N-benzyl tryptanthrin derivative, and preparation method and application thereof

Technical Field

The present invention belongs to the technical field of pharmaceutical chemistry and specifically relates to an N-benzyl tryptanthrin derivative, and preparation method and application thereof.

Prior Art

Indole-2,3-dioxygenase (abbreviated as IDO; MW 48,000; EC 1.13.11.42) is a heme-containing enzyme which constitutes the first enzyme, as well as a rate-limiting enzyme in the mammalian tryptophan metabolism pathway. IDO catalyzes an oxidation reaction in which the essential amino acid tryptophan undergoes double oxidation to produce N-formylkynurenine and is responsible for the physiological clean-up of tryptophan *in vivo*. By degrading tryptophan, IDO produces a tryptophan-poor microenvironment *in vivo*, thereby resulting in the occurrence of various diseases which are closely linked to the lack of tryptophan, such as cancer, cataracts and neurological disorders. Therefore, the search for a highly efficient inhibitor which targets IDO has become a focus of drug research and development in recent years.

IFN-γ is one of a number of potential inducers of IDO expression. During continued activation under a high level of IFN-γ stimulation, IDO reduces the availability of free serum tryptophan, thus reducing 5-hydroxytryptamine production. These changes, in conjunction with the accumulation of neurologically active kynurenine metabolites, such as quinolinic acid (also induced by IDO), promote the onset of neurological/psychiatric disorders and act as the cause of a variety of psychological disorders, while also inducing symptoms associated with chronic diseases characterized by IDO activity and tryptophan degradation. IDO activity also relates to the onset of age-related nuclear cataracts. IDO is the first enzyme involved in UV filter biosynthesis in the lens and is also the rate-

limiting enzyme. Modifications to UV filter compounds derived from the degradation of tryptophan (3-hydroxykynurenine and kynurenine glucoside) are present in human lens proteins. The amount of these UV filter compounds increases with age, gradually causing the lens to become cloudy, which further leads to so-called age-related nuclear cataracts.

IDO expression also relates to the suppression of an immune response caused by blocking local T-lymphocyte proliferation. T-lymphocytes are very sensitive to a lack of tryptophan and T-lymphocytes will be arrested in the G1 phase of the cell cycle in the absence of tryptophan. This T cell-mediated immune response suppression is the cause of many diseases, including autoimmune diseases, allograft rejection, neurodegenerative disorders, depression, bacterial or viral infections (e.g. the human immunodeficiency virus (HIV)) and cancer (Swanson et al. Am. J. Respir. Cell Mol. Biol. 2003, 30, 311).

It has been found that most human tumors constitutively express IDO. Murine tumor cells obtained from previously immunized mice have shown that expression of IDO can provide protection from rejection, and administration of 1-MT eliminates the aforementioned effect. Furthermore, concomitant administration of an IDO inhibitor improved the effectiveness of cancer treatment.

IDO inhibitors may be used in the control of mental disorders, as well as the treatment of other diseases which include the IDO-mediated metabolism of tryptophan as a pathological feature; said diseases including viral infections such as AIDS, bacterial infection such as Lyme disease and streptococcal infection, neurodegenerative disorders (e.g., Alzheimer's disease, Huntington's disease and Parkinson's disease), depression, cancer (including T-cell leukemia and colon cancer), ocular diseases (such as cataracts and age-related yellowing) and autoimmune diseases. A variety of different *in vitro* assays (Takikawa, et al. J. Biol. Chem. 1998, 263, 2041) can be used to filter (for example, via high throughput

screening) and measure the IDO inhibition activity of a reaction reference or extracts obtained from natural sources, or determine IDO inhibition-related kinetic constants.

IDO is closely linked to the pathogenesis of many different diseases and has already been shown to be a target for cancer, Alzheimer's disease, depression, cataracts and other serious diseases. IDO inhibitors have broad prospects for application as drugs, but so far no suitable IDO inhibitor has been marketed as a drug; therefore, the search for a novel, efficient IDO inhibitor has important theoretical significance and potential applied value.

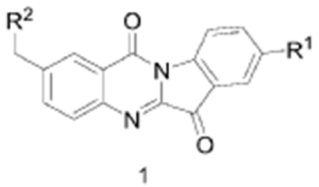
Existing studies have shown that the IDO inhibitor 1-MT (1-methyl-tryptophan) can enhance the sensitivity of tumor cells to T-cell immune stimulation *in vitro;* and the same agent is capable of retarding the growth of tumor cells and enhancing chemotherapy drug anti-tumor efficacy *in vivo* in animal models, with effects observed for virtually all spontaneously occurring tumors. Unfortunately, the inhibition efficiency of most existing IDO inhibitors is low, and the inhibition constant (Ki) of the most commonly used IDO inhibitor in *in vivo* and *in vitro* trials, 1-MT, is a mere 34 μM.

Thus, there is an urgent need in this field to develop a novel, efficient IDO inhibitor.

Description of the Invention

The objective of the present invention is the provision of an N-benzyl tryptanthrin derivative, and preparation method and application thereof.

The first aspect of the present invention provides an N-benzyl tryptanthrin derivative, wherein said derivative has the general structure as shown in Formula 1 below.



