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DESIGN, SYNTHESIS, PHYSICAL PROPERTIES AND INDOLEAMINE 2, 3-DIOXYGENASE 1 INHIBITORY ACTIVITY OF SUBSTITUED INDOLE DERIVATIVES WITH *N*-H, *N*-METOXYMETHYL, OR *N*-MEHYLTHIOMETHYL GROUPS TOWARD FRAGMENT-BASED DRUG DISCOVERY§

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Abstract – We designed and prepared *N*-H, *N*-CH_2OMe (MOM), and *N*-CH_2SMe (MTM) forms of various substituted indoles, using a docking study, as a small fragment library toward fragment-based drug discovery (FBDD) and evaluated their indoleamine 2, 3-dioxygenase 1 (IDO1) inhibitory activities and physical properties.

Indoleamine 2, 3-dioxygenase 1 (IDO1), a well-characterized immunosuppressive enzyme, has attracted growing attention as a potential target for cancer immunotherapy.[1] However, current literature indicates that we are far from understanding the biological relevance of IDO1 expression during tumorigenesis.[2] Further, 1-methyl-D-tryptophan (also known as indoximod or NLG8189), epacadostat (also known as INCB24360), NLG919 (also known as GDC-0919), and F001287 have entered clinical development (Figure 1).[3] However, the failure of clinical trials of several IDO1 inhibitors, including INCB24360, NLG919, and F001287, has been known since the winter of 2017. Thus, discovery of new IDO1 inhibition strategies is extremely urgent.

Some IDO1 inhibitors reported thus far have indole or indole-like structures (Figure 1). In general, the indole scaffold is widely distributed in natural products and bioactive molecules. In view of its unique

physico-chemical and biological properties, it has been used as a privileged scaffold in medicinal chemistry. To date, many natural and synthetic indole derivatives have been discovered as promising agents used in clinical evaluations. On the other hand, current research has not yet reported the effect of *N*-CH_2OMe (MOM) or *N*-CH_2SMe (MTM) substituents on the 1-position nitrogen of poly-substituted indoles on IDO1 inhibitory activity.

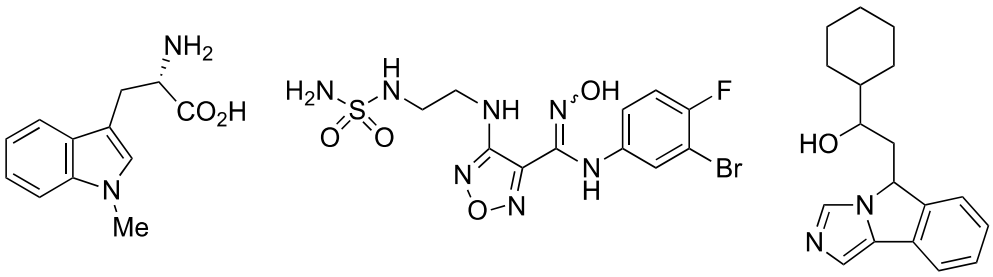
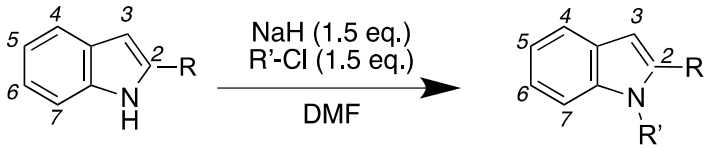


Figure 1. Reported IDO1 inhibitors

Therefore, we prepared *N*-H, *N*-MOM, and *N*-MTM forms of various substituted indoles as a small fragment library toward fragment-based drug discovery (FBDD)[4] and evaluated their IDO1 inhibitory activities and physical properties. In addition, the docking score between each indole derivative obtained and IDO1 was determined by computational chemistry. The correlation between this docking score and IDO1 inhibitory activity was analyzed to design new molecules, which were also synthesized to evaluate their IDO1 inhibitory activities and physical properties.

First, *N*-MOM and *N*-MTM forms of 2-methylindole and 2-phenylindole were synthesized using standard *N*-alkylation conditions of indole derivatives (Table 1). IDO1 inhibitory activity, solubility, and artificial membrane permeability tests (PAMPA) of these synthesized compounds (including the starting material with the NH moiety) were evaluated (Table 2). An IDO1 cell-based assay, kynurenine production, showed the ratio of tryptophan converted to kynurenine by IDO1. Smaller percentages of kynurenine production indicate higher IDO1 inhibitory activity. Solubility describes the solubility in the second fluid for dissolution test, with higher values indicating higher solubility. The 2-methylindole derivative showed almost no IDO1 inhibition (runs 1–3), whereas the *N*-H, *N*-MOM, and *N*-MTM forms of 2-phenylindole showed kynurenine production rates (runs 4–6) of 31, 44, and 27%, respectively, where the kynurenine production rate is an index of IDO1 inhibitory activity. Compound **1f** showed the highest IDO1 inhibitory activity, though almost unsoluble in this solubility experiment (run 6).

