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Synthesis of novel tryptanthrin derivatives as dual inhibitors of indoleamine 2,3-dioxygenase 1 and tryptophan 2,3-dioxygenase

Yuanyuan Li[a], Shengnan Zhang[b], Rong Wang[a], Menghan Cui[a], Wei Liu[c], Qing Yang[b], Chunxiang Kuang[a,*]

[a] Shanghai Key Lab of Chemical Assessment and Sustainability, School of Chemical Science and Engineering, Tongji University, 1239 Siping Road, 200092 Shanghai, China
[b] State Key Laboratory of Genetic Engineering, Department of Biochemistry, School of Life Sciences, Fudan University, 2005 Songhu Road, 200438 Shanghai, China
[c] School of Pharmacy, Nantong University, 19 Qixiu Road, 226001 Nantong, China

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ABSTRACT

Indoleamine 2,3-dioxygenase 1 (IDO1) and tryptophan 2,3-dioxygenase (TDO) are promising drug development targets due to their implications in pathologies such as cancer and neurodegenerative diseases. The search for IDO1 inhibitor has been intensely pursued but there is a paucity of potent TDO and IDO1/TDO dual inhibitors. Natural product tryptanthrin has been confirmed to bear IDO1 and/or TDO inhibitory activities. Herein, twelve novel tryptanthrin derivatives were synthesized and evaluated for the IDO1 and TDO inhibitory potency. All of the compounds were found to be IDO1/TDO dual inhibitors, in particular, compound **9a** and **9b** bore IDO1 inhibitory activity similar to that of INCB024360, and compound **5a** and **9b** had remarkable TDO inhibitory activity superior to that of the well-known TDO inhibitor LM10. This work enriches the collection of IDO1/TDO dual inhibitors and provides chemical molecules for potential development into drugs.

The kynurenine pathway (KP) is the major route of metabolism of the essential amino acid L-tryptophan (Trp), degrading ~95% of the dietary Trp into nicotinamide adenine dinucleotide (NAD).[1] Indoleamine 2,3-dioxygenase 1 (IDO1) and tryptophan 2,3-dioxygenase (TDO) initiate the first rate-limiting step in KP.[2] IDO1 is widely expressed in various organs and cells while TDO is constitutively expressed in the liver and brain[1,3].

IDO1 and/or TDO are overactivated or overexpressed in many human cancers, which is associated with poor patient outcomes.[4,5] IDO1 and TDO mediated depletion of Trp and production of kynurenine (Kyn) can provide an immunosuppressive tumor micro-environment, in which effector T cells (Teff) and natural killer (NK) cells are suppressed, T regulatory cells (Treg) are activated, and myeloid-derived suppressor cells (MDSCs) are expanded.[6,7] Furthermore, some KP metabolites have immunosuppressive ablitities. For example, quinolinic acid (QA) can inhibit the responses, proliferation and survival of Teff and promote the survival and metastasis of tumor cells.[7–9] 3-Hydroxykynurenine (3-HK) and 3-hydroxyanthranilic acid (3-HAA) can reduce proliferation and increase preferential apoptosis of both T helper 1 (TH1) lymphocytes and natural killer (NK) cells.[10,11] In addition, QA, 3-HK and 3-HAA are neurotoxic which is related to the death and/or apoptosis of neuronal.[8,12] The dysregulation of KP is strongly associated with neurological diseases such as Alzheimer's disease (AD) and Huntington's disease.[2,13–16] Hence, IDO1 and TDO have been regarded as important targets for the treatment of cancer and AD.[17]

At present, a variety of IDO1 inhibitors including epacadostat (INCB024360), BMS-986205 and PF-06840003 have been subjected to clinical trials.[18–20] Some TDO inhibitors including LM10, 680C91 and NSC36398 are evaluated in animal experiments.[4,21,22] Besides, several IDO1/TDO dual inhibitors are also under clinical or preclinical studies, such as navoximod (GDC-0919, NLG919), RG-70099 and SHR9146 (NCT03208959, HTI-1090), although the structures of RG-70099 and SHR9146 have not been disclosed (Fig. 1).[23,24] However, new and viable IDO1/TDO dual inhibitor skeletons are severely lacking. Thus, it is urgent to develop IDO1/TDO dual inhibitors.

