Bioorganic & Medicinal Chemistry xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and biological evaluation of novel tryptoline derivatives as indoleamine 2,3-dioxygenase (IDO) inhibitors

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ARTICLE INFO

Article history:
Received 6 December 2012
Revised 23 December 2012
Accepted 24 December 2012
Available online xxxx

Keywords:
Indoleamine 2,3-dioxygenase
IDO
Tryptoline derivatives
Tryptophan
Biomimetic synthesis

ABSTRACT

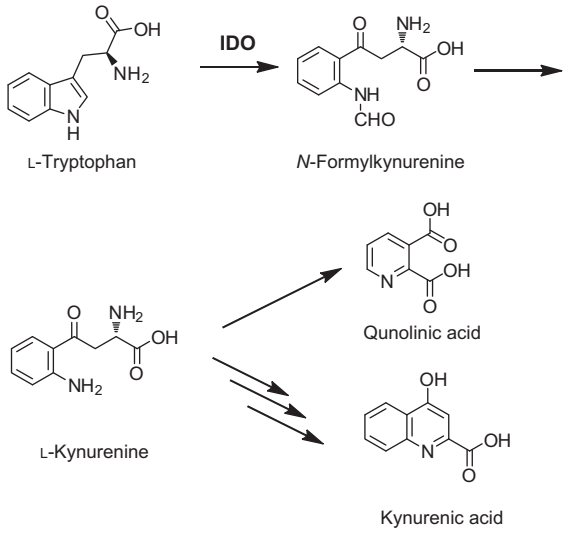
Indoleamine 2,3-dioxygenase (IDO) plays a significant role in several disorders such as Alzheimer's disease, age-related cataracts and tumors. A series of novel tryptoline derivatives were synthesized and evaluated for their inhibitory activity against IDO. Substituted tryptoline derivatives (**11a**, **11c**, **11e**, **12b** and **12c**) were demonstrated to be more potent than known inhibitor MTH-Trp. Suzuki–Miyaura cross-coupling reaction of **11a**–**d** with phenylboronic acid proceeded in high yields. In most cases, C5 and C6 substitutions on the corresponding indole ring were well tolerated. The tryptoline derivative **11c** is a promising chemical lead for the discovery of novel IDO inhibitors.

1. Introduction

Catabolism of tryptophan, one of the least abundant of the essential amino acids, is tightly controlled in the human body.[1] Tryptophan is converted to kynurenine through the kynurenine pathway, the major metabolic route.[2] Kynurenine is further metabolized into neurotoxic quinolinic acid or neuroprotective kynurenine acid (Scheme 1). Indoleamine 2,3-dioxygenase (IDO) catalyzes the initial and rate-limiting step of the pathway.[3] A large body of evidence has shown that tryptophan and its metabolites play significant roles in several biological processes[4–7] and disorders, including Alzheimer's disease,[8] age-related cataracts[9] and cancer.[10]

Since tryptophan is essential for T-lymphocyte proliferation, IDO is deeply related to immune system. IDO is constitutively expressed in various types of tumor cells, thus escaping from immune detection and attack.[11] High expression of IDO predicts poor prognosis of serous ovarian cancer,[12] endometrial cancer,[13] colorectal cancer[14] and acute myeloid leukemia,[15] suggesting IDO as an attractive therapeutic target for these diseases.[11,16] The IDO inhibitors 1-methyl-D-tryptophan and INCB024360 (structure undisclosed) are currently being studied in phase 1 clinical trials in patients with advanced malignancies.[17,18]

1-Methyl-DL-tryptophan (1-MT; **1**), the first IDO inhibitor was reported in 1991[19] and has been used as a chemical tool for studying biological roles of IDO. A more potent inhibitor, methylthiohydantoin-DL-tryptophan (MTH-Trp; **2**), was discovered by library screening in 2005.[20] The same group also identified a moderately active inhibitor brassinin (**3**) in a subsequent screening



Scheme 1. The kynurenine pathway of tryptophan metabolism.

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0968-0896/$ - see front matter © 2013 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.bmc.2012.12.028

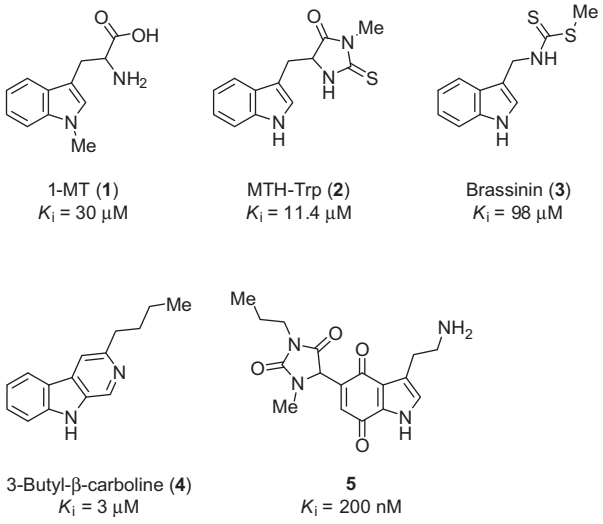


Figure 1. Structures of representative IDO inhibitors.

campaign.[21] 3-Butyl-β-carboline (**4**)[22] and a synthetic analogue of marine sponge natural product (**5**)[23] were both discovered as potent inhibitors during the lead optimization effort. All of these inhibitors possess an indole moiety as a common structural feature (Fig. 1). Very recently, hydroxyamidine inhibitors have also been reported.[24]

Substituted tryptophans themselves also function as IDO inhibitors.[25] We have developed biomimetic syntheses of tryptophan derivatives utilizing direct coupling of indole units and serine equivalent.[26,27] The reaction of indole and serine in the presence of acetic anhydride was the first practical synthesis of tryptophan derivatives having substituents on the benzene ring. During the course of this study, we developed a practical synthesis of benztryptophans and attempted to synthesize analogues of these to find new IDO inhibitors. As a result of this study, we discovered tryptoline derivatives (MTH-benz[*e*]tryptoline, **6a**; MTH-benz[*f*]tryptoline, **6b**; MTH-benz[*g*]tryptoline, **6c**; Fig. 2) as novel IDO inhibitors.

Herein, we present the synthesis and biological evaluation of these MTH-benztryptoline derivatives and MTH-tryptoline analogues, which were more easily synthesized than the benz analogues. These compounds should be promising chemical leads in the discovery of novel IDO inhibitors.

2. Chemistry

2.1. Synthesis of MTH-benztryptoline (6a–c)

The synthesis of MTH-benztryptolines is shown in Scheme 2. As we previously reported,[26] DL-*N*-acetylserine reacted with acetic anhydride to give an intermediate that reacted in one-pot with benzindoles[28] (**7**) to afford *N*-acetyl-DL-benztryptophans by Michael addition. The resulting compounds were subsequently protected with trimethylsilyldiazomethane to yield the corresponding methyl esters **8**.