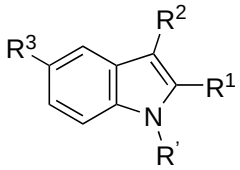
Table 1. Preparation of *N*-MOM and *N*-MTM derivatives from substituted *N*-H indole



1a: R = 2-Me, **1d**: 2-Ph R' = MOM or MTM

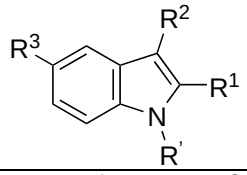
run	substrate	product		yield (%)
			R' =	
1	**1a**	**1b**	MOM	97
2	**1a**	**1c**	MTM	63
3	**1d**	**1e**	MOM	99
4	**1d**	**1f**	MTM	55

Table 2. IDO1 inhibitory activity and physical properties of compounds **1a** – **1f**

run						IDO cell based assay (50 μM)	solubility (μM)	PAMPA (X 10^{-6} cm/sec)	Glide docking score (kcal/mol)
		R^1 =	R^2 =	R^3 =	R' =	Kynurenine production (%)			
1	**1a**	Me	H	H	H	92	42	31.9	-7.262
2	**1b**	Me	H	H	MOM	86	25	34.7	-6.654
3	**1c**	Me	H	H	MTM	79	16	39.7	-7.120
4	**1d**	Ph	H	H	H	31	23	3.9	-6.492
5	**1e**	Ph	H	H	MOM	44	6.3	42.9	-5.235
6	**1f**	Ph	H	H	MTM	27	<5	-	-6.227

Based on these results, we designed and synthesized indole derivatives with various aryl groups at the 2-position and investigated their functions (Table 3). Specifically, derivatives with fluorine groups as electron-withdrawing moieties on the phenyl groups (*para* position) at the 2-position of indole were prepared (runs 1–3). In addition to derivatives having electron-donating methyl or methoxy groups on the phenyl group (*para* position; Table 2, runs 4–6 vs. Table 3, runs 4–9), compounds with both a phenyl group at the 2-position and a methoxycarbonyl group at another position (3- or 5-position; runs 10-13) on the indole were also prepared. *N*-MOM and *N*-MTM derivatives were synthesized from the corresponding *N*-H indole compound using the reaction conditions in Table 1.

Table 3. IDO1 inhibitory activity and physical properties of compounds **1g** – **1s**

run						IDO cell based assay (50 μM)	solubility (μM)	PAMPA (X 10^{-6} cm/sec)	Glide docking score (kcal/mol)
		R^1 =	R^2 =	R^3 =	R' =	Kynurenine production (%)			
1	**1g**	C_6H_4-*p*F	H	H	H	50	3.1	11.5	-6.099
2	**1h**	C_6H_4-*p*F	H	H	MOM	27	5.5	2.6	-6.139
3	**1i**	C_6H_4-*p*F	H	H	MTM	31	0.8	0	-6.016
4	**1j**	C_6H_4-*p*Me	H	H	H	63	1.1	1.7	-6.009
5	**1k**	C_6H_4-*p*Me	H	H	MOM	19	1.9	0	-5.883
6	**1l**	C_6H_4-*p*Me	H	H	MTM	18	<0.5	0	-5.55
7	**1m**	C_6H_4-*p*OMe	H	H	H	111	<0.5	2.6	-6.13
8	**1n**	C_6H_4-*p*OMe	H	H	MOM	14	3.2	4.2	-5.556
9	**1o**	C_6H_4-*p*OMe	H	H	MTM	21	<0.5	0	-5.059
10	**1p**	Ph	CO_2Me	H	MOM	11	20	25.4	-5.987
11	**1q**	Ph	CO_2Me	H	MTM	11	2	5.2	-5.316
12	**1r**	Ph	H	CO_2Me	MOM	51	0.7	3.9	-6.032
13	**1s**	Ph	H	CO_2Me	MTM	22	<0.5	0.4	-5.526

Considering biological activity, focusing on the phenyl group at the 2-position, the biological activity of the *N*-MOM and *N*-MTM dervatives tends to improve as the aromatic ring becomes electron-rich. On the other hand, the opposite tendency is seen in the N-H derivative. Interestingly, the activity of the 2-phenyl compound with a methoxycarbonyl group at the 3-position instead of the 5-position was improved (runs 1–13). Regarding solubility and PAMPA, the MOM form tended to yield better results than the MTM form, and the MOM group was found to be suitable as a substituent at the indole 1-position.

Next, we designed indole derivatives, **1t**–**1ab**, having polar substituents at the 5-position, such as OH, CN, and CH_2OH, as the indole derivatives in Table 3 exhibited poor solubility.

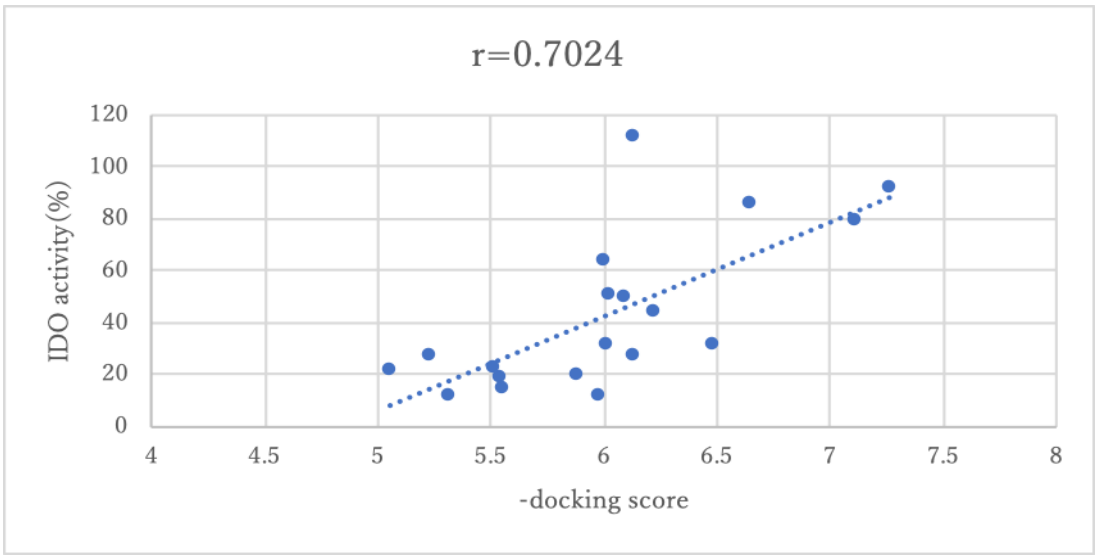
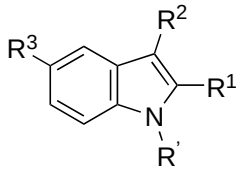