Abbreviations: Kyn, kynurenine; KP, kynurenine pathway; Trp, L-tryptophan; NAD, nicotinamide adenine dinucleotide; IDO1, indoleamine 2,3-dioxygense1; TDO, tryptophan 2,3-dioxygenase; QA, quinolinic acid; PA, picolinic acid; Teff, effector T cells; NK, natural killer; Treg, T regulatory cells; MDSCs, myeloid-derived suppressor cells; AD, Alzheimer's disease; 3-HK, 3-hydroxy kynurenine; KA, kynurenic acid; *m*-CPBA, *meta*-chloroperbenzonic acid; NMO, *N*-methylmorpholine-*N*-oxide; NBS, *N*-bromosuccinimide; AIBN, azobisisobutyronitrile; r.t., room temperature; DCM, dichloromethane; DMA, *N*,*N*-dimethylacetamide; DMF, *N*,*N*-dimethylformamide; THF, tetrahydrofuran

* Corresponding author.
E-mail addresses: 1731041@tongji.edu.cn (Y. Li), 17110700084@fudan.edu.cn (S. Zhang), 1831059@tongji.edu.cn (R. Wang), 1931000@tongji.edu.cn (M. Cui), weiliu2015@ntu.edu.cn (W. Liu), yangqing68@fudan.edu.cn (Q. Yang), kuangcx@tongji.edu.cn (C. Kuang).

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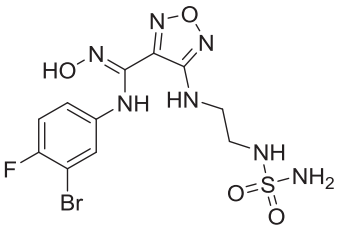
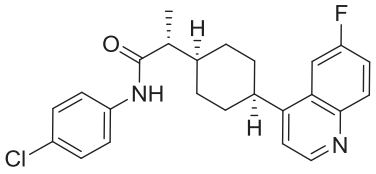
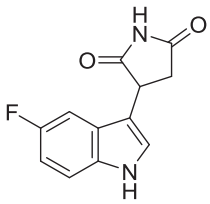


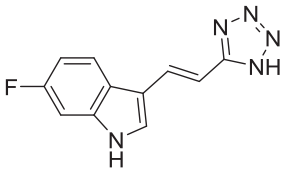
Epacadostat (INCB024360)
IDO1 IC_{50} = 0.0718 μM



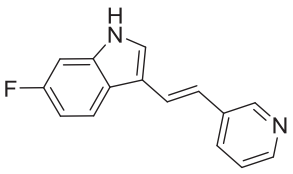
BMS-986205
IDO1 IC_{50} = 0.0011 μM



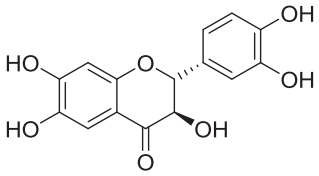
PF-06840003
IDO1 IC_{50} = 0.41 μM



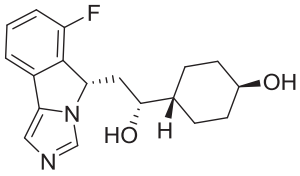
LM10
TDO IC_{50} = 11.58 μM



680C91
TDO IC_{50} = 0.937 μM



NSC36398
TDO IC_{50} = 16.3 μM



Navoximod
IDO1 IC_{50} = 0.075 μM
TDO IC_{50} =10-20 fold of IDO1

Fig. 1. Structures of IDO1 selective inhibitors, TDO selective inhibitors and IDO1/TDO dual inhibitors in clinical or preclinical trials.

