCl_3): δ 3.5, 20.8, 30.7, 58.7, 67.8, 74.8, 77.5, 82.7, 93.2, 108.3 (d, $J_{\text{C-F}} = 9.6$ Hz), 109.3 (d, $J_{\text{C-F}} = 8.4$ Hz), 116.0 (d, $J_{\text{C-F}} = 22.8$ Hz), 117.9 (d, $J_{\text{C-F}} = 22.8$ Hz), 146.8, 154.2 (d, $J_{\text{C-F}} = 233.9$ Hz); HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{15}\text{H}_{17}\text{FNO}$, 246.1289; found, 246.1286.

2-[4-(But-2-yn-1-yloxy)but-1-yn-1-yl]-4-chloro-*N*-methylaniline (7d)

According to the procedure described for the preparation of **7c**, compound **6d** (320 mg, 1.20 mmol) was converted to the title compound **7d** (112 mg, 36%) by the reaction with **8a** (161 mg, 1.32 mmol), $\text{PdCl}_2(\text{PPh}_3)_2$ (42.0 mg, 0.0598 mmol), CuI (11.4 mg, 0.0598 mmol), and Et_3N (0.52 mL) in DMF (4.0 mL) at room temperature for 10.5 h under Ar: yellow oil; IR (neat cm^{-1}): 3418 (NH); ^1H NMR (500 MHz, CDCl_3): δ 1.86 (t, $J = 2.3$ Hz, 3H), 2.76 (t, $J = 6.9$ Hz, 2H), 2.86 (d, $J = 2.9$ Hz, 3H), 3.71 (t, $J = 6.6$ Hz, 2H), 4.18 (q, $J = 2.3$ Hz, 2H), 4.70–4.72 (br m, 1H), 6.45 (d, $J = 8.6$ Hz, 1H), 7.12 (dd, $J = 8.6$, 2.3 Hz, 1H), 7.19 (d, $J = 2.3$ Hz, 1H); $^{13}\text{C}\{^1\text{H}\}$ NMR (125 MHz, CDCl_3): δ 3.6, 20.8, 30.3, 58.7, 67.8, 74.8, 77.2, 82.7, 93.5, 109.0, 109.7, 120.1, 129.2, 131.0, 148.6; HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{15}\text{H}_{17}\text{ClNO}$, 262.0993; found, 262.0993.

2-[4-(But-2-yn-1-yloxy)but-1-yn-1-yl]-*N*,4-dimethylaniline (7e)

According to the procedure described for the preparation of **7c**, compound **6e** (158 mg, 0.641 mmol) was converted to the title compound **7e** (41.2 mg, 27%) by the reaction with **8a** (86.2 mg, 0.705 mmol), $\text{PdCl}_2(\text{PPh}_3)_2$ (45.0 mg, 0.0641 mmol), CuI (6.10 mg, 0.0321 mmol), and Et_3N (0.28 mL) in DMF (2.1 mL) at room temperature for 5 h under Ar: brown oil; IR (neat cm^{-1}): 3404 (NH); ^1H NMR (500 MHz, CDCl_3): δ 1.86 (t, $J = 2.3$ Hz, 3H), 2.19 (s, 3H), 2.76 (t, $J = 6.9$ Hz, 2H), 2.87 (s, 3H), 3.71 (t, $J = 6.9$ Hz, 2H), 4.17 (q, $J = 2.3$ Hz, 2H), 4.53 (s, 1H), 6.48 (d, $J = 8.0$ Hz, 1H), 6.99 (dd, $J = 8.0$, 1.7 Hz, 1H), 7.07 (d, $J = 1.7$ Hz, 1H); $^{13}\text{C}\{^1\text{H}\}$ NMR (125 MHz, CDCl_3): δ 3.6, 20.1, 20.9, 30.5, 58.7, 68.1, 74.9, 78.4, 82.6, 91.9, 107.7, 108.9, 125.0, 130.0, 132.1, 147.9; HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{16}\text{H}_{20}\text{NO}$, 242.1539; found, 242.1543.