Figure 2. Correlation between docking score and IDO1 activity (%) of indole derivatives in Tables 2 and 3

Furthermore, we proceeded to design and synthesize new compounds by computational chemistry using the previously introduced data. Specifically, using docking software Glide,[5] we calculated docking scores[6] of the newly synthesized indole derivatives (Tables 2 and 3) and IDO1 and created a database to show the correlation between docking score and IDO1 inhibitory activities. As a result (Figure 2), the correlation coefficient (r) between the biological activity of the evaluated compound and the docking score[7] was good (0.7024). From the graph, the activity is predicted to increase as the docking score decreases, and compounds with a docking scores of 5 or less have high activity. We calculated the same docking score of various substituted indoles and found that the indole having a phenyl group at the 2-position and ethoxycarbonyl group at the 3-position and the compound having an ethyl group at the 2-position and methoxycarbonyl group at the 3-position showed the desired docking scores (molecular design), so we prepared these indole derivatives (**1ac**–**1ah**). In Table 4, we summarize chemical structures of our designed compounds (**1t**–**1ah**) and their IDO1 inhibitory activities and physical properties.

Table 4. IDO1 inhibitory activity and physical properties of compounds **1t** – **1ah**

run						IDO cell based assay (50 μM)	solubility (μM)	PAMPA (X 10^{-6} cm/sec)	Glide docking score (kcal/mol)
		R^1 =	R^2 =	R^3 =	R' =	Kynurenine production (%)			
1	**1t**	H	H	OH	H	107	-	-	-7.506
2	**1u**	H	H	OH	MOM	112	>95	23.1	-6.054
3	**1v**	H	H	OH	MTM	118	>95	32.4	-5.232
4	**1w**	H	H	CN	H	106	94	39.4	-6.505
5	**1x**	H	H	CN	MOM	104	95	53.3	-5.518
6	**1y**	H	H	CN	MTM	72	73	49.5	-5.284
7	**1z**	H	H	CH_2OH	H	107	>95	4.4	-7.536
8	**1aa**	H	H	CH_2OH	MOM	110	>95	28.8	-5.921
9	**1ab**	H	H	CH_2OH	MTM	107	>95	34.2	-6.551
10	**1ac**	Ph	CO_2Et	H	H	32	1.9	-	-4.446
11	**1ad**	Ph	CO_2Et	H	MOM	40	1.1	-	-4.428
12	**1ae**	Ph	CO_2Et	H	MTM	-2	6	-	-4.587
13	**1af**	Et	CO_2Me	H	H	14	81	37.3	-4.659
14	**1ag**	Et	CO_2Me	H	MOM	53	43.2	-	-4.815
15	**1ah**	Et	CO_2Me	H	MTM	82	<0.5	-	-5.075

Derivatives (**1t**–**1ab**) with polar functional groups showed good solubility and PAMPA values but had little IDO1 inhibitory activity, as predicted by docking scores. On the other hand, indole derivatives **1ac**–**1ah** exhibited IDO1 inhibitory activity, as expected from the docking scores. Figure 3 indicates the relationship between biological activity of the indole compound in Table 4 and the docking score. The correlation coefficient was good (0.74).

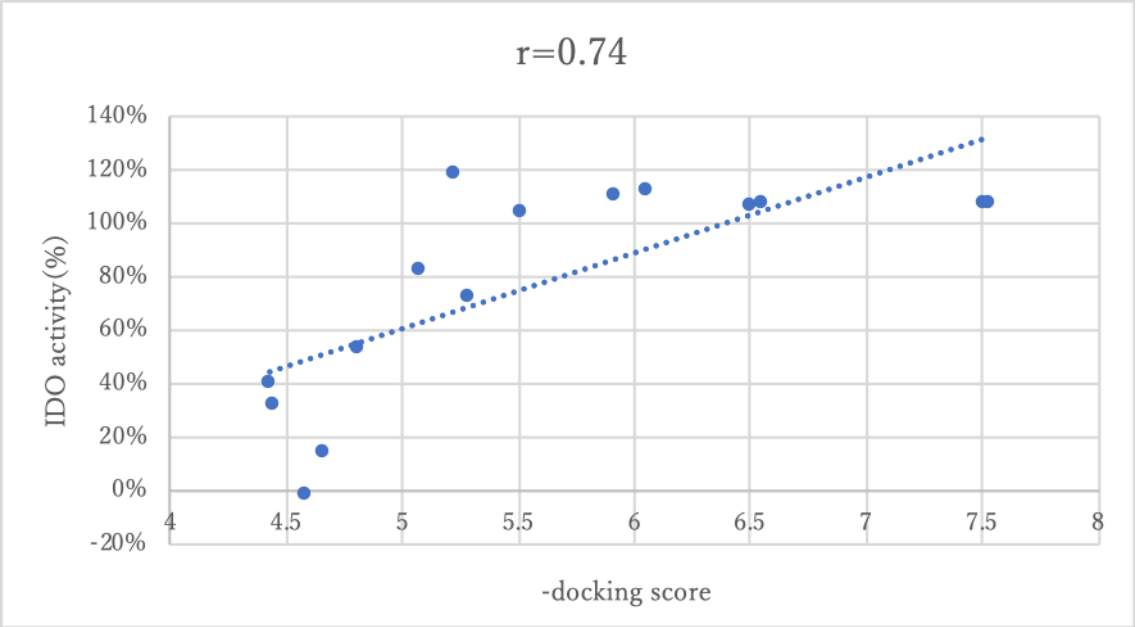


Figure 3. Correlation between docking score and IDO1 activity(%) of indole derivatives in Table 4

In this study, the indole compounds showing satisfactory values for IDO1 inhibitory activity, solubility, and PAMPA were determined to be **1p** and **1af**.[8] Regarding solubility and PAMPA, the MOM form tended to yield better results than the MTM form, and the MOM group was suitable as a 1-position indole substituent. We believe the results of these studies are useful, not only for the development of IDO1 inhibitors, but also for molecular design using computational chemistry and substituents at the 1-position of indole.