In general, the selective removal of amide protecting groups such as acetyl groups is difficult, because strongly acidic or basic conditions at high temperature are required to hydrolyze the amide bond. This sometimes results in racemization and decomposition of the indole ring. We have reported[29] the conversion of optically active 4-bromo-*N*-acetyltryptophan to *N*-Boc-tryptophan under mild conditions by using Boc_2O. According to this method, simultaneous deacetylation and protection with $(Boc)_2O$ were accomplished to provide **9**. Removal of the Boc-groups using trifluoroacetic acid in dichloromethane, followed by Pictet–Spengler reaction gave tryptoline carboxylic acid methyl esters **10** as an inseparable diastereomixture (*cis*-major).[30] Cyclization of thiohydantoin was accomplished by treatment with methyl isothiocyanate and triethylamine in dichloromethane to afford MTH-benztryptolines **6** as a more stable *trans*-racemate.[31]

2.2. Synthesis of substituted MTH-tryptoline derivatives

Next, we turned our attention to simplified analogues of **6** to acquire synthetic tractability. Commercially available bromo- or chloro-substituted indoles were converted to the corresponding halogen-substituted tryptoline derivatives **11a–h** in the same way as for **6** (Scheme 3). Suzuki–Miyaura cross-coupling reaction of bromo-substituted analogues **11a–d** with phenylboronic acid proceeded smoothly to give the corresponding adducts **12a–d** in high yield.

The chiral analogues **15a** and **15b** were prepared to confirm the importance of the absolute configuration (Scheme 4).[30,31] Starting from L-tryptophan, Pictet–Spengler reaction gave a mixture of *cis* and *trans* compounds (4:1), which were separable to give the pure *cis*-diastereomer **13** as the major product. Esterification of **13** followed by cyclization with methyl isothiocyanate proceeded smoothly to afford the more stable *trans*-diastereomer **15a** as a single product by inverting the asymmetric center of the amino acid to give the D-enantiomer. The enantiomer **15b** (L-series) was prepared from D-tryptophan in the same manner. The stereochemistry was determined by NOE experiments and ^{13}C NMR chemical shifts,[31] and the ee's of **15a** and **15b** were determined to be 96.2% and 95.3%, respectively, by chiral HPLC analysis.

3. Measurement of IDO inhibitory activity

The synthetic compounds were evaluated for IDO inhibitory activity using the literature method.[32] Briefly, the reaction mixture contained potassium phosphate buffer, ascorbic acid, methylene blue, catalase, human recombinant IDO,[33] L-tryptophan and testing materials diluted in DMSO. The reaction was conducted at 37 °C and stopped by addition of trichloroacetic acid. After centrifugation, kynurenine in the supernatant was measured by HPLC and ultraviolet absorbance at 360 nm. The IC_{50} values were the means of more than two independent experiment sets calculated by linear interpolation.

4. Results and discussion

The results are shown in Table 1. MTH-benztryptolines (**6a–c**) showed comparable activity with **2**.[34] Simplified tryptoline analogues (**11a–h**, **12a–d**) retained comparable activity with **6a–c**, indicating that synthesis of benztryptoline derivatives were not essential. All of these analogues were synthesized from commercially available substituted indoles, demonstrating synthetic tractability. Five of them (**11a**, **11c**, **11e**, **12b** and **12c**) were more potent than **2** or **6a–c**. Interestingly, substitution at the C4′ position

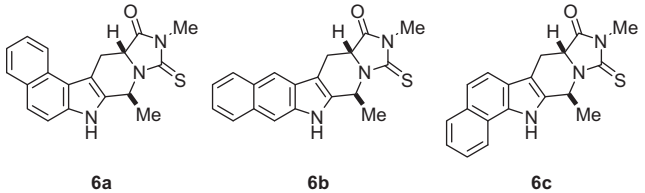
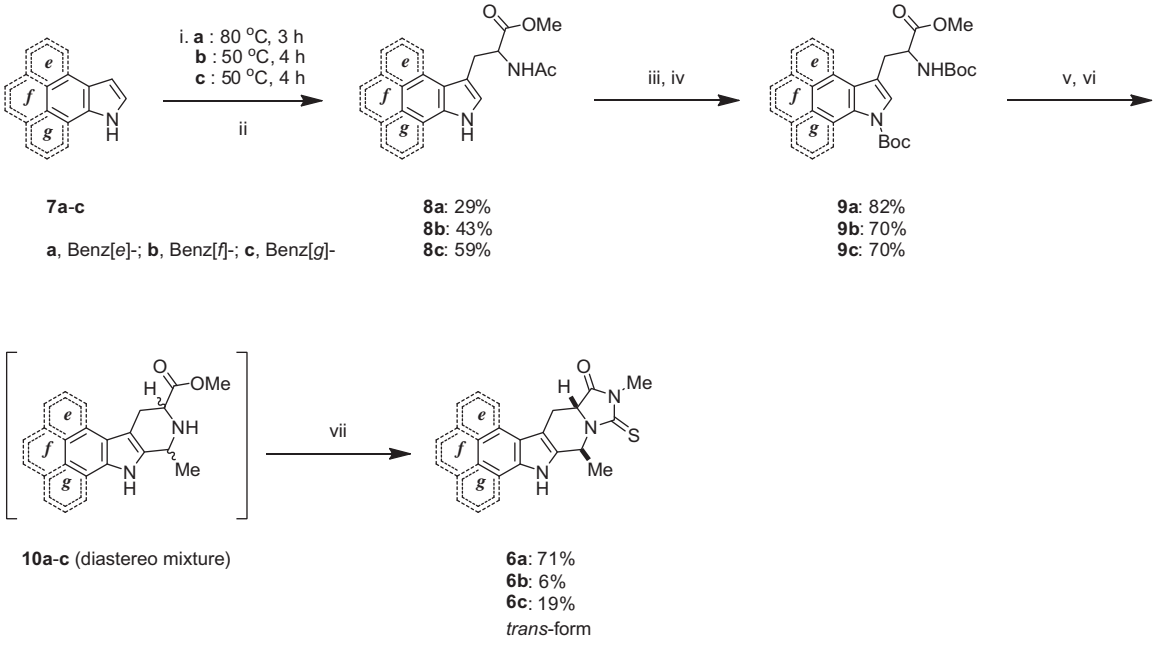
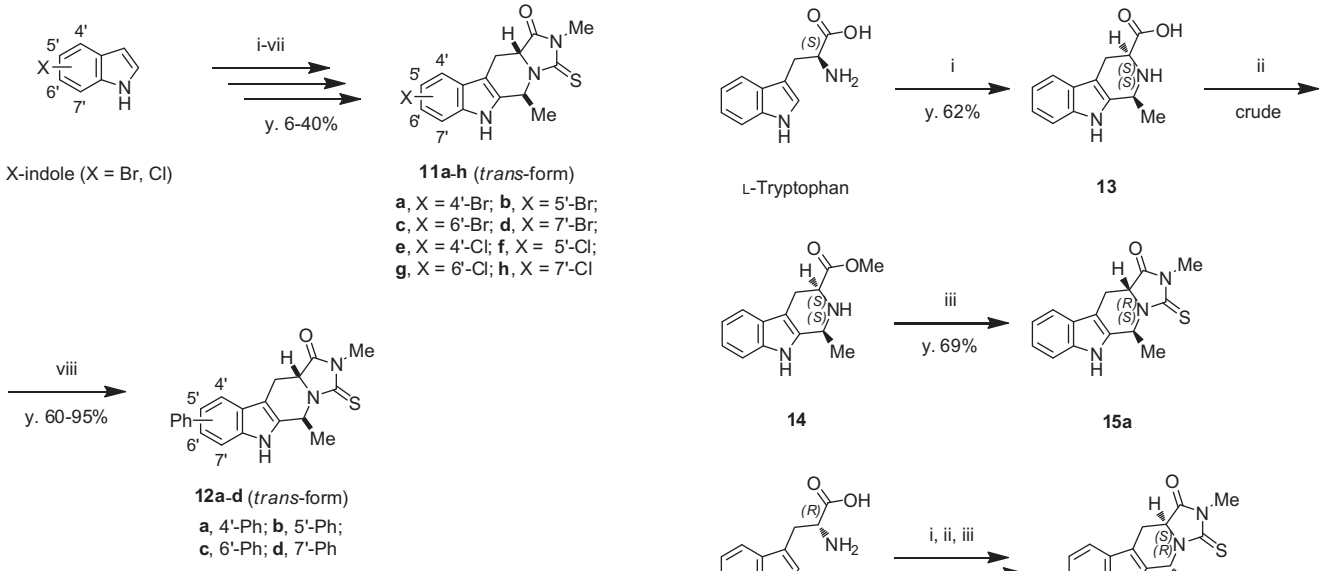