Tryptanthrin (indolo[2,1-*b*]quinazoline-6,12-dione), an indolequinazoline alkaloid, possesses a wide range of biological effects such as antibacterial, anti-inflammatory, antileishmanial, antimalarial and antitumor activities.[25–29] However, tryptanthrin is poorly soluble in water, which greatly affects its pesticide effect.[30] In our previous studies, a series of tryptanthrin derivatives were synthesized and evaluated for IDO1 inhibitory activity.[31] Subsequently, some of these tryptanthrin derivatives have been proven to bear TDO inhibitory activity.[32] Afterwards, several novel tryptanthrin derivatives have been synthesized and evaluated, the testing results demonstrated that some of these tryptanthrin derivatives were IDO1/TDO dual inhibitors.[33] With the continuous interest in tryptanthrins, we designed and synthesized twelve novel tryptanthrin derivatives and evaluated their IDO1 and TDO inhibitory activities on enzymatic levels, IDO1 inhibitory activity on cellular level and water solubility.

Twelve novel tryptanthrin derivatives containing aldehyde group (**5a**), triazole (**5b**), *N*-benzylnaphthenate (**5c**, **5e**, **5g**), *N*-benzylnaphthenic acid (**5d**, **5f**, **5h**), cinnamic acid ester (**9a**), cinnamic acid (**9b**), boric acid ester (**9c**) and boric acid (**9d**) were designed and synthesized (Fig. 2). The syntheses of tryptanthrin derivatives were described in Schemes 1 and 2. 5-Methylisatoic anhydride **2** or 5-bromoisatoic anhydride **7** were synthesized by the Baeyer-Villiger reaction of 5-methylisatin **1** or 5-bromoisatin **6** with *meta*-chloroperbenzonic acid (*m*-CPBA), respectively. 2-Methyl-8-fluorotryptanthrin **3** or 2-bromo-8-fluorotryptanthrin **8** were severally synthesized through the reaction of compound **2** or **7** with 5-fluoroisatin in the presence of triethylamine. 2-Bromomethyl-8-fluorotryptanthrin **4** was obtained by reacting compound **3** with *N*-bromosuccinimide (NBS) and azobisisobutyronitrile (AIBN).

Compound **5a**-**5h** were synthesized by utilizing compound **4** as a reactant, while compound **9a**-**9d** were synthesized by the reaction of compound **8** as a substrate (Scheme 2). Compound **4** was oxidized to compound **5a** with *N*-methylmorpholine-*N*-oxide (NMO). Compound **4** was reacted with sodium azide to introduce an azide group, which was further reacted with propiolic acid under the catalysis of cuprous iodide and sodium ascorbate to yield compound **5b**. Compound **5c**, **5e** and **5g** were synthesized through the reaction of compound **4** with methyl 4-piperidinecarboxylate, methyl 3-piperidinecarboxylate and proline methyl ester hydrochloride, respectively, in the presence of triethylamine and potassium iodide. Compound **5d** and **5f** were obtained by reacting compound **4** with 4-piperidinecarboxylic acid and 3-piperidinecarboxylic acid, respectively, in the presence of potassium iodide. Compound **5h** was afforded by the hydrolysis of compound **5g** in the alcohol solution of sodium hydroxide. Compound **9a** was synthesized through the Heck reaction of compound **8** with ethyl acrylate in the presence of potassium phosphate and catalyzed by palladium(II) acetate in *N*,*N*-dimethylacetamide (DMA). Compound **9b** was obtained through the hydrolysis of compound **9a** in the ethanolic solution of sodium hydroxide. Compound **9c** was synthesized through the Miyaura reaction[34] of compound **8** with bis(pinacolato)diboron under the alkaline condition of potassium acetate and catalyzed by [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) ($PdCl_2$(dppf)) in *N*,*N*-dimethylformamide (DMF). Compound **9c** was hydrolyzed in the aqueous solution of tetrahydrofuran (THF) under the effect of sodium periodate and hydrochloric acid to obtain compound **9d**.

Twelve tryptanthrin derivatives we synthesized were subjected to the enzymatic IDO1 inhibition assay (Fig. S1). Under the same conditions, the IC_{50} value of INCB024360 against IDO1 was determined to be