EXPERIMENTAL

Chemistry

• *General Information*

All reagents were purchased from commercial sources and used without further purification, unless otherwise noted. Reactions were performed under a nitrogen atmosphere using purchased anhydrous solvent. All reactions were monitored by thin-layer chromatography (TLC) using Merck silica gel $60F_{254}$ (0.25 mm). The products were purified by column chromatography over silica gel Kieselgel 60 (60-210 μm, spherical neutral) purchased from Kanto Chemical or Silica Gel 60N (40-50 μm, spherical neutral) purchased from Kanto Chemical. ^{1}H, ^{13}C and ^{19}F NMR spectra were recorded at 25 °C on a JEOL JNM-AL300 (at 300 MHz, 75 MHz and 282 MHz respectively), a JEOL JNM-ECS 400 (at 400 MHz, 100 MHz and 376 MHz, respectively) or a JEOL JNM-LA 500 (at 500 MHz, 125 MHz and 470 MHz, respectively), and the chemical shifts are reported relative to an internal standard, tetramethylsilane (TMS) (^{1}H, *δ:* = 0.00 ppm), $CDCl_3$ (^{1}H, *δ:* = 7.26 ppm, ^{13}C, *δ:* = 77.0 ppm) or C_6F_6 (^{19}F, *δ:* = -162.0 ppm). Data for ^{1}H NMR spectra are reported as follows: chemical shift (*δ:* ppm) (multiplicity, coupling constant

(Hz), integration). Multiplicity and qualifier abbreviations are as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. High-resolution mass spectra (MALDI-TOF) were performed on JEOL JMS-3000. Compounds **1a**, **1d**, **1g**, **1j**, **1m**, **1t**, **1w** and **1z** are commercially available. Compounds **1b**,[9] **1e**,[10] **1ac**,[11] **1af**,[12] Methyl 2-phenyl-1*H*-indole-5-carboxylate[13] and methyl 2-phenyl-1*H*-indole-3-carboxylate [13] are known compounds.

• *General procedure for the synthesis of N-MOM or N-MTM indoles*

NaH (1.5 eq.) was added to a solution of indole compounds in DMF at 0 °C. After stirring for 30 min at the same temperature, MOMCl or MTMCl (1.5 eq.) was added dropwise to the solution. The resulting mixture was stirred for 5 h and slowly warmed to room temperature. After removal of the solvent under vacuo. The residue was partitioned between AcOEt and water. The combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4, filtered, concentrated and purified on silica gel chromatography using *n*-hexane/AcOEt to give the corresponding product.

1-(Methoxymethyl)-2-methyl-1*H*-indole (1b)[9] Following general procedures, compound **1a** (350 mg, 2.0 mmol) was converted to **1b** (340 mg, 97%) using column eluent (*n*-hexane : AcOEt = 20 : 1). Red oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.40-7.37 (1H, m), 7.22 (1H, d, *J* = 8.3 Hz), 7.06-6.95 (2H, m), 6.14 (1H, s), 5.17 (2H, s), 3.05 (3H, s), 2.28 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 137.44, 136.58, 128.17, 120.96, 119.93, 119.58, 108.95, 101.59, 73.50, 55.37, 12.31.

2-Methyl-1-((methylthio)methyl)-1*H*-indole (1c) Following general procedures, compound **1a** (382 mg, 2.0 mmol) was converted to **1c** (241 mg, 63%) using column eluent (*n*-hexane : AcOEt = 30 : 1). Colorless oil. ^{1}H MMR (400 MHz, $CDCl_3$) δ: 7.49 (1H, d, *J* = 7.8 Hz), 7.35 (1H, d, *J* = 8.2 Hz), 7.14 (1H, dd, *J* = 7.1, 3.5 Hz), 7.07 (1H, dd, *J* = 7.3, 3.7 Hz), 6.27 (1H, br s), 5.15 (2H, s), 2.47 (3H, s), 2.01 (3H, s). ^{13}C MMR (100 MHz, $CDCl_3$) δ: 137.0, 136.3, 128.1, 120.8, 119.8, 119.7, 109.3, 101.4, 45.6, 14.1, 12.8. HRMS (MALDI-TOF) calcd for $C_{11}H_{14}NS$ $(M+H)^+$: 191.0768, found: 191.0758.

1-(Methoxymethyl)-2-phenyl-1*H*-indole (1e)[10] Following general procedures, compound **1d** (474 mg, 2.0 mmol) was converted to **1e** (470 mg, 99%). using column eluent (*n*-hexane : AcOEt = 30 : 1). Colorless oil. ^{1}H MMR (400 MHz, $CDCl_3$) δ: 7.57-7.52 (3H, m), 7.44 (1H, d, *J* = 7.3 Hz), 7.41-7.29 (3H, m), 7.18 (1H, ddd, *J* = 8.2, 1.4, 1.4 Hz), 7.09 (1H, *J* = ddd, *J* = 6.9, 0.9, 0.9 Hz), 6.53 (1H, s), 5.33 (2H, s), 3.21 (3H, s). ^{13}C MMR (100 MHz, $CDCl_3$) δ: 141.8, 138.3, 132.4, 129.5, 128.6, 128.3, 128.1, 122.3, 120.7, 120.6, 110.2, 103.3, 74.7, 55.9.