Figure 2. Structures of MTH-benztryptolines (**6a–c**).



Scheme 2. Reagents and conditions: (i) 2.0 mol equiv of DL-serine, 9.5 mol equiv of Ac_2O in acetic acid; (ii) 2.6 mol equiv of trimethyl-silyldiazomethane, ethyl acetate/MeOH, rt, 1 h; (iii) 5.0 mol equiv of $(Boc)_2O$, 1.0 mol equiv of DMAP in THF, rt, 3 h; (iv) 11.0 mol equiv of $H_2NNH_2{\cdot}H_2O$ in dichloroethane/MeOH, rt, 3 h; (v) 10.0 mol equiv of trifluoroacetic acid in dichloromethane, rt, 8 h; (vi) 10.0 mol equiv of acetaldehyde, rt, 5 h; (vii) 5.0 mol equiv of methyl isothiocyanate in triethylamine–dichloromethane (3:1), rt, 3 h.



Scheme 3. Reagents and conditions: (i) 2.0 mol equiv of DL-serine, 9.5 mol equiv of Ac_2O in acetic acid; (ii) 2.6 mol equiv of trimethyl-silyldiazomethane, ethyl acetate/MeOH, rt, 1 h; (iii) 5.0 mol equiv of $(Boc)_2O$, 1.0 mol equiv of DMAP in THF, rt, 3 h; (iv) 11.0 mol equiv of $H_2NNH_2{\cdot}H_2O$ in dichloroethane/MeOH, rt, 3 h; (v) 10.0 mol equiv of trifluoroacetic acid in dichloromethane, rt, 8 h; (vi) 10.0 mol equiv of acetaldehyde, rt, 5 h; (vii) 5.0 mol equiv of methyl isothiocyanate in triethylamine–dichloromethane (3:1), rt, 3 h; (viii) 1.5 mol equiv of phenylboronic acid, 0.10 mol equiv of $PdCl_2(PCy_3)_2$, 1.5 mol equiv of K_3PO_4, 1,4-dioxane, reflux, 4 h.

Scheme 4. Reagents and conditions: (i) 3.6 mol equiv of acetaldehyde, H_2SO_4, rt, 6 h; (ii) 1.5 mol equiv of thionyl chloride, MeOH, rt, 8 h; (iii) 5.0 mol equiv of methyl isothiocyanate in triethylamine-dichloromethane (3:1), rt, 3 h.

of the corresponding indole was more favored than C6′ in chloro derivatives (**11e** vs **11g**), whereas the opposite was true in the larger bromo substituents (**11a** vs **11c**). In both cases, C7′ substitution was the least favored (**11d** and **11h**), suggesting that the indole NH is essential. Recently, docking studies of L-Trp with IDO showed the importance of the indolic nitrogen atom, which is involved in a ternary complex with Fe(III) and superoxide.[35] Our results accorded well with the literature except for the phenyl derivative **12d**. One possibility is that the binding mode of **12d** is different from those of others because of steric clash between the phenyl substituent and the heme iron. In contrast, the phenyl group at the C4′ position was more disfavored than at C7′ (**12a** vs **12d**), demonstrating intolerance of bulky substituents at C4′. This was in line with MTH-benz[g]tryptoline **6c** which showed stronger inhibitory

Table 1
IDO inhibitory activity of methylthiohydantoin derivatives.

Compound	Substituent	IC_{50} (μM)
MTH-Trp (**2**)		76.9
MTH-Benz[*e*]tryptoline (**6a**)		82.0
MTH-Benz[*f*]tryptoline (**6b**)		58.9
MTH-Benz[*g*]tryptoline (**6c**)		55.0
11a	X = 4′-Br	68.2
11b	X = 5′-Br	74.6
11c	X = 6′-Br	46.1
11d	X = 7′-Br	109.3
11e	X = 4′-Cl	46.4
11f	X = 5′-Cl	113.9
11g	X = 6′-Cl	85.5
11h	X = 7′-Cl	119.8
12a	X = 4′-Ph	95.4
12b	X = 5′-Ph	63.5
12c	X = 6′-Ph	62.0
12d	X = 7′-Ph	81.1
15a		39% Inhibition at 200 μM
15b		174.6

activity than MTH-benz[*e*]tryptoline **6a**. Phenyl substitutions at both C5′ and C6′ were well tolerated (**12b** and **12c**). According to the docking model, substituents on the C5′ or C6′ positions are directed toward the flexible loop. Consequently, the C6′ position is one of the most preferred by every substituent, predicting the existence of chemical space in this area. The bromide **11c** is one of the best inhibitors and is being used as a chemical lead in further discovery efforts.

The L-enantiomer **15b** exhibited more potent inhibitory activity than the D-enantiomer **15a**. This is well correlated with the fact that the L-enantiomer is the eutomer of 1-MT (**1**). We have recently reported the convenient synthesis of chiral tryptophan derivatives using Negishi cross-coupling between substituted indoles and iodoalanine derived from chiral serine.[27] According to this method, enantiomerically pure substituted tryptoline analogues could be obtained.

5. Conclusion

A series of substituted tryptoline derivatives were synthesized and evaluated for their inhibitory activity against IDO. Five compounds revealed better activity than MTH-Trp (**2**), suggesting that the template has sufficient chemical space. The most potent inhibitor **11c** was easily reacted under cross-coupling conditions, demonstrating synthetic tractability. It was shown that substitution at C5′ or C6′ was preferred. In the present study, we further revealed that the L-enantiomer was the eutomer. We have already established a synthetic method of chiral tryptophan analogues having substituents on the benzene ring. Chiral substituted tryptoline derivatives can be prepared according to the reported method.