2-[4-(But-2-yn-1-yloxy)but-1-yn-1-yl]-4-methoxy-*N*-methylaniline (7f)

According to the procedure described for the preparation of **7c**, compound **6f** (551 mg, 2.55 mmol) was converted to the title compound **7f** (124 mg, 19%) by the reaction with **8a** (464 mg, 3.80 mmol), $\text{PdCl}_2(\text{PPh}_3)_2$ (179 mg, 0.255 mmol), CuI (48.6 mg, 0.255 mmol), and Et_3N (1.1 mL) in DMF (8.5 mL) at 80 °C for 5 h under Ar: brown oil; ^1H NMR (500 MHz, CDCl_3): δ 1.87 (t, $J = 2.3$ Hz, 3H), 2.77 (t, $J = 6.9$ Hz, 2H), 2.87 (s, 3H), 3.70–3.74 (m, 5H), 4.18 (q, $J = 2.3$ Hz, 2H), 4.38 (s, 1H), 6.52 (d, $J = 8.6$ Hz, 1H), 6.82 (dd, $J = 8.6$, 2.9 Hz, 1H), 6.85 (d, $J = 2.9$ Hz, 1H); $^{13}\text{C}\{^1\text{H}\}$ NMR (125 MHz, CDCl_3): δ 3.6, 20.9,

30.9, 55.9, 58.7, 68.0, 74.8, 78.2, 82.7, 92.3, 108.3, 110.1, 116.2, 116.8, 145.0, 150.6; HRMS (ESI-TOF) m/z : $[M + H]^+$ calcd for $C_{16}H_{20}NO_2$, 258.1489; found, 258.1487.

5-Bromo-2-[4-(but-2-yn-1-yloxy)but-1-yn-1-yl]-*N*-methylaniline (7g)

According to the procedure described for the preparation of **7c**, compound **6g** (214 mg, 0.686 mmol) was converted to the title compound **7g** (62.2 mg, 30%) by the reaction with **8a** (92.2 mg, 0.755 mmol), $PdCl_2(PPh_3)_2$ (24.1 mg, 0.0343 mmol), CuI (6.53 mg, 0.0343 mmol), and Et_3N (0.30 mL) in DMF (2.3 mL) at room temperature for 10.5 h under Ar: yellow oil; IR (neat cm^{-1}): 3397 (NH); 1H NMR (500 MHz, $CDCl_3$): δ 1.86 (t, $J = 2.3$ Hz, 3H), 2.75 (t, $J = 6.9$ Hz, 2H), 2.87 (d, $J = 5.2$ Hz, 3H), 3.70 (t, $J = 6.9$ Hz, 2H), 4.17 (q, $J = 2.3$ Hz, 2H), 4.78–4.82 (br m, 1H), 6.66 (d, $J = 2.3$ Hz, 1H), 6.70 (dd, $J = 8.0, 2.3$ Hz, 1H), 7.06 (d, $J = 8.0$ Hz, 1H); $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$): δ 3.6, 20.9, 30.1, 58.7, 67.8, 74.8, 77.5, 82.7, 93.4, 106.6, 111.6, 118.6, 123.5, 132.6, 150.9; HRMS (ESI-TOF) m/z : $[M + H]^+$ calcd for $C_{15}H_{17}BrNO$, 306.0488; found, 306.0482.

(*Z*)-8-Fluoro-6,11-dimethyl-1,2,4,11-tetrahydrooxocino[4,5-*b*]indole (5c)