1-((Methylthio)methyl)-2-phenyl-1*H*-indole (1f) Following general procedures, compound **1d** (507 mg,

2.0 mmol) was converted to **1f** (279 mg, 55%) using column eluent (*n*-hexane : AcOEt = 30 : 1). Colorless oil. ^{1}H MMR (400 MHz, $CDCl_3$) δ: 7.56 (1H, d, *J* = 7.8 Hz), 7.49-7.46 (2H, m), 7.43-7.38 (3H, m), 7.35-7.34 (1H, m), 7.19 (1H, dd, *J* = 7.3, 3.7 Hz), 7.10 (1H, dd, *J* = 7.3, 3.7 Hz), 6.48 (1H, s), 5.19 (2H, s), 1.77 (3H, s). ^{13}C MMR (100 MHz, $CDCl_3$) δ: 141.5, 137.4, 132.5, 131.0, 129.6, 128.7, 128.2, 122.0, 120.7, 120.62, 110.8, 103.5, 46.9, 14.6. HRMS (MALDI-TOF) calcd for $C_{16}H_{16}NS$ $(M+H)^+$: 253.09252, found: 253.0914

2-(4-Fluorophenyl)-1-(methoxymethyl)-1*H*-indole (1h) Following general procedures, compound **1g** (724 mg, 2.8 mmol) was converted to **1h** (594 mg, 82%) using column eluent (*n*-hexane : AcOEt = 10 : 1). Yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.65-7.61 (m, 3H), 7.50 (d, *J* = 8.2 Hz, 1H), 7.27 (d, *J* = 6.6 Hz, 1H), 7.21-7.14 (m, 3H), 6.59 (s, 1H), 5.40 (s, 2H) , 3.34 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 162.7 (d, *J* = 249 Hz), 140.6, 138.2, 131.2 (d, *J* = 8.8 Hz), 128.4, 128.1, 122.4, 120.8, 120.5, 115.6 (d, *J* = 21.3 Hz), 110.0, 103.4, 74.5, 56.0. ^{19}F NMR ($CDCl_3$, 375 MHz) δ: -113.7. HRMS (MALDI-TOF) calcd for $C_{16}H_{14}FNO$ $(M)^+$: 255.10592, found: 255.10574. mp 57-58 °C (*n*-hexane /AcOEt).

2-(4-Fluorophenyl)-1-((methylthio)methyl)-1*H*-indole (1i) Following general procedures, compound **1g** (1.18 g, 4.4 mmol) was converted to **1i** (531 mg, 45%) using column eluent (*n*-hexane : AcOEt = 30 : 1). Pale red powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.70 (d, *J* = 7.8 Hz, 1H), 7.63-7.56 (m, 3H), 7.33 (dd, *J* = 8.9 Hz, 1H), 7.24 (m, 3H), 6.61 (s, 1H), 5.30 (s, 2H), 1.96 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 162.7(d, *J* = 238 Hz), 140.2, 137.3, 131.4 (d, *J* = 12.5 Hz), 128.6, 128.5, 128.3, 122.1, 120.6, 115.7 (d, *J* = 25 Hz), 110.7, 103.6, 46.8, 14.7. ^{19}F NMR ($CDCl_3$, 375 MHz) δ: -113.7. HRMS (MALDI-TOF) calcd for $C_{16}H_{14}FNS(M)^+$: 271.08314, found: 271.08327. mp 81-86 °C (*n*-hexane /AcOEt).

1-(Methoxymethyl)-2-(*p*-tolyl)-1*H*-indole (1k) Following general procedures, compound **1j** (382 mg, 1.5 mmol) was converted to **1k** (333 mg, 87%) using column eluent (*n*-hexane : AcOEt = 10 : 1). White powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.60 (d, *J* = 7.8 Hz, 1H), 7.49 (d, *J* = 7.2 Hz, 2H), 7.47-7.46 (m, 1H), 7.23 (d, *J* = 7.2 Hz, 2H), 7.20-7.16 (m, 1H), 7.13 (dd, *J* = 7.3, 7.3 Hz, 1H), 6.55 (s, 1H), 5.39 (s, 2H), 3.26 (s, 3H), 2.39 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 141.8, 138.1, 138.0, 129.4, 129.3, 129.2, 128.3, 122.1, 120.6, 120.4, 110.1, 102.9, 74.6, 55.8, 21.2. HRMS (MALDI-TOF) calcd for $C_{17}H_{17}NO(M)^+$: 251.13104, found: 251.13122. mp 60-61 °C (*n*-hexane /AcOEt).

1-((Methylthio)methyl)-2-(*p*-tolyl)-1*H*-indole (1l) Following general procedures, compound **1j** (418 mg, 1.5 mmol) was converted to **1l** (180 mg, 43%) using column eluent (*n*-hexane : AcOEt = 10 : 1). Pale red powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.62 (d, *J* = 7.8 Hz, 1H), 7.49 (d, *J* = 7.9 Hz, 1H), 7.29 (d, *J* = 8.8

Hz, 2H), 7.24 (m, *J* = 8.0 Hz, 3H), 7.17 (dd, *J* = 7.6, 7.6 Hz, 1H), 6.52 (s, 1H), 5.26 (s, 2H), 2.43 (s, 3H), 1.85 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 141.5, 138.1, 137.2, 129.53, 129.51, 129.3, 128.5, 121.8, 120.58, 120.53, 110.7, 103.1, 46.8, 21.2, 14.6. HRMS (MALDI-TOF) calcd for $C_{17}H_{17}NS(M)^+$: 267.10825, found: 267.10834. mp 60-61 °C (*n*-hexane /AcOEt).