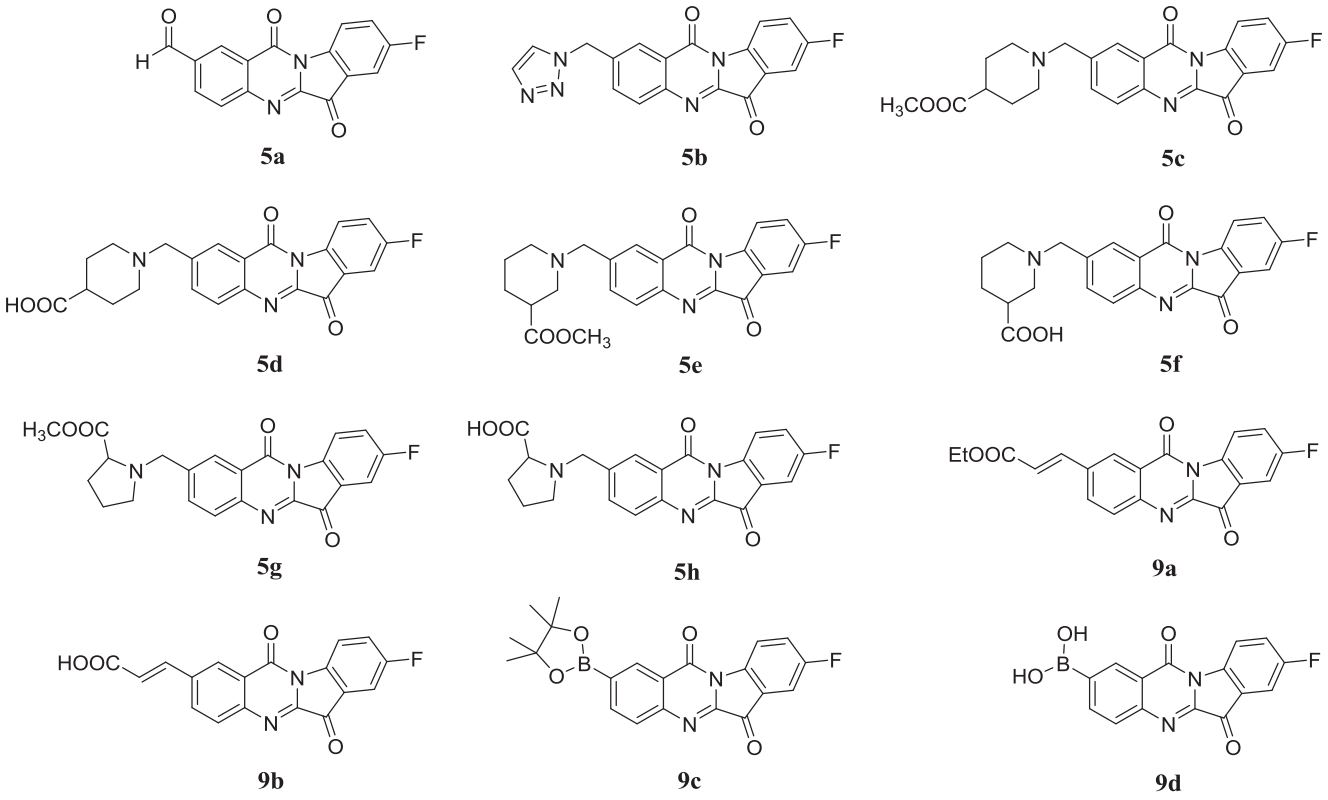
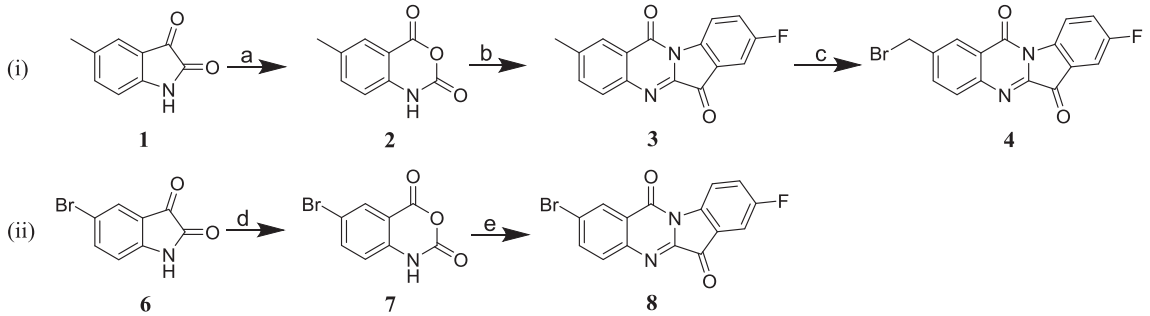


Fig. 2. Twelve tryptanthrin derivatives designed and synthesized in this work.