To a stirred mixture of **7c** (51.0 mg, 0.208 mmol) including impurities in *i*-PrOH (2.1 mL) was added MS 3 Å at room temperature. After the mixture was stirred at this temperature for 30 min, $IPrAuNTf_2$ (9.01 mg, 0.0104 mmol) was added to the mixture. After being stirred at 80 °C for 10 h, the reaction mixture was filtered through a short pad of celite and silica gel, and the filtrate was evaporated in vacuo to give a crude product, which was purified by PTLC twice (hexane/acetone = 5/1; toluene/EtOAc = 5/1) to give **5c** (2.8 mg, *ca.* 5.5%) as yellow oil; 1H NMR (500 MHz, $CDCl_3$): δ 2.26 (s, 3H), 2.68–2.83 (br m, 1H), 3.04–3.26 (br m, 2H), 3.27–3.42 (br m, 1H), 3.71 (s, 3H), 4.00–4.16 (br m, 1H), 4.17–4.33 (br m, 1H), 5.83 (dd, $J = 7.2, 7.2$ Hz, 1H), 6.95 (ddd, $J = 9.0, 9.0, 2.5$ Hz, 1H), 7.21 (dd, $J = 9.0, 4.4$ Hz, 1H), 7.24–7.28 (m, 1H); $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$): δ 23.0, 28.5, 29.9, 63.7, 67.6, 104.8 (d, $J_{C-F} = 23.1$ Hz), 109.3 (d, $J_{C-F} = 26.0$ Hz), 109.7 (d, $J_{C-F} = 10.1$ Hz), 113.5 (d, $J_{C-F} = 4.3$ Hz), 122.7, 126.2 (d, $J_{C-F} = 11.6$ Hz), 133.5, 136.6, 138.4, 157.7 (d, $J_{C-F} = 234.1$ Hz); HRMS (ESI-TOF) m/z : $[M + H]^+$ calcd for $C_{15}H_{17}FNO$, 246.1289; found, 246.1290.

(*Z*)-8-Chloro-6,11-dimethyl-1,2,4,11-tetrahydrooxocino[4,5-*b*]indole (5d)

According to the procedure described for the preparation of **5c**, **7d** (50.9 mg, 0.194 mmol) was converted into **5d** (2.3 mg, 4.5%) by the reaction with $IPrAuNTf_2$ (8.43 mg, 0.00972 mmol) in *i*-PrOH (1.9 mL) at 80 °C for 6 h: white solid; mp 140–142 °C; 1H NMR (500 MHz, $CDCl_3$): δ 2.27 (s, 3H), 2.70–2.80 (br m, 1H), 3.06–3.26 (br m, 2H), 3.27–3.39 (br m, 1H), 3.70 (s, 3H), 4.00–4.16 (br m, 1H), 4.18–4.33 (br m, 1H), 5.84 (dd, $J = 7.7, 7.7$ Hz, 1H), 7.15 (dd, $J = 8.6, 1.7$ Hz, 1H), 7.22 (d, $J = 8.6$ Hz, 1H), 7.57 (d, $J = 1.7$ Hz, 1H); $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$): δ 23.2, 28.4, 29.9, 63.6, 67.5, 110.2, 113.2, 119.2, 121.4, 123.0, 125.2, 127.0, 135.4, 136.5, 138.1; HRMS (ESI-TOF) m/z : $[M + H]^+$ calcd for $C_{15}H_{17}ClNO$, 262.0993; found, 262.0991.

(Z)-6,8,11-Trimethyl-1,2,4,11-tetrahydrooxocino[4,5-*b*]indole (5e)

According to the procedure described for the preparation of **5c**, **7e** (11.8 mg, 0.0488 mmol) was converted into **5e** (3.85 mg, 33%) by the reaction with IPrAuNTf₂ (2.11 mg, 0.00244 mmol) in *i*-PrOH (0.49 mL) at 80 °C for 5 h: yellow oil; ¹H NMR (500 MHz, CDCl₃): δ 2.29 (s, 3H), 2.45 (s, 3H), 2.67–2.78 (br m, 1H), 3.07–3.23 (br m, 2H), 3.28–3.38 (br m, 1H), 3.69 (s, 3H), 4.01–4.13 (br m, 1H), 4.18–4.31 (br m, 1H), 5.83 (dd, *J* = 8.0, 8.0 Hz, 1H), 7.04 (d, *J* = 8.6 Hz, 1H), 7.20 (d, *J* = 8.6 Hz, 1H), 7.40 (s, 1H); ¹³C{¹H} NMR (150 MHz, CDCl₃): δ 21.5, 23.3, 28.4, 29.7, 63.7, 67.6, 109.0, 112.9, 119.4, 122.3, 122.7, 126.2, 128.8, 135.3, 136.9, 137.5; HRMS (ESI-TOF) *m/z*: [M + H]⁺ calcd for C₁₆H₂₀NO, 242.1539; found, 242.1537.