In conclusion, tryptoline derivatives are considered to be promising chemical leads in the discovery of novel IDO inhibitors.

6. Experimental

6.1. General experimental

Silica gel column chromatography was performed using Fuji Silysia PSQ100B. ^{1}H NMR spectra were recorded on a Bruker Avance 400 (400 MHz) spectrometer. Chemical shifts are expressed in ppm relative to TMS as an internal standard. Mass spectra were measured in a combination with a Waters Acquity UPLC system (0.05% trifluoroacetic acid in acetonitrile: 0.05% trifluoroacetic acid in H_2O) and a Micromass ZQ (ESI) spectrometer. Melting or decomposition points (Mp or Dp, respectively) were obtained on a Büchi 535 melting point apparatus and are uncorrected. Elemental analyses were performed on a Perkin Elmer 2400 II CHN Analyzer. High performance liquid chromatography (HPLC) was performed on a Waters 2795 Series chromatographs using Chiralpak IC-3 column (15 cm) in mixture of hexane/ethanol/diethylamine (85:15:0.1).

6.2. General procedure for the synthesis of *N*-acetyl-benztryptophan methyl ester (8)

DL-Serine (2.0 mol equiv) was added to acetic acid (0.2 M) and acetic anhydride (9.5 mol equiv), and the mixture was stirred for 3 h at 45 °C. To the reaction mixture, compound **7** (1.0 mol equiv) was added and stirred under the conditions shown in Scheme 2. After pouring into water, the mixture was concentrated in vacuo, the residue was extracted with ethyl acetate. The combined organic layers were washed with 30% NaOH aq and brine, dried over sodium sulfate, and concentrated in vacuo. The residue was dissolved in methanol (0.37 M) and ethyl acetate (0.16 M), 2.0 M trimethylsilyldiazomethane in toluene solution (2.6 mol equiv) was added dropwise, and the mixture was stirred for 1 h. After the addition of acetic acid (4.0 mol equiv), the reaction mixture was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–ethyl acetate) to give the product.

6.2.1. *N*-Acetyl-benz[*e*]tryptophan methyl ester (8a)

Yield 29%. Pale yellow powder. Mp 178–183 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.42 (d, J = 8.4 Hz, 1H), 8.40 (br s, 1H), 7.90 (d, J = 8.0 Hz, 1H), 7.58 (d, J = 9.2 Hz, 1H), 7.55 (ddd, J = 8.4, 6.8, 1.6 Hz, 1H), 7.48 (d, J = 8.8 Hz, 1H), 7.40 (ddd, J = 8.0, 6.8, 1.2 Hz, 1H), 7.05 (d, J = 2.0 Hz, 1H), 5.98 (d, J = 8.0 Hz, 1H), 5.12 (dt, J = 6.8, 5.2 Hz, 1H), 3.76 (dd, J = 15.2, 5.6 Hz, 1H), 3.69 (s, 3H), 3.59 (dd, J = 15.2, 6.4 Hz, 1H), 1.82 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.7, 169.9, 133.3, 129.9, 129.0, 128.9, 126.0, 123.9, 123.2, 122.9, 121.2, 120.2, 113.0, 112.9, 53.0, 52.3, 30.3, 23.2. MS (EI) m/z 310 $[M]^+$. Anal. Calcd for $C_{18}H_{18}N_2O_3$: C, 69.66; H, 5.85; N, 9.03. Found: C, 69.86; H, 5.86; N, 8.71. IR (KBr): 3409, 3258, 1749, 1644 cm^{-1}.

6.2.2. *N*-Acetyl-benz[*f*]tryptophan methyl ester (8b)

Yield 43%. Colorless needles. Mp 191–194 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.38 (br s, 1H), 8.10 (s, 1H), 7.93 (d, J = 7.6 Hz, 1H), 7.87 (d, J = 8.0 Hz, 1H), 7.81 (s, 1H), 7.35–7.42 (m, 2H), 7.20 (d, J = 2.4 Hz, 1H), 6.12 (d, J = 3.6 Hz, 1H), 5.03 (dd, J = 5.2, 4.8 Hz, 1H), 3.70 (s, 3H), 3.47 (dd, J = 9.6, 5.6 Hz, 1H), 3.40 (dd, J = 14.8, 5.6 Hz, 1H), 1.99 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 172.5, 169.3, 136.9, 129.4, 129.3, 128.3, 127.8, 127.5, 127.2, 123.1, 122.0, 115.0, 108.5, 106.3, 52.8, 51.8, 27.2, 22.3. MS (EI) m/z 310 $[M]^+$. Anal. Calcd for $C_{18}H_{18}N_2O_3$: C, 69.66; H, 5.85; N, 9.03. Found: C, 69.71; H, 5.89; N, 9.01. IR (KBr): 3392, 1752, 1642 cm^{-1}.

6.2.3. *N*-Acetyl-benz[*g*]tryptophan methyl ester (8c)

Yield 59%. Colorless needles. Mp 194–197 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.60 (br s, 1H), 8.23 (d, J = 8.0 Hz, 1H), 8.01 (d. J = 7.6 Hz, 1H), 7.89 (d, J = 8.0 Hz, 1H), 7.63 (d, J = 8.8 Hz, 1H), 7.50 (t, J = 6.8 Hz, 1H), 7.44 (d, J = 8.1 Hz, 1H), 7.37 (t, J = 6.8 Hz, 1H), 7.19 (d, J = 1.6 Hz, 1H), 4.59 (dd, J = 9.2, 6.0 Hz, 1H), 3.59 (s, 3H), 3.23 (dd, J = 8.8, 6.0 Hz, 1H), 3.13–3.16 (m, 1H), 1.83 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 172.1, 169.0, 130.2, 129.4, 128.0, 124.9, 123.2, 122.6, 121.8, 121.6, 120.3, 119.0, 118.5, 111.4, 53.4, 51.7, 27.2, 22.3. MS (EI) m/z 310 $[M]^+$.

Anal. Calcd for $C_{18}H_{18}N_2O_3$: C, 69.66; H, 5.85; N, 9.03. Found: C, 69.62; H, 5.93; N, 8.87. IR (KBr): 3401, 3296, 1725, 1648 cm^{-1}.

6.3. General procedure for the synthesis of N1,N2-di-Boc-benztryptophan methyl ester (9)

Boc_2O (5.0 mol equiv) was added to a stirred solution of compound **8** (1.0 mol equiv) and DMAP (1.0 mol equiv) in tetrahydrofuran (0.1 M), and the mixture was stirred for 3 h. After pouring into water, the mixture was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and concentrated in vacuo, the residue was purified by silica gel column chromatography (hexane–ethyl acetate) to give a yellow solid. The solid was dissolved in 1,2-dichloroethane (0.24 M) and methanol (1 M). Hydrazine monohydrate (11 mol equiv) was added dropwise to the solution. After stirring for 1 h, water was added to the reaction mixture. The mixture was extracted with ethyl acetate, and the combined organic layers were washed with brine, dried over sodium sulfate, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–ethyl acetate) to give the product.