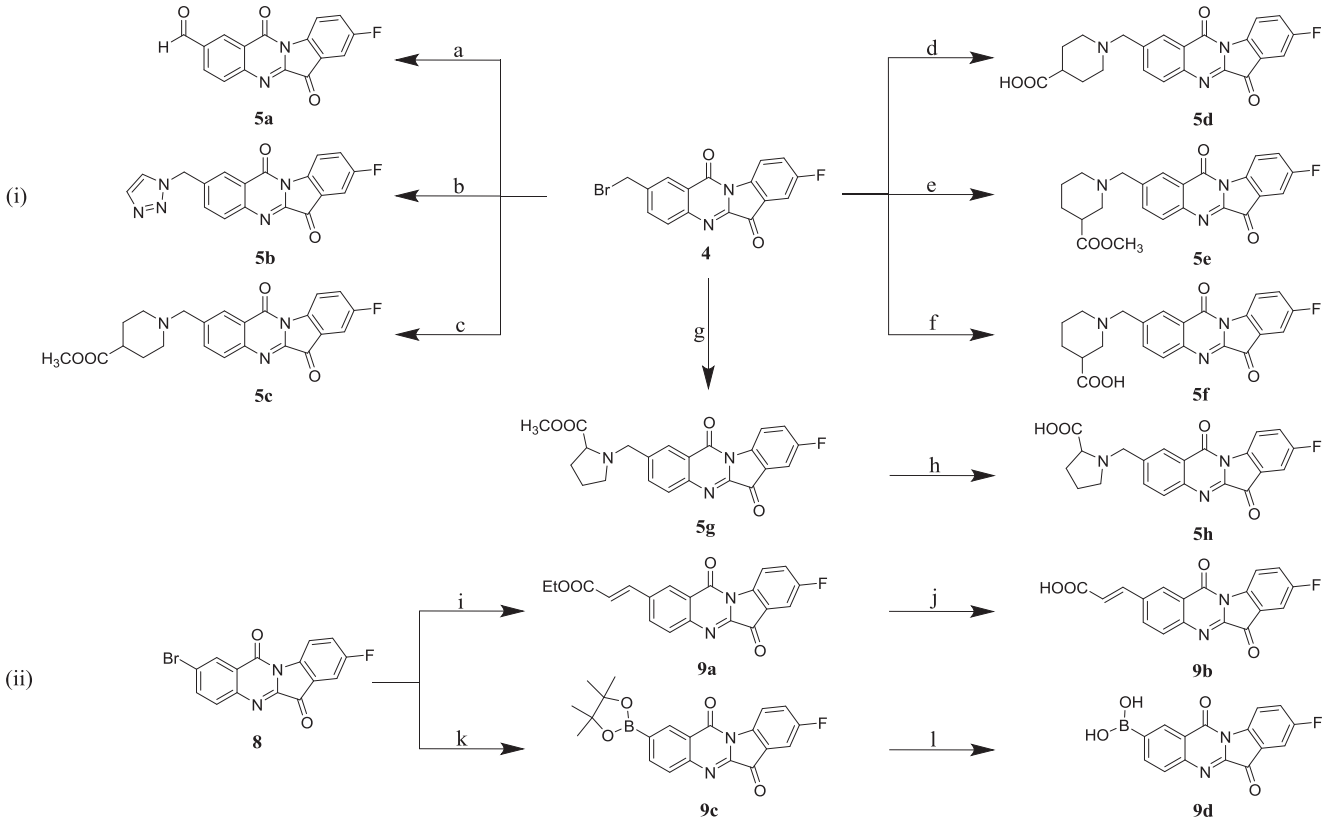
0.09 μM (see Table 1), which was consistent with that in literature.[35] All of the tested tryptanthrin derivatives exhibited IDO1 inhibitory activity. Furthermore, compound **9a** (IC_{50} = 0.19 μM) and **9b** (IC_{50} = 0.12 μM) with cinnamic acid ester and cinnamic acid group, respectively, showed much better IDO1 inhibitory activity than with 8-fluorotryptanthrin (IC_{50} = 0.534 μM)[31] and showed comparable potency with INCB024360. The IDO1 inhibitory activity of compound **5a**, **5b**, **5d**, **5f**, **5g** and **5h** was similar to that of 8-fluorotryptanthrin. Whereas compound **5c**, **5e** and **9d** led to about 2- to 3-fold drop of potency in the IDO1 inhibitory assays than 8-fluorotryptanthrin. However, the IDO1 inhibitory activity shown by compound **9c** (IC_{50} = 13.09 μM) which contained pinacol borate group at the 2-substituent of tryptanthrin exceeded 10 μM.