(Z)-8-Methoxy-6,11-dimethyl-1,2,4,11-tetrahydrooxocino[4,5-*b*]indole (5f)

According to the procedure described for the preparation of **5c**, **7f** (14.7 mg, 0.0571 mmol) was converted into **5f** (2.66 mg, 18%) by the reaction with IPrAuNTf₂ (2.47 mg, 0.00286 mmol) in *i*-PrOH (0.57 mL) at 80 °C for 3.5 h: white solid; mp 104–105 °C; ¹H NMR (500 MHz, CDCl₃): δ 2.29 (s, 3H), 2.67–2.78 (br m, 1H), 3.05–3.26 (br m, 2H), 3.29–3.42 (br m, 1H), 3.69 (s, 3H), 3.86 (s, 3H), 4.02–4.12 (br m, 1H), 4.20–4.32 (br m, 1H), 5.84 (dd, *J* = 7.7, 7.7 Hz, 1H), 6.87 (dd, *J* = 8.6, 2.3 Hz, 1H), 7.07 (d, *J* = 2.3 Hz, 1H), 7.20 (d, *J* = 8.6 Hz, 1H); ¹³C{¹H} NMR (150 MHz, CDCl₃): δ 23.1, 28.5, 29.8, 56.0, 63.8, 67.6, 102.1, 109.9, 110.9, 113.1, 122.4, 126.3, 132.2, 137.2, 137.5, 154.0; HRMS (ESI-TOF) *m/z*: [M + H]⁺ calcd for C₁₆H₂₀NO₂, 258.1489; found, 258.1486.

(Z)-9-Bromo-6,11-dimethyl-1,2,4,11-tetrahydrooxocino[4,5-*b*]indole (5g)

According to the procedure described for the preparation of **5c**, **7g** (42.3 mg, 0.138 mmol) was converted into **5g** (1.6 mg, 3.8%) by the reaction with IPrAuNTf₂ (5.99 mg, 0.00691 mmol) in *i*-PrOH (1.4 mL) at 80 °C for 11 h: colorless amorphous solid; ¹H NMR (500 MHz, CDCl₃): δ 2.26 (s, 3H), 2.67–2.81 (br m, 1H), 3.07–3.25 (br m, 2H), 3.26–3.40 (br m, 1H), 3.68 (s, 3H), 4.01–4.16 (br m, 1H), 4.18–4.31 (br m, 1H), 5.84 (dd, *J* = 7.2, 7.2 Hz, 1H), 7.21 (dd, *J* = 8.6, 1.7 Hz, 1H), 7.44–7.48 (m, 2H); ¹³C{¹H} NMR (150 MHz, CDCl₃): δ 23.2, 28.3, 29.8, 63.5, 67.5, 112.3, 113.6, 114.8, 120.9, 122.7, 123.0, 124.9, 136.5, 137.3, 137.8; HRMS (ESI-TOF) *m/z*: [M + H]⁺ calcd for C₁₅H₁₇BrNO, 306.0488; found, 306.0479.

2-Bromo-5-methyl-6,7,8,9,10,11-hexahydro-5*H*-cycloocta[*b*]indole (5m)

According to the procedure described for the preparation of **5b**, compound **5q** (10.8 mg, 0.0388 mmol) was converted to the title compound **5m** (8.57 mg, 76%) by the reaction with MeI (48 μL, 0.776 mmol) and NaH (31.0 mg, 0.776 mmol) in THF (1.9 mL) at room temperature for 3.5 h under Ar. ¹H NMR (500 MHz, CDCl₃): δ 1.36–1.46 (m, 2H), 1.64–1.77 (m, 4H), 2.79–2.84 (m, 2H), 2.86–2.90 (m, 2H), 3.64 (s, 3H), 7.11 (d, *J* = 8.6 Hz, 1H), 7.20 (dd, *J* = 8.6, 1.7 Hz, 1H), 7.60 (d, *J* = 1.7 Hz, 1H). The ¹H NMR spectrum was in good agreement with that reported.²⁸