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where

R^1 corresponds to hydrogen or fluorine;

R^2 corresponds to $-NR^3R^4$;

R^3 and R^4 are each independently selected from the following set: H, substituted or unsubstituted C1 – C4 alkyl groups, substituted or unsubstituted C2 – C4 alkenyl groups, substituted or unsubstituted C2 – C4 alkynyl groups, and substituted or unsubstituted C3 – C6 cycloalkyl groups;

or R^3 and R^4 forms a substituted or unsubstituted 5 – 7 membered heterocyclic ring with an adjacent nitrogen atom, where said 5 – 6 membered saturated ring includes 1 – 2 nitrogen atoms as well as 0 – 2 heteroatoms selected from the following set: O and S;

here, substitution refers to cases in which one or more hydrogen atoms present on a given group (preferably hydrogen atoms present on a nitrogen atom) are substituted by a substituent selected from the following set: C1 – C4 alkyl groups, C1 – C4 haloalkyl groups, amino-protecting groups (preferably t-butoxycarbonyl) and halogens.

In another preferred embodiment, when R^3 and R^4 together with an adjacent nitrogen atom form a substituted or unsubstituted 5 – 6 membered saturated ring, the nitrogen atom present on the ring may optionally bear an amino-protecting group.

In another preferred embodiment, said amino-protecting group is selected from the following set:

In another preferred embodiment, said 5 – 7 membered heterocyclic ring is not a heteroaryl ring.

In another preferred embodiment, said 5 – 7 membered heterocyclic ring is a saturated heterocyclic ring, preferably a 5 – 6 membered saturated heterocyclic ring.

In another preferred embodiment, said 5 – 7 membered heterocyclic ring contains only one or two heteroatoms.

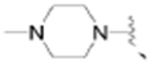
In another preferred embodiment, all of the heteroatoms of said 5 – 7 membered heterocyclic ring are constituted by N.

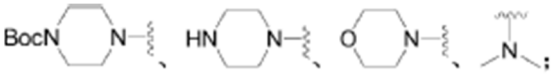
In another preferred embodiment, R^3 and R^4 together are each independently selected from the following set: C1-C4 alkyl groups; or, R^3 and R^4 together with an adjacent nitrogen atom form a substituted or unsubstituted 5 - 6 membered saturated ring, where said 5 - 6 membered saturated ring includes 1 - 2 nitrogen atoms, as well as an optional one heteroatom selected from the following set: O.

In another preferred embodiment, R^3 and R^4 are not simultaneously constituted by H.

In another preferred embodiment, R^2 is a cyclic imine.

In another preferred embodiment, R^2 is a substituted or unsubstituted group selected from the following set:



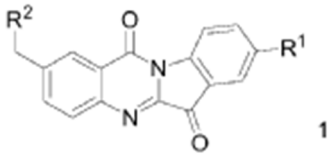


where "〰" indicates a connection site;

here, substitution refers to cases in which one or more hydrogen atoms present on a given group are substituted by a substituent selected from the following set: C1 - C4 alkyl groups and halogens.

In another preferred embodiment, R^1 corresponds to F.

The second aspect of the present invention provides an N-benzyl tryptanthrin derivative, wherein the general structure of said derivative is as shown below:



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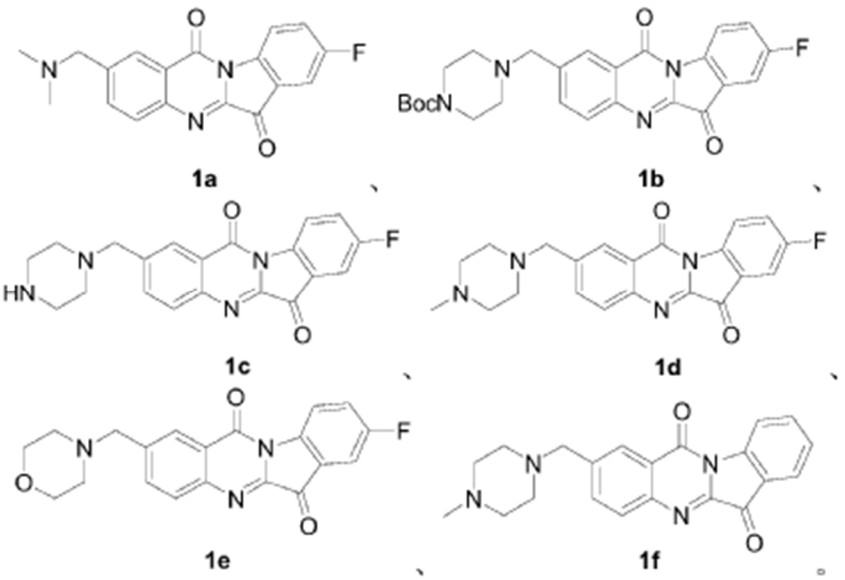
R^1 = H or F

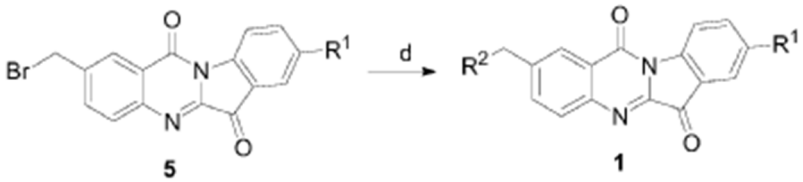
R^2 =

where the substituent R^2 corresponds to a cyclic imine or dialkyl-substituted amine and R^1 corresponds to hydrogen or fluorine.

In another preferred embodiment, said derivative is a compound selected from the following set:



The third aspect of the present invention provides a method for the preparation of an N-benzyl tryptanthrin derivative as given in the first or second aspects of the present invention, where said method includes the following steps:



(d) The compound given in Formula 5 (2-bromomethyl-tryptanthrin) is reacted with R^2H in an inert solvent in the presence of triethylamine to obtain the compound shown in Formula 1;

where R^1 and R^2 are as defined above.