The twelve tryptanthrin derivatives were tested for the ability to inhibit TDO on enzymatic levels (Fig. S2). Under the same conditions, the IC_{50} value of the well-known TDO inhibitor LM10 was determined to be 11.58 μM (see Table 1), which was consistent with the value reported by Dolušić.[36] All of the twelve tryptanthrin derivatives showed TDO inhibitory activity and were superior to LM10. Particularly, in compound **5a** (IC_{50} = 0.06 μM) and **9b** (IC_{50} = 0.03 μM), the increase of 193- and 386-fold in TDO inhibitory potency with respect to LM10, and the increase of 16- and 32-fold in TDO inhibitory potency with respect to 8-fluorotryptanthrin (IC_{50} = 0.937 μM),[32] was attributed to the aldehyde and cinnamic acid group, respectively. Compound **5d**, **5f**, **5g**, **5h** and **9a** were better TDO inhibitors than 8-fluorotryptanthrin. The TDO inhibitory activity of compound **5b**, **5c** and **9d** was found to be equipotent with that of 8-fluorotryptanthrin. The TDO inhibitory activity of compound **5e** (IC_{50} = 2.87 μM) and **9c** (IC_{50} = 6.02 μM) was about 4- and 2-fold higher than that of LM10, although it was lower than that of 8-fluorotryptanthrin. Thus, all of the twelve tryptanthrin derivatives we synthesized were IDO1/TDO dual inhibitors.

To further study the IDO1 inhibitory activity of tryptanthrin derivatives, the cellular IDO1 inhibitory activity of the twelve compounds was tested using HeLa cells (Fig. S3). Under the same conditions, the IC_{50} value of INCB024360 was determined to be 0.02 μM (see Table 2), which was consistent with that in the literature.[18] The cellular inhibitory activity of most of the compounds (**5a**-**5c**, **5e** and **9a**-**9d**) were better than enzymatic inhibitory activity, which is probably due to the complexity of the enzyme.[4,37,38] In particular, compound **5b**, **9a** and **9b** severally containing triazole, cinnamic acid ester and cinnamic acid



Scheme 1. Synthesis of 2-bromomethyl-8-fluorotryptanthrin **4** and 2-bromo-8-fluorotryptanthrin **8**. Reaction conditions: (a, d) *m*-CPBA, DCM, 4 **h**, r.t.; (b, e) 5-fluoroisatin, Et_3N, CH_3CN, 4 **h**, 85 °C; (c) NBS, AIBN, CCl_4, N_2, 12 **h**, 80 °C.



Scheme 2. Synthesis of compound **5a**-**5h** and **9a**-**9d**. Reaction conditions: (a) 1) NMO, CH_3CN, 2 **h**, r.t.; 2) 3 **h**, 85 °C; (b) 1) NaN_3, CH_3COCH_3, H_2O, 10 h, r.t.; 2) CuI, Na ascorbate, propiolic acid, 8 h, 100 °C; (c, e, g) KI, Et_3N, CH_3CN, *N*-benzylnaphthenate, 4 h, r.t.; (d, f) KI, CH_3CN, *N*-benzylnaphthenic acid, 4 h, 80 °C; (h) NaOH, CH_3OH, H_2O, 5 h, r.t.; (i) ethyl acrylate, $Pd(OAc)_2$, K_3PO_4, DMA, N_2, 8 h, 140 °C; (j) NaOH, EtOH, H_2O, 5 h, r.t.; (k) $PdCl_2$(dppf), KOAc, DMF, bis(pinacolato)diboron, N_2, 18 h, 80 °C; (l) 1)$NaIO_4$, THF, H_2O, 15 min, r.t.; 2) HCl, 5 h, r.t.