4-Bromo-2-[3-(but-2-yn-1-yloxy)prop-1-yn-1-yl]aniline (7h)

According to the procedure described for the preparation of **7c**, compound **6b** (333 mg, 1.12 mmol) was converted to the title compound **7h** (245 mg, 79%) by the reaction with **8b** (133 mg, 1.23 mmol), PdCl₂(PPh₃)₂ (39.2 mg, 0.0558 mmol), CuI (10.6 mg, 0.0558 mmol) and Et₃N (0.49 mL) in DMF (3.7 mL) at room temperature for 22 h under Ar: orange oil; IR (neat cm⁻¹): 3369 (NH), 3477 (NH); ¹H NMR (500 MHz, CDCl₃): δ 1.88 (t, *J* = 2.3 Hz, 3H), 4.22–4.24 (br m, 2H), 4.27 (q, *J* = 2.3 Hz, 2H), 4.50 (s, 2H), 6.57 (d, *J* = 8.6 Hz, 1H), 7.20 (dd, *J* = 8.6, 2.3 Hz, 1H), 7.39 (d, *J* = 2.3 Hz, 1H); ¹³C {¹H} NMR (125 MHz, CDCl₃): δ 3.6, 57.1, 57.2, 74.2, 81.9, 83.4, 90.9, 108.7, 108.9, 115.7, 132.7, 134.5, 147.1; HRMS (ESI-TOF) *m/z*: [M + H]⁺ calcd for C₁₃H₁₃BrNO, 278.0175; found, 278.0171.

4-Bromo-2-[3-(but-2-yn-1-yloxy)prop-1-yn-1-yl]-N-methylaniline (7i)

To a stirred solution of **7h** (94.3 mg, 0.339 mmol) in THF (1.1 mL) was added MeLi (1.09 M in Et₂O; 0.370 mL, 0.407 mmol) at –78 °C under argon. After the mixture was stirred at this temperature for 1 h, MeI (27.5 μL, 0.441 mmol) was added to the mixture. The mixture was gradually warmed to room temperature and stirred for 4 h at this temperature. The reaction was quenched with saturated aqueous NH₄Cl. The resulting mixture was extracted with EtOAc twice. The combined organic layer was washed with brine, dried over Na₂SO₄, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (gradient 4 to 15% EtOAc in hexane) to give **7i** (42.8 mg, 43%) as orange oil; IR (neat cm⁻¹): 3412 (NH); ¹H NMR (500 MHz, CDCl₃): δ 1.88 (t, *J* = 2.3 Hz, 3H), 2.87 (d, *J* = 5.2 Hz, 3H), 4.26 (q, *J* = 2.3 Hz, 2H), 4.50 (s, 2H), 4.60–4.66 (br m, 1H), 6.44 (d, *J* = 9.2 Hz, 1H), 7.29 (dd, *J* = 9.2, 2.3 Hz, 1H), 7.38 (d, *J* = 2.3 Hz, 1H); ¹³C {¹H} NMR (125 MHz, CDCl₃): δ 3.6, 30.2, 57.15, 57.25, 74.2, 82.0, 83.4, 91.2, 107.0, 108.2, 110.5, 132.9, 134.4, 149.1; HRMS (ESI-TOF) *m/z*: [M + H]⁺ calcd for C₁₄H₁₅BrNO, 292.0332; found, 292.0325.

7-Bromo-5,10-dimethyl-3,10-dihydro-1H-oxepino[3,4-*b*]indole (5n)