1-(Methoxymethyl)-2-(4-methoxyphenyl)-1*H*-indole (1n) Following general procedures, compound **1m** (382 mg, 1.4 mmol) was converted to **1n** (321 mg, 84%) using column eluent (*n*-hexane : AcOEt = 10 : 1). White powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.62 (d, *J* = 7.8 Hz, 1H), 7.56 (d, *J* = 7.6 Hz, 2H), 7.51 (d, *J* = 7.5 Hz, 1H), 7.25 (dd, *J* = 7.4, 7.4 Hz, 1H), 7.17 (dd, *J* = 7.6, 7.6 Hz, 1H), 7.01 (d, *J* = 7.6 Hz, 2H), 6.56 (s, 1H), 5.41 (s, 2H), 3.87 (s, 3H), 3.31 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 159.6, 141.6, 138.1, 130.7, 128.3, 124.7, 122.0, 120.6, 120.3, 114.0, 110.0, 102.6, 74.6, 55.9, 55.3. HRMS (MALDI-TOF) calcd for $C_{17}H_{17}NO_2$ $(M)^+$: 267.12592, found: 267.12585. mp 65-66 °C (*n*-hexane /AcOEt).

2-(4-Methoxyphenyl)-1-((methylthio)methyl)-1*H*-indole (1o) Following general procedures, compound **1m** (192 mg, 0.7 mmol) was converted to **1o** (73 mg, 38%) using column eluent (*n*-hexane : AcOEt = 10 : 1). Pale red powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.61 (d, *J* = 7.2 Hz, 1H), 7.50-7.47 (m, 3H), 7.22(d, *J* = 7.5Hz, 2H), 7.21-7.19(m,1H), 7.16-7.11(m,1H), 6.55 (s, 1H), 5.39 (s, 2H), 3.29 (s, 3H), 2.40 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 141.5, 138.2, 137.2, 129.53, 129.51, 129.3, 128.7, 121.8, 120.6, 120.5, 103.1, 55.3, 46.8, 21.2, 14.6. HRMS (MALDI-TOF) calcd for $C_{17}H_{17}NOS(M)^+$: 283.10314, found: 283.10327. mp 80-81 °C (*n*-hexane /AcOEt).

Methyl 1-(methoxymethyl)-2-phenyl-1*H*-indole-3-carboxylate (1p) Following general procedures, methyl 2-phenyl-1*H*-indole-3-carboxylate (155 mg, 0.5 mmol)[11] was converted to **1p** (120 mg, 77%) using column eluent (*n*-hexane : AcOEt = 10 : 1). Orange oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.23-8.19 (m, 1H), 7.55-7.52 (m, 1H), 7.49-7.41 (m, 5H), 7.34-7.30 (m, 2H), 5.26 (s, 2H), 3.74 (s, 3H), 3.14 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 165.5, 146.9, 136.4, 130.8, 130.5, 129.1, 128.0, 126.6, 123.4, 122.6, 122.0, 110.6, 106.5, 74.6, 56.0, 50.8. HRMS (MALDI-TOF) calcd for $C_{18}H_{17}NO_3$ $(M)^+$: 295.12085, found: 295.12077.

Methyl 1-((methylthio)methyl)-2-phenyl-1*H*-indole-3-carboxylate (1q) Following general procedures, methyl 2-phenyl-1*H*-indole-3-carboxylate (329 mg, 1.0 mmol)[11] was converted to **1q** (135 mg, 41%) using column eluent (*n*-hexane : AcOEt = 10 : 1). Orange oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.24-8.19 (m, 1H), 7.54-7.45 (m, 4H), 7.44-7.41 (m, 2H), 7.36-7.30 (m, 2H), 5.05 (s, 2H), 3.73 (s, 3H), 1.86 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 165.3, 146.5, 135.8, 130.8, 130.7, 129.2, 128.1, 126.8, 123.1, 122.6,

122.1, 111.0, 106.3, 50.8, 46.8, 14.9. HRMS (MALDI-TOF) calcd for $C_{18}H_{17}NO_2S(M)^+$: 311.09807, found: 311.09824.

Methyl 1-(methoxymethyl)-2-phenyl-1*H*-indole-5-carboxylate (1r) Following general procedures, methyl 2-phenyl-1*H*-indole-5-carboxylate (139 mg, 0.5 mmol)[11] was converted to **1r** (104 mg, 75%) using column eluent (*n*-hexane : AcOEt = 10 : 1). Beige powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.32-8.31 (m, 1H), 7.92-7.88 (m, 1H), 7.56-7.53 (m, 2H), 7.47-7.38 (m, 4H), 6.61 (s, 1H), 5.36 (s, 2H), 3.87 (s, 3H), 3.23 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 168.0, 143.2, 140.7, 131.8, 129.5, 128.7, 128.5, 128.0, 123.7, 123.4, 122.8, 110.0, 104.3, 74.8, 56.1, 51.9. HRMS (MALDI-TOF) calcd for $C_{18}H_{17}NO_3$ $(M)^+$: 295.1208, found: 295.1219. mp 78-79 °C (*n*-hexane /AcOEt).

Methyl 1-((methylthio)methyl)-2-phenyl-1*H*-indole-5-carboxylate (1s) Following general procedures, methyl 2-phenyl-1*H*-indole-5-carboxylate (780 mg, 2.5 mmol)[11] was converted to **1s** (312 mg, 40%) using column eluent (*n*-hexane : AcOEt = 10 : 1). Orange oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ:8.32-8.31 (m, 1H), 7.91-7.89 (m, 1H), 7.50-7.37 (m, 6H), 6.57 (s, 1H), 5.20 (s, 2H), 3.88 (s, 3H), 1.79 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 168.1, 142.8, 140.0, 131.9, 129.7, 128.8, 128.6, 128.1, 123.5, 123.3, 122.6, 110.5, 104.5, 51.9, 47.0, 14.7. HRMS (MALDI-TOF) :calcd for $C_{18}H_{17}NO_2S(M)^+$: 311.09804, found: 311.09821.