6.3.1. N1,N2-di-Boc-benz[*e*]tryptophan methyl ester (9a)

Yield 82%. Colorless solid. Mp 155–157 °C. ^1H NMR (400 MHz, acetone-d_6): δ = 8.53 (d, J = 8.4 Hz, 1H), 8.41 (d, J = 8.8 Hz, 1H), 8.01 (d, J = 7.6 Hz, 1H), 7.80 (d, J = 8.8 Hz, 1H), 7.70 (s, 1H), 7.63 (t, J = 8.4 Hz, 1H), 7.50 (t, J = 8.0 Hz, 1H), 6.45 (d, J = 8.1 Hz, 1H), 4.72 (s, 1H), 3.71–3.76 (m, 4H), 3.43 (dd, J = 15.2, 10.8 Hz, 1H), 1.70 (s, 9H), 1.33 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 172.5, 155.5, 148.9, 132.4, 130.2, 128.9, 127.6, 126.6, 125.3, 124.3, 123.7, 122.9, 117.8, 115.0, 84.1, 78.3, 53.0, 52.1, 29.2, 28.0, 27.6, 27.4. MS (EI) m/z 468 $[M]^+$. Anal. Calcd For $C_{26}H_{32}N_2O_6$: C, 66.65; H, 6.88; N, 5.98. Found: C, 66.66; H, 6.94; N, 5.74. IR (KBr): 3364, 1744, 1709 cm^{-1}.

6.3.2. N1,N2-di-Boc-benz[*f*]tryptophan methyl ester (9b)

Yield 70%. Colorless powder. Mp 158–165 °C. ^1H NMR (400 MHz, acetone-d_6): δ = 8.62 (s, 1H), 8.13 (s, 1H), 8.02 (d, J = 7.2 Hz, 1H), 8.01 (d, J = 5.6 Hz, 1H), 7.71 (s, 1H), 7.45 (ddd, J = 10.8, 6.0, 3.2 Hz, 2H), 6.34 (d, J = 7.6 Hz, 1H), 4.61 (q, J = 5.2 Hz, 1H), 3.71 (s, 3H), 3.38 (dd, J = 15.2, 5.2 Hz, 1H), 3.23 (dd, J = 15.2, 9.6 Hz, 1H), 1.71 (s, 9H), 1.33 (s, 9H). MS (EI) m/z 468 $[M]^+$. Anal. Calcd For $C_{26}H_{32}N_2O_6$: C, 66.65; H, 6.88; N, 5.98. Found: C, 66.83; H, 6.96; N, 5.78. IR (KBr): 3445, 1725 cm^{-1}.

6.3.3. N1,N2-Di-Boc-benz[*g*]tryptophan methyl ester (9c)

Yield 70%. Colorless oil. ^1H NMR (400 MHz, $CDCl_3$): δ = 8.82 (d, J = 8.1 Hz, 1H), 7.90 (d, J = 8.0 Hz, 1H), 7.69 (d, J = 8.1 Hz, 1H), 7.51–7.61 (m, 2H), 7.43–7.46 (m, 2H), 3.69 (s, 3H), 3.32 (dd, J = 6.4, 4.2 Hz, 1H), 3.26 (dd, J = 6.4, 4.2 Hz, 1H), 1.72 (s, 9H), 1.44 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.3, 155.1, 150.3, 132.5, 130.9, 128.7, 126.0, 125.3, 125.2, 124.9, 124.5, 123.4, 117.6, 115.4, 84.1, 80.0, 53.8, 52.3, 29.7, 28.3, 28.1, 27.7. MS (ESI) m/z 491 $[M+Na]^+$. HRMS (EI): $C_{26}H_{32}N_2O_6$ requires m/z 468.2260. Found m/z 468.2261. IR (neat): 1740, 1160 cm^{-1}.

6.4. General procedure for the synthesis of MTH-tryptoline derivatives (6, 11)

Trifluoroacetic acid (10.0 mol equiv) was added to a stirred solution of compound **9** (1 mol equiv) in dichloromethane (0.17 M). After stirring for 30 min, acetaldehyde (10.0 mol equiv) was added to the reaction mixture. The mixture was stirred for another 8 h, concentrated in vacuo, the residue was extracted with ethyl acetate. The combined organic layers were washed with saturated $NaHCO_3$ aq and brine, dried over sodium sulfate, and concentrated in vacuo to give benztryptoline derivative (**10**), which was used in the next step without purification.

Compound **10** (1 mol equiv) was dissolved in dichloromethane (0.17 M) and triethylamine (0.3 M). To a stirred solution, methyl isothiocyanate (5.0 mol equiv) was added. The mixture was stirred for 3 h at 30 °C. After pouring into water, the mixture was concentrated in vacuo, the residue was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–ethyl acetate) to give the product.

6.4.1. *trans*-9,11-Dimethyl-10-thioxo-7,7a,9,10,11,12-hexahydro-9,10a,12-triaza-benzo[*c*]cyclopenta[*h*]fluoren-8-one (MTH-benz[*e*]tryptoline; 6a)

Yield 71%. Colorless powder. Dp 260–263 °C. ^1H NMR (400 MHz, $CDCl_3$): δ = 8.25 (s, 1H), 8.22 (d, J = 8.4 Hz, 1H), 7.90 (d, J = 8.0 Hz, 1H), 7.60 (d, J = 8.8 Hz, 1H), 7.54 (ddd, J = 8.4, 6.8, 1.6 Hz, 1H), 7.48 (d, J = 8.4 Hz, 1H), 7.42 (ddd, J = 8.0, 7.2, 0.8 Hz, 1H), 5.93 (q, J = 6.4 Hz, 1H), 4.46 (dd, J = 10.8, 6.0 Hz, 1H), 3.92 (dd, J = 14.8, 5.6 Hz, 1H), 3.35 (s, 3H), 3.22 (dd, J = 15.2, 11.2 Hz, 1H), 1.69 (d, J = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 179.7, 173.4, 132.7, 132.2, 129.0, 128.6, 128.3, 125.7, 122.7, 122.3, 118.7, 113.5, 106.1, 55.1, 48.2, 27.4, 25.1, 19.2. MS (ESI) m/z 336 $[M+H]^+$. HRMS (EI): $C_{19}H_{17}N_3OS$ requires m/z 335.1092, found m/z 335.1095. IR (KBr): 3380, 1722 cm^{-1}.