To a stirred mixture of **7i** (24.0 mg, 0.0828 mmol) in *i*-PrOH (0.83 mL) was added IPrAuNTf₂ (3.59 mg, 0.00414 mmol) at room temperature. After being stirred at 80 °C for 7 h, the reaction mixture was filtered through a short pad of celite and silica gel, and the filtrate was evaporated in vacuo to give a crude product, which was purified by PTLC (hexane/acetone = 5/1) on amine silica gel to give **5n** (6.94 mg, 29%) as yellow oil; ¹H NMR (500 MHz, CDCl₃): δ 2.40 (d, *J* = 1.1 Hz, 3H), 3.70 (s, 3H), 4.01 (d, *J* = 6.2 Hz, 2H), 4.76 (s, 2H), 5.85 (tq, *J* = 6.2, 1.1 Hz, 1H), 7.20 (d, *J* = 8.9 Hz, 1H), 7.32 (dd, *J* = 8.9, 1.7 Hz, 1H), 7.95 (d, *J* = 1.7 Hz, 1H); ¹³C {¹H} NMR (125 MHz, CDCl₃): δ 23.1, 29.9, 62.9, 66.1, 110.9, 113.3, 115.1, 122.9, 123.3, 124.6, 127.2, 135.9, 137.6, 139.2; HRMS (ESI-TOF) *m/z*: [M + H]⁺ calcd for C₁₄H₁₅BrNO, 292.0332; found, 292.0336.

tert-Butyl {3-[5-bromo-2-(methylamino)phenyl]prop-2-yn-1-yl}(but-2-yn-1-yl)carbamate (7j)

According to the procedure described for the preparation of **7c**, compound **6h** (243 mg, 0.777 mmol) was

converted to the title compound **7j** (248 mg, 81%) by the reaction with **8c** (177 mg, 0.855 mmol), PdCl₂(PPh₃)₂ (27.3 mg, 0.0389 mmol), CuI (7.40 mg, 0.0389 mmol) and Et₃N (0.34 mL) in DMF (2.6 mL) at room temperature for 10 h under Ar: yellow oil; IR (neat cm⁻¹): 3417 (NH), 1695 (C=O); ¹H NMR (500 MHz, DMSO-*d*₆ 100 °C): δ 1.50 (s, 9H), 1.80–1.84 (m, 3H), 2.86 (d, *J* = 4.0 Hz, 3H), 4.07–4.17 (br m, 2H), 4.39 (s, 2H), 5.21–5.28 (br m, 1H), 6.43 (d, *J* = 8.6 Hz, 1H), 7.25–7.29 (m, 1H), 7.34 (d, *J* = 2.3 Hz, 1H); ¹³C{¹H} NMR (125 MHz, DMSO-*d*₆, 100 °C): δ 2.4, 27.5 (3C), 29.3, 35.7, 36.1, 74.3, 78.3, 79.1, 79.6, 91.6, 104.9, 107.9, 110.5, 131.9, 132.9, 149.0, 153.4; HRMS (ESI-TOF) *m/z*: [M + H]⁺ calcd for C₁₉H₂₄BrN₂O₂, 391.1016; found, 391.1014.

***tert*-Butyl 7-bromo-5,10-dimethyl-3,10-dihydroazepino[3,4-*b*]indole-2(1*H*)-carboxylate (5o)**

According to the procedure described for the preparation of **5n**, **7j** (217 mg, 0.554 mmol) was converted into **5o** (134 mg, 62%) by the reaction with JohnPhosAuNTf₂ (21.5 mg, 0.0277 mmol) in *i*-PrOH (5.5 mL) at 80 °C for 4 h: yellow solid; mp 129–132 °C; IR (neat cm⁻¹): 1691 (C=O); ¹H NMR (500 MHz, DMSO-*d*₆, 100 °C): δ 1.38 (s, 9H), 2.30 (d, *J* = 1.1 Hz, 3H), 3.74 (s, 3H), 3.86 (d, *J* = 5.2 Hz, 2H), 4.65 (s, 2H), 5.64–5.69 (m, 1H), 7.25 (dd, *J* = 8.6, 2.0 Hz, 1H), 7.42 (d, *J* = 8.6 Hz, 1H), 7.86 (d, *J* = 2.0 Hz, 1H); ¹³C{¹H} NMR (125 MHz, DMSO-*d*₆, 100 °C): δ 23.0, 27.6 (3C), 29.3, 42.4, 45.0, 78.8, 111.4, 111.7, 111.8, 121.2, 121.9, 123.1, 126.8, 132.5, 135.1, 138.2, 153.6; HRMS (ESI-TOF) *m/z*: [M + H]⁺ calcd for C₁₉H₂₄BrN₂O₂, 391.1016; found, 391.1007.