Table 1
Enzymatic IDO1 and TDO inhibitory activities of tryptanthrin derivatives.

Compound	IC_{50} (μM)	
	IDO1	TDO
5a	0.46	0.06
5b	0.78	1.13
5c	1.20	0.99
5d	0.48	0.45
5e	1.43	2.87
5f	0.57	0.42
5g	0.46	0.30
5h	0.55	0.18
9a	0.19	0.37
9b	0.12	0.03
9c	13.09	6.02
9d	1.79	1.21
8-fluorotryptanthrin	0.534[a]	0.937[b]
INCB024360	0.09	ND[c]
LM10	ND[c]	11.58

[a] The enzymatic IDO1 inhibitory activity of 8-fluorotryptanthrin was tested in ref.[31]

[b] The enzymatic TDO inhibitory activity of 8-fluorotryptanthrin was assessed in ref.[32]

[c] ND: not detected.

Table 2
Cellular IDO1 inhibitory activity of tryptanthrin derivatives.

Compound	IDO1
	HeLa IC_{50} (μM)
5a	0.16
5b	0.08
5c	0.15
5d	3.10
5e	0.37
5f	1.84
5g	0.70
5h	3.76
9a	0.02
9b	0.06
9c	0.75
9d	0.23
INCB024360	0.02

group displayed excellent cellular IDO1 inhibitory activity (IC_{50} = 0.08, 0.02, and 0.06 μM, respectively). The cellular IDO1 inhibitory activity of compound **5a** (IC_{50} = 0.16 μM) and **5c** (IC_{50} = 0.15 μM) was also satisfactory. Surprisingly, compound **9c** exhibited good cellular IDO1 inhibitory activity (IC_{50} = 0.75 μM) although it bore poor enzymatic inhibitory activity. In contrast to the compounds mentioned above, the cellular inhibitory activity against IDO1 of compound **5d**, **5f** and **5h** was weaker than the enzymatic inhibitory activity. Compound **5d**, **5f** and **5h** were all 2-*N*-benzylnaphthenic acid substituent derivatives. The cellular IDO1 inhibitory activity of compound **5g** was similar to its enzymatic inhibitory activity.

The water solubility of these tryptanthrin derivatives was tested (Table 3). Compared with tryptanthrin (1.339 μg/mL),[30] 8-fluorotryptanthrin (0.741 μg/mL) was a little more difficult to dissolve due to the imputing of fluorine group. Compound **5d**, **5f** and **5h** containing *N*-naphthenic acid group showed an increase of 20-fold boost toward water solubility than 8-fluorotryptanthrin. The water solubility of other compounds was similar to that of 8-fluorotryptanthrin and tryptanthrin.

Table 3
Water solubility of tryptanthrin derivatives.

Compound	Water solubility (μg/mL)
5a	0.862
5b	1.282
5c	1.053
5d	16.667
5e	1.087
5f	14.286
5g	0.893
5h	15.385
9a	0.735
9b	1.124
9c	0.625
9d	0.690
8-fluorotryptanthrin	0.741
tryptanthrin	1.339[a]

[a] The water solubility of tryptanthrin was reported in ref.[30]

In summary, twelve tryptanthrin derivatives were synthesized and found to bear IDO1 and TDO inhibitory activities. Compound **9a** and **9b** displayed excellent enzymatic and cellular inhibitory activities against IDO1 suggesting that the cinnamic acid ester and cinnamic acid group might contribute to the IDO1 inhibitory activity of these compounds. Compound **5a** and **9b** exhibited perfect inhibitory activity against TDO demonstrating that the aldehyde and cinnamic acid group were benefit to the TDO inhibitory activity of these compounds. In addition, the water solubility of tryptanthrins containing amino and carboxyl groups (**5d**, **5f** and **5h**) was increasing. Further investigations on water solubility and biological activities of tryptanthrin derivatives are still in progress.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2020.127159.

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