6.4.2. *trans*-9,11-Dimethyl-10-thioxo-7,7a,9,10,11,12-hexahydro-9,10a,12-triaza-benzo[*b*]cyclopenta[*h*]fluoren-8-one (MTH-benz[*f*]tryptoline; 6b)

Yield 6%. Colorless powder. Dp 237–242 °C. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.92 (d, J = 9.6 Hz, 1H), 7.91 (s, 1H), 7.86 (d, J = 8.8 Hz, 1H), 7.78 (br s, 1H), 7.73 (s, 1H), 7.33–7.40 (m, 2H), 5.90 (q, J = 6.8 Hz, 1H), 4.42 (dd, J = 10.8, 5.6 Hz, 1H), 3.50 (dd, J = 15.2, 5.6 Hz, 1H), 3.34 (s, 3H), 2.89 (dd, J = 15.2, 10.8 Hz, 1H), 1.68 (d, J = 6.8 Hz, 3H). HRMS (EI): $C_{19}H_{17}N_3OS$ requires m/z 335.1092, found m/z 335.1081. IR (KBr): 3355, 1726 cm^{-1}.

6.4.3. *trans*-9,11-Dimethyl-10-thioxo-7,7a,9,10,11,12-hexahydro-9,10a,12-triaza-benzo[*a*]cyclopenta[*h*]fluoren-8-one (MTH-benz[*g*]tryptoline; 6c)

Yield 19%. Colorless powder. Dp 247 °C. ^1H NMR (400 MHz, $CDCl_3$): δ = 8.58, (s, 1H), 7.98 (d, J = 8.0 Hz, 1H), 7.92 (d, J = 8.4 Hz, 1H), 7.52–7.58 (m, 3H), 7.44 (d, J = 8.0 Hz, 1H), 5.98 (q, J = 6.4 Hz, 1H), 4.41 (dd, J = 11.2, 5.6 Hz, 1H), 3.50 (dd, J = 14.8, 5.6 Hz, 1H), 3.33 (s, 3H), 2.90 (ddd, J = 14.8, 11.2, 2.0 Hz, 1H), 1.71 (d, J = 6.8 Hz, 3H). HRMS (EI): $C_{19}H_{17}N_3OS$ requires m/z 335.1092, found m/z 335.1095. IR (KBr): 3354, 1726 cm^{-1}.

6.4.4. *trans*-10-Bromo-2,5-dimethyl-1-oxo-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pydiro[3,4-*b*]indole-3(2*H*)-thione (11a)

Yield 28%. Pale brown solid. Dp 285–288 °C. ^1H NMR (400 MHz, $CDCl_3$): δ = 11.56 (s, 1H), 7.38 (d, J = 8.0 Hz, 1H), 7.19 (d, J = 7.0 Hz, 1H), 7.02 (d, J = 8.0 Hz, 1H), 5.64 (q, J = 6.7 Hz, 1H), 4.81 (dd, J = 10.8, 5.6 Hz, 1H), 3.73 (dd, J = 15.4, 6.1 Hz, 1H), 3.16 (s, 3H), 3.00–3.05 (m, 1H), 1.58 (d, J = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 179.7, 173.2, 137.4, 135.7, 124.3, 122.7, 112.5, 111.0, 104.3, 54.9, 48.1, 27.4, 24.0, 18.9. MS (ESI) m/z 364 $[M+H]^+$. HRMS (EI): $C_{15}H_{14}BrN_3OS$ requires m/z 363.0041, found m/z 363.0043. IR (KBr): 3329, 1729, 1485 cm^{-1}.

6.4.5. *trans*-9-Bromo-2,5-dimethyl-1-oxo-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pydiro[3,4-*b*]indole-3(2*H*)-thione (11b)

Yield 32%. Pale brown solid. Mp 210–212 °C. ^1H NMR (400 MHz, $CDCl_3$): δ = 11.39 (s, 1H), 7.71 (d, J = 2.1 Hz, 1H), 7.33 (d, J = 8.7 Hz,

1H), 7.21 (dd, J = 8.4, 1.8 Hz, 1H), 5.67 (q, J = 6.7 Hz, 1H), 4.79 (dd, J = 10.8, 5.6 Hz, 1H), 3.29 (dd, J = 10.2, 5.1 Hz, 1H), 3.16 (s, 3H), 2.69–2.85 (m, 1H), 1.57 (d, J = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 179.8, 173.2, 135.7, 135.0, 127.6, 124.0, 120.6, 113.2, 111.4, 104.0, 55.2, 48.3, 27.4, 22.3, 18.9. MS (ESI) m/z 364 $[M+H]^+$. HRMS (EI): $C_{15}H_{14}BrN_3OS$ requires m/z 363.0041, found m/z 363.0042. IR (KBr): 3345, 1739, 1476, 1312 cm^{-1}.

6.4.6. *trans*-8-Bromo-2,5-dimethyl-1-oxo-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pydiro[3,4-*b*]indole-3(2*H*)-thione (11c)

Yield 11%. Pale brown solid. Mp 254–256 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 11.35 (s, 1H), 7.55 (d, J = 1.5 Hz, 1H), 7.47 (d, J = 8.7 Hz, 1H), 7.14–7.16 (m, 1H), 5.66 (q, J = 6.3 Hz, 1H), 4.79 (dd, J = 11.0, 5.9 Hz, 1H), 3.28 (dd, J = 14.8, 5.6 Hz, 1H), 3.16 (s, 3H), 3.09 (dd, J = 7.2, 4.6 Hz, 1H), 1.57 (d, J = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 179.8, 173.2, 137.1, 135.1, 124.9, 121.8, 120.0, 114.2, 113.8, 104.5, 55.2, 48.2, 27.4, 22.3, 18.8. MS (ESI) m/z 364 $[M+H]^+$. HRMS (EI): $C_{15}H_{14}BrN_3OS$ requires m/z 363.0041, found m/z 363.0038. IR (KBr): 3331, 1727, 1468, 1313 cm^{-1}.

6.4.7. *trans*-7-Bromo-2,5-dimethyl-1-oxo-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pydiro[3,4-b]indole-3(2*H*)-thione (11d)

Yield 6%. Pale brown solid. Mp 265–267 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 11.30 (s, 1H), 7.53 (d, J = 7.7 Hz, 1H), 7.33 (d, J = 7.7 Hz, 1H), 6.98 (t, J = 7.7 Hz, 1H), 5.71 (q, J = 6.7 Hz, 1H), 4.83 (dd, J = 11.0, 5.9 Hz, 1H), 3.29 (dd, J = 15.1, 5.9 Hz, 1H), 3.16 (s, 3H), 3.07–3.09 (m, 1H), 1.60 (d, J = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 179.7, 173.2, 135.6, 134.7, 127.5, 124.1, 120.4, 117.8, 105.6, 103.9, 54.9, 48.3, 27.4, 22.4, 19.1. MS (ESI) m/z 364 $[M+H]^+$. HRMS (EI): $C_{15}H_{14}BrN_3OS$ requires m/z 363.0041, found m/z 363.0040. IR (KBr): 3364, 1736, 1474, 1312 cm^{-1}.