7-Bromo-5,10-dimethyl-1,2,3,10-tetrahydroazepino[3,4-*b*]indole (5p)

To a solution of **5o** (63.0 mg, 0.161 mmol) in CH₂Cl₂ (1.6 mL) was added TFA (1.6 mL). After being stirred at room temperature for 40 min, the mixture was diluted with saturated aqueous NaHCO₃. The resulting mixture was extracted with CH₂Cl₂ twice. The combined organic layer was washed with brine, dried over Na₂SO₄, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (CH₂Cl₂/MeOH = 10/1) to give **5p** (33.3 mg, 71%) as orange oil; ¹H NMR (500 MHz, CDCl₃): δ 2.35 (s, 3H), 3.38 (d, *J* = 6.3 Hz, 2H), 3.85 (s, 3H), 4.19 (s, 2H), 5.89 (t, *J* = 6.3 Hz, 1H), 7.23 (d, *J* = 8.6 Hz, 1H), 7.37 (dd, *J* = 8.6, 1.4 Hz, 1H), 7.86 (d, *J* = 1.4 Hz, 1H); ¹³C{¹H} NMR (125 MHz, CDCl₃): δ 22.4, 30.4, 39.2, 42.4, 111.5, 113.8, 116.5, 118.1, 123.0, 125.7, 126.4, 133.9, 136.2, 142.0; HRMS (ESI-TOF) *m/z*: [M + H]⁺ calcd for C₁₄H₁₆BrN₂, 291.0491; found, 291.0488.

Biology

Inhibitory activity for cellular kynurenine production and cell viability

The kynurenine production in A431 cells was determined as follows. In brief, A431 cells (3.0 × 10⁵ cells/mL) were seeded in a 96-well culture plate (100 μL/well) and grown overnight. Serial DMSO dilutions of compounds in a total volume of 100 μL culture medium including tryptophan and human IFN-γ (10 ng/mL final concentration) per well were added into wells containing the cells. After an

additional 24 h of incubation, 200 μL /well of a mixed solution of 7% (v/v) aqueous CCl_3COOH and 2% (w/v) *p*-dimethylaminobenzaldehyde in acetic acid (2:5) was added into each well. The yellow color derived from kynurenine was measured at 460 nm using a SpectraMax M5 multi-mode microplate reader (Molecular Devices). The cell viability of A431 cells was assessed using CCK-8 assay.

rhIDO inhibitory activity

The rhIDO activity was determined as follow. In brief, the standard 200 μL reaction mixture contained 50 mM potassium phosphate buffer (pH 6.5), 20 mM ascorbic acid (neutralized with NaOH and HCl), 100 $\mu\text{g}/\text{mL}$ catalase, 10 μM methylene blue, 0.2 mM L-tryptophan, 0.26 $\mu\text{g}/\text{mL}$ rhIDO (R & D systems), and 2% DMSO. The reaction was carried out at 37 $^\circ\text{C}$ for 120 min and stopped by the addition of 40 μL of 30% (w/v) $\text{CCl}_3\text{CO}_2\text{H}$. After heating at 50 $^\circ\text{C}$ for 15 min, the reaction mixture was centrifuged at 15000g for 5 min. The supernatant (150 μL) was transferred into a well of a 96-well microplate and mixed with 150 μL of 2% (w/v) *p*-dimethylaminobenzaldehyde in acetic acid. The yellow pigment derived from kynurenine was measured at 490 nm using a SpectraMax M5 multi-mode microplate reader (Molecular Devices).

ACKNOWLEDGEMENTS

This work was supported by the JSPS KAKENHI (grant numbers JP18H04615, JP18H04408 and JP18H02559 and JP17H03971), by the Japan Agency for Medical Research and Development (AMED) (grant numbers JP18gm1010007 and JP18ak0101072), by the Platform Project for Supporting Drug Discovery and Life Science Research (Platform for Drug Discovery, Informatics, and Structural Life Science) from the AMED, and by the Uehara Memorial Foundation.

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