1-(Methoxymethyl)-1*H*-indol-5-ol (1u) Following general procedures, compound **1t** (48 mg, 0.3 mmol) was converted to **1u** (40 mg, 84%) using column eluent (*n*-hexane : AcOEt = 5 : 1). Brown powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.15 (d, *J* = 8.7 Hz, 1H), 6.95 (d, *J* = 3.6 Hz, 1H), 6.84(d, *J* = 2.4 Hz, 1H), 6.62 (d, *J* = 2.4 Hz, 1H), 6.22 (d, *J* = 3.3 Hz, 1H), 5.21 (s, 2H), 3.04 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 149.9, 131.7, 129.9, 129.1, 111.8, 110.5, 105.4, 101.8, 76.7, 55.8. HRMS (MALDI-TOF) calcd for $C_{10}H_{11}NO_2$ $(M+H)^+$: 177.07830, found: 177.07843. mp 48-50 °C (*n*-hexane /AcOEt).

1-((Methylthio)methyl)-1*H*-indol-5-ol (1v) Following general procedures, compound **1t** (115 mg, 0.6 mmol) was converted to **1v** (47 mg, 41%) using column eluent (*n*-hexane : AcOEt = 5 : 1). Brown oil. ^{1}H NMR (300 MHz, CD_3OD) δ: 8.77 (s, 1H), 7.33 (d, *J* = 3.0 Hz, 2H), 6.86 (d, *J* = 2.4 Hz, 1H), 6.66 (dd, *J* = 8.9, 2.4 Hz, 1H), 5.30 (s, 2H), 1.97 (s, 3H). ^{13}C NMR (75 MHz, CD_3OD) δ: 152.0, 130.8, 130.3, 130.1, 112.2, 111.8, 105.3, 101.2, 49.5, 14.9. HRMS (MALDI-TOF) calcd for $C_{10}H_{11}NOS$ $(M+H)^+$: 194.06318, found: 194.06341.

1-(Methoxymethyl)-1*H*-indole-5-carbonitrile (1x) Following general procedures, compound **1w** (803

mg, 4.3 mmol) was converted to **1x** (651 mg, 81%) using column eluent (*n*-hexane : AcOEt = 10 : 1). Brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.97 (s, 1H), 7.54 (d, *J* = 8.4 Hz, 1H), 7.47 (d, *J* = 8.2 Hz, 1H), 7.30 (d, *J* = 3.0 Hz, 1H), 6.61 (d, *J* = 3.0 Hz, 1H), 5.47 (s, 2H), 3.24 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 137.8, 130.2, 128.8, 126.4, 125.1, 120.5, 110.8, 103.4, 103.3, 76.6, 56.0. HRMS (MALDI-TOF) calcd for $C_{11}H_{11}N_2O$ $(M)^+$: 187.08660, found : 187.08659.

1-((Methylthio)methyl)-1*H*-indole-5-carbonitrile (1y) Following general procedures, compound **1w** (1.41 g, 7.0 mmol) was converted to **1y** (537 mg, 38%) using column eluent (*n*-hexane : AcOEt = 10 : 1). Orange powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.96 (s, 1H), 7.46-7.45 (m, 2H), 7.30 (d, *J* = 3.0 Hz, 1H), 6.59 (d, *J* = 3.3 Hz, 1H), 5.16 (s, 2H), 1.98 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 137.4, 130.0, 128.8, 126.6, 124.8, 120.5, 110.7, 103.21, 103.19, 49.6, 14.5. HRMS (MALDI-TOF) calcd for $C_{11}H_{11}N_2S$ $(M)^+$: 203.06353, found: 203.06357. mp 54-55 °C (*n*-hexane /AcOEt).

(1-(Methoxymethyl)-1*H*-indol-5-yl)-methanol (1aa) Following general procedures, compound **1z** (147 mg, 0.8 mmol) was converted to **1aa** (122 mg, 83%) using column eluent (*n*-hexane : AcOEt = 5 : 1). Brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.61 (s, 1H), 7.47 (d, *J* = 8.4 Hz, 1H), 7.27 (d, *J* = 2.9 Hz, 1H), 7.18 (d, *J* = 3.3 Hz, 1H), 6.52 (dd, *J* = 3.5, 1.0 Hz, 1H), 5.44 (s, 2H), 4.76 (s, 2H), 3.21 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 135.9, 132.9, 129.1, 128.6, 122.0, 119.8, 110.0, 102.6, 76.7, 66.1, 55.8. HRMS (MALDI-TOF) calcd for $C_{11}H_{13}NO_2(M)^+$: 191.09421, found: 191.09408.

(1-((Methylthio)methyl)-1*H*-indol-5-yl)-methanol (1ab) Following general procedures, compound **1z** (327 mg, 1.5 mmol) was converted to **1aa** (121 mg, 37%) using column eluent (*n*-hexane : AcOEt = 5 : 1). Brown powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.60 (s, 1H), 7.42 (d, *J* = 8.7 Hz, 1H), 7.27 (d, *J* = 8.7 Hz, 1H), 7.19 (d, *J* = 3.0 Hz, 1H), 6.50 (d, *J* = 3.6 Hz, 1H), 5.16 (s, 2H), 4.75 (s, 2H), 1.97 (s, 3H). ^{13}C NMR (75 MHz $CDCl_3$) δ: 135.5, 132.7, 129.1, 128.3, 121.8, 120.1, 110.0, 102.4, 66.1, 49.4, 14.4. HRMS (MALDI-TOF) calcd for $C_{11}H_{14}NOS$ $(M+H)^+$: 208.07881, found: 208.07906. mp 49-50 °C (*n*-hexane /AcOEt).