6.4.8. *trans*-10-Chloro-2,5-dimethyl-1-oxo-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pydiro[3,4-*b*]indole-3(2*H*)-thione (11e)

Yield 28%. Colorless solid. Dp 260–263 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 11.54 (s, 1H), 7.34 (d, J = 7.7 Hz, 1H), 7.01–7.09 (m, 2H), 5.66 (q, J = 6.3 Hz, 1H), 4.82 (dd, J = 11.0, 5.9 Hz, 1H), 3.64 (dd, J = 15.1, 5.9 Hz, 1H), 3.16 (s, 3H), 3.03 (ddd, J = 15.1, 11.0, 1.5 Hz, 1H), 1.58 (d, J = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 179.7, 173.2, 137.5, 135.5, 124.3, 122.8, 122.3, 119.4, 110.6, 103.7, 55.0, 48.1, 27.4, 24.0, 18.9. MS (ESI) m/z 320 $[M+H]^+$. Anal. Calcd For $C_{15}H_{14}ClN_3OS{\cdot}0.1H_2O$: C, 56.02; H, 4.45; N, 13.07. Found: C, 55.89; H, 4.56; N, 12.86. IR (KBr): 3332, 1729, 1484, 1314 cm^{-1}.

6.4.9. *trans*-9-Chloro-2,5-dimethyl-1-oxo-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pydiro[3,4-*b*]indole-3(2*H*)-thione (11f)

Yield 32%. Pale brown solid. Dp 241–243 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 11.38 (s, 1H), 7.57 (d, J = 2.0 Hz, 1H), 7.37 (d, J = 8.7 Hz, 1H), 7.10 (dd, J = 8.4, 1.8 Hz, 1H), 5.67 (q, J = 6.7 Hz, 1H), 4.79 (dd, J = 11.3, 5.6 Hz, 1H), 3.27–3.33 (m, 1H), 3.16 (s, 3H), 2.78 (dd, J = 14.1, 11.0 Hz, 1H), 1.57 (d, J = 6.7 Hz, 3H). ^{13}CNMR (100 MHz, DMSO-d_6): δ = 179.8, 173.2, 135.9, 134.8, 127.0, 123.5, 121.4, 117.6, 112.7, 104.1, 55.2, 48.3, 27.4, 22.3, 18.9. MS (ESI) m/z 320 $[M+H]^+$. Anal. Calcd for $C_{15}H_{14}ClN_3OS{\cdot}0.4H_2O$: C, 55.09; H, 4.56; N, 12.85. Found: C, 54.95; H, 4.60; N, 12.58. IR (KBr): 3341, 1730, 1475, 1311 cm^{-1}.

6.4.10. *trans*-8-Chloro-2,5-dimethyl-1-oxo-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pydiro[3,4-*b*]indole-3(2*H*)-thione (11g)

Yield 40%. Pale brown solid. Dp 237–239 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 11.35 (s, 1H), 7.52 (d, J = 8.2 Hz, 1H), 7.41 (d, J = 1.5 Hz, 1H), 7.03 (dd, J = 8.4, 1.8 Hz, 1H), 5.66 (q, J = 6.5 Hz, 1H), 4.79 (dd, J = 11.3, 5.6 Hz, 1H), 3.29 (dd, J = 15.1, 5.9 Hz, 1H), 3.16 (s, 3H), 2.77–2.83 (m, 1H), 1.57 (d, J = 6.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 179.8, 173.2, 136.7, 135.2, 126.1, 124.6, 119.6, 119.2, 110.9, 104.4, 55.2, 48.3, 27.4, 22.3, 18.9. MS (ESI) m/z 320 $[M+H]^+$. Anal. Calcd for $C_{15}H_{14}ClN_3OS{\cdot}0.4H_2O$: C, 55.09; H, 4.56; N, 12.85. Found: C, 55.29; H, 4.73; N, 12.64. IR (KBr): 3333, 1728, 1466, 1313 cm^{-1}.

6.4.11. *trans*-7-Chloro-2,5-dimethyl-1-oxo-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pydiro[3,4-*b*]indole-3(2*H*)-thione (11h)

Yield 21%. Pale brown solid. Dp 283–285 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 11.44 (s, 1H), 7.49 (d, J = 7.7 Hz, 1H), 7.19 (d, J = 7.7 Hz, 1H), 7.03 (dd, J = 7.7 Hz, 1H), 5.70 (q, J = 6.1 Hz, 1H), 4.82 (dd, J = 10.8, 5.6 Hz, 1H), 3.30 (dd, J = 15.1, 5.9 Hz, 1H), 3.17 (s, 3H), 2.83 (ddd, J = 15.0, 11.1, 1.5 Hz, 1H), 1.61 (d, J = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 179.7, 173.2, 135.6, 133.2, 127.7, 121.1, 120.0, 117.3, 115.6, 105.5, 54.9, 48.3, 27.4, 22.4, 19.0. MS (ESI) m/z 320 $[M+H]^+$. Anal. Calcd. for $C_{15}H_{14}ClN_3OS{\cdot}0.8H_2O$: C, 53.90; H, 4.70; N, 12.57. Found: C, 54.09; H, 4.57; N, 12.35. IR (KBr): 3367, 1725, 1480, 1308 cm^{-1}.

6.5. General procedure for the synthesis of MTH-phenyltryptoline (12)

A mixture of MTH-bromotryptoline (1 mol equiv), phenylboronic acid (1.5 mol equiv), and $PdCl_2(PCy_3)_2$ (0.1 mol equiv) in 1,4-dioxane (0.5 M) and 1.27 M K_3PO_4 aq (1.5 mol equiv) was refluxed for 4 h. After pouring into water, the mixture was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–ethyl acetate) to give the product.

6.5.1. *trans*-10-Phenyl-2,5-dimethyl-1-oxo-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pydiro[3,4-*b*]indole-3(2*H*)-thione (12a)

Yield 60%. Colorless solid. Dp 263–265 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 11.39 (s, 1H), 7.38–7.46 (m, 6H), 7.16 (t, J = 7.4 Hz, 1H), 6.88 (dd, J = 7.2 Hz, 1H), 5.66 (q, J = 6.7 Hz, 1H), 4.56 (dd, J = 11.0, 5.4 Hz, 1H), 3.11 (s, 3H), 2.54–2.61 (m, 1H), 2.37 (dd, J = 15.4, 5.6 Hz, 1H), 1.57 (d, J = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 179.5, 173.2, 140.9, 136.8, 134.8, 134.2, 129.2, 127.9, 127.0, 123.4, 121.4, 120.3, 110.6, 103.7, 55.2, 48.2, 27.3, 25.1, 18.8. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 179.5, 173.2, 140.9, 136.8, 134.8, 134.2, 129.2, 127.9, 127.0, 123.4, 121.4, 120.3, 110.6, 103.7, 55.2, 48.2, 27.3, 25.1, 18.8. MS (ESI) m/z 362 $[M+H]^+$. HRMS (EI): $C_{21}H_{19}N_3OS$ requires m/z 361.1249, found m/z 361.1247. IR (KBr): 3342, 1735, 1478, 1318 cm^{-1}.

6.5.2. *trans*-9-Phenyl-2,5-dimethyl-1-oxo-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pydiro[3,4-*b*]indole-3(2*H*)-thione (12b)

Yield 92%. Colorless solid. Mp 180–182 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 11.23 (s, 1H), 7.80 (s, 1H), 7.69 (d, J = 7.7 Hz, 2H), 7.43–7.46 (m, 4H), 7.28–7.34 (m, 1H), 5.69 (q, J = 6.7 Hz, 1H), 4.82 (dd, J = 10.8, 5.6 Hz, 1H), 3.18 (s, 3H), 2.82–2.88 (m, 1H), 1.59 (d, J = 6.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 179.8, 173.3, 141.6, 135.9, 134.9, 131.4, 128.8, 126.6, 126.4, 126.2, 120.8, 116.4, 115.6, 111.6, 104.6, 67.3, 55.3, 48.4, 27.4, 22.8, 18.9. MS (ESI) m/z 362 $[M+H]^+$. HRMS (EI): $C_{21}H_{19}N_3OS$ requires m/z 361.1249, found m/z 361.1246. IR (KBr): 3361, 1737, 1471, 1315 cm^{-1}.

6.5.3. *trans*-8-Phenyl-2,5-dimethyl-1-oxo-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pydiro[3,4-*b*]indole-3(2*H*)-thione (12c)

Yield 95%. Colorless solid. Mp 288–290 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 11.26 (s, 1H), 7.68 (d, J = 7.2 Hz, 2H), 7.57–7.59 (m, 2H), 7.46 (t, J = 7.7 Hz, 2H), 7.30–7.35 (m, 2H), 5.70 (q, J = 6.1 Hz, 1H), 4.82 (dd, J = 11.0, 5.9 Hz, 1H), 3.18 (s, 3H), 2.84 (dd, J = 14.3, 11.8 Hz, 1H), 1.60 (d, J = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 179.8, 173.3, 141.4, 137.0, 135.0, 134.0, 128.8, 126.7, 126.6, 125.3, 118.6, 118.3, 109.2, 104.1, 55.3, 48.4, 27.4, 22.5, 19.0. MS (ESI) m/z 362 $[M+H]^+$. HRMS (EI): $C_{21}H_{19}N_3OS$ requires m/z 361.1249, found m/z 361.1250. IR (KBr): 3319, 2927, 1730, 1469, 1318 cm^{-1}.

6.5.4. *trans*-7-Phenyl-2,5-dimethyl-1-oxo-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pydiro[3,4-*b*]indole-3(2*H*)-thione (12d)

Yield 75%. Colorless solid. Mp 223–225 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.91 (s, 1H), 7.64 (d, J = 7.2 Hz, 2H), 7.55 (t, J = 7.7 Hz, 2H), 7.50 (t, J = 4.6 Hz, 1H), 7.42–7.45 (m, 1H), 7.12–7.13 (m, 1H), 5.72 (q, J = 6.5 Hz, 1H), 4.85 (dd, J = 11.0, 5.9 Hz, 1H), 3.17 (s, 3H), 2.85 (dd, J = 14.3, 10.8 Hz, 1H), 1.56 (d, J = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 179.6, 173.4, 138.6, 135.1, 133.6, 128.9, 128.3, 127.3, 126.7, 125.2, 121.9, 119.6, 117.5, 104.6, 54.9, 48.3, 29.0, 27.4, 22.4, 19.2. MS (ESI) m/z 362 $[M+H]^+$. HRMS (EI): $C_{21}H_{19}N_3OS$ requires m/z 361.1249, found m/z 361.1249. IR (KBr): 3376, 2925, 1730, 1474, 1308 cm^{-1}.

6.6. Synthesis of *trans*-2,5-dimethyl-1-oxo-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-3(2*H*)-thione (15)

The title compounds were prepared according to the literature method[28,29] starting from L- or D-tryptophan. The reaction conditions and yields were shown in Scheme 4.

6.6.1. (5*S*,11a*R*)-2,5-Dimethyl-1-oxo-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-3(2*H*)-thione (15a)

Colorless solid. Mp 284–287 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 11.14 (s, 1H), 7.49 (d, J = 7.7 Hz, 1H), 7.35 (d, J = 7.7 Hz, 1H), 7.10 (t, J = 7.7 Hz, 1H), 7.01 (t, J = 7.7 Hz, 1H), 5.67 (q, J = 6.5, 1H), 4.79 (dd, J = 11.0, 5.9 Hz, 1H), 3.28 (dd, J = 15.1, 5.9 Hz, 1H), 3.17 (s, 3H), 2.81 (m, 1H), 1.58 (d, J = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 179.7, 173.3, 136.3, 134.0, 125.8, 121.5, 118.9, 118.1, 111.2, 104.0, 55.3, 48.3, 27.3, 22.4, 18.9. MS (ESI) m/z 286 $[M+H]^+$. HRMS (EI): $C_{15}H_{15}N_3OS$ requires m/z 285.0936, found m/z 285.0935. IR (KBr): 3331, 1733, 1473, 1316 cm^{-1}. 96.2% ee.

6.6.2. (5*R*,11a*S*)-2,5-Dimethyl-1-oxo-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-b]indole-3(2*H*)-thione (15b)

Colorless solid. Mp 284–287 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 11.14 (s, 1H), 7.49 (d, J = 7.7 Hz, 1H), 7.35 (d, J = 8.2 Hz, 1H), 7.10 (t, J = 7.2 Hz, 1H), 7.01 (t, J = 7.2 Hz, 1H), 5.67 (q, J = 6.5, 1H), 4.79 (dd, J = 10.8, 5.6 Hz, 1H), 3.29 (m, 1H), 3.17 (s, 3H), 2.81 (m, 1H), 1.58 (d, J = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 180.0, 173.3, 136.3, 134.0, 125.8, 121.5, 118.9, 118.1, 111.2, 104.0, 55.3, 48.4, 27.3, 22.4, 18.9. MS (ESI) m/z 286 $[M+H]^+$. HRMS (EI): $C_{15}H_{15}N_3OS$ requires m/z 285.0936, found m/z 285.0935. IR (KBr): 3332, 1733, 1475, 1316 cm^{-1}. 95.3% ee.

6.6.3. Enzymatic assay

The standard assay mixture (200 μL) contained 50 mM potassium phosphate buffer (pH 6.5), 10 mM ascorbate, 10 μM methylene blue, 100 μg/ml catalase, 200 μM L-tryptophan, and 10 μg/mL rhIDO. The reaction, at 37 °C, was started by the addition of the substrate and terminated after 60 min by adding 40 μl of 30% (w/v) trichloroacetic acid and further incubated at 50 °C for 15 min to hydrolyze *N*-formylkynurenine produced by indoleamine 2,3-dioxygenase to kynurenine. After centrifugation at 1500 × *g* for 5 min at 20 °C, kynurenine in the supernatant was measured by HPLC with a reversed phase column (4.6 mm × 15 cm) of TSK-100Z. The mobile phase was 10 mM ammonium acetate containing 10% (w/v) methanol, and kynurenine was detected by absorbance at 360 nm. All determinations were carried out in triplicate. The data presented are average values.

References and notes

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