Ethyl 1-(methoxymethyl)-2-phenyl-1*H*-indole-3-carboxylate (1ad) Following general procedures, compound **1ac** (246 mg, 0.8 mmol)[13] was converted to **1ad** (202 mg, 82%) using column eluent (*n*-hexane : AcOEt = 7 : 1). White powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.27-8.24 (m, 1H), 7.56-7.53 (m, 1H), 7.49-7.43 (m, 5H), 7.35-7.25 (m, 2H), 5.28 (s, 2H), 4.19 (q, *J* = 7.5 Hz, 2H), 3.16 (s, 3H), 1.17 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 165.0, 146.7, 136.3, 131.0, 130.6, 129.1, 127.9, 126.8, 123.4, 122.6, 122.0, 110.5, 106.7, 74.6, 59.5, 56.0, 14.1. HRMS (MALDI-TOF) calcd for $C_{19}H_{19}NO_3$

$(M)^+$: 309.13648, found: 309.13634. mp 108-109 °C (*n*-hexane /AcOEt).

Ethyl 1-((methylthio)methyl)-2-phenyl-1*H*-indole-3-carboxylate (1ae) Following general procedures, compound **1ac** (278 mg, 0.9 mmol)[13] was converted to **1ae** (125 mg, 45%) using column eluent (*n*-hexane : AcOEt = 15 : 1). White powder. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.20-8.19 (m, 1H), 7.47-7.45 (m, 1H), 7.43-7.42 (m, 3H), 7.38-7.36 (m, 2H), 7.28-7.26 (m, 2H), 5.00 (s, 2H), 4.12 (q, *J* = 7.2 Hz, 2H), 1.81 (s, 3H), 1.10 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 165.0, 146.3, 135.8, 131.1, 130.7, 129.1, 128.0, 127.0, 123.1, 122.5, 122.1, 110.9, 106.5, 59.4, 46.7, 14.8, 14.1. HRMS (MALDI-TOF) calcd for $C_{19}H_{19}NO_2S$ $(M)^+$: 325.11367, found: 325.11354. mp 97-98 °C (*n*-hexane /AcOEt).

Methyl 2-ethyl-1-(methoxymethyl)-1*H*-indole-3-carboxylate (1ag) Following general procedures, compound **1af** (50 mg, 0.2 mmol)[12] was converted to **1ag** (39 mg, 77%) using column eluent (*n*-hexane : AcOEt = 6 : 1). White powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.14-8.11 (m, 1H), 7.46-7.43 (m, 1H), 7.27-7.23 (m, 2H), 5.49 (s, 2H), 3.94 (s, 3H), 3.29 (s, 3H), 3.27 (q, *J* = 7.7 Hz, 2H), 1.29 (t, *J* = 7.7 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 166.0, 151.2, 136.5, 126.6, 122.6, 122.1, 121.6, 109.6, 104.3, 73.6, 56.0, 50.8, 18.9, 14.1. HRMS (MALDI-TOF) calcd for $C_{14}H_{17}NO_3$ $(M)^+$: 247.12085, found: 247.12072. mp 58-59 °C (*n*-hexane /AcOEt).

Methyl 2-ethyl-1-((methylthio)methyl)-1*H*-indole-3-carboxylate (1ah) Following general procedures, compound **1af** (40 mg, 0.2 mmol)[12] was converted to **1ah** (17 mg, 43%) using column eluent (*n*-hexane : AcOEt = 8 : 1). Yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.14-8.11 (m, 1H), 7.43-7.40 (m, 1H), 7.27-7.24 (m, 2H), 5.20 (s, 2H), 3.94 (s, 3H), 3.28 (q, *J* = 7.5 Hz, 2H), 2.06 (s, 3H), 1.30 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 165.9, 150.6, 136.0, 126.7, 122.3, 122.1, 121.7, 110.0, 104.3, 50.8, 45.4, 19.0, 14.5, 13.9. HRMS (MALDI-TOF) calcd for $C_{14}H_{17}NO_2S$ $(M)^+$: 263.09805, found: 263.09822.

Biology

Inhibitory activity for cellular kynurenine production and cell viability

The kynurenine production in A431 cells was determined as follows. In brief, A431 cells (3.0×10^5 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_3CO_2H 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. The result of this experiment is the average performed at n = 3.

Solubility

Solubility of each compound in the 2nd fluid for dissolution test (pH 6.8), was measured at a final concentration of 100 μM in 1% DMSO. After shaking for 5hr, each solution was filtered using MultiScreen HTS MSSLBPC10 (Millipore) and filtrates were analyzed via HPLC (Waters) with photodiode array detection (wavelength was 280 nm). This is a one-time experiment. However, it is a routine experiment, and it is analyzed together with the control compound every time, and it is confirmed from this result that the experiment is established.

Permeability

Parallel artificial membrane permeability assay was done using Gentest™ Pre-coated PAMPA Plate System (CORNING). Procedure was initiated by adding donor solutions, 100 μM in 0.1 M phosphate buffer (pH 6.5) containing 5% DMSO, to each well of the donor plate. The receiver plate was then placed onto the donor plate and phosphate buffer (pH 7.4) containing 5% of DMSO was added to the acceptor well. After 5 h incubation, plates were separated and compounds were analyzed via HPLC (Waters) with photodiode array detection (wavelength was 280 nm). Correlation of permeability coefficients (Papp) was calculated according to manufacturer's procedure. This is a one-time experiment. However, it is a routine experiment, and it is analyzed together with the control compound every time, and it is confirmed from this result that the experiment is established.

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REFERENCES AND NOTES

[§]This paper is dedicated to Dr. Yasuyuki Kita on the occasion of his 77th birthday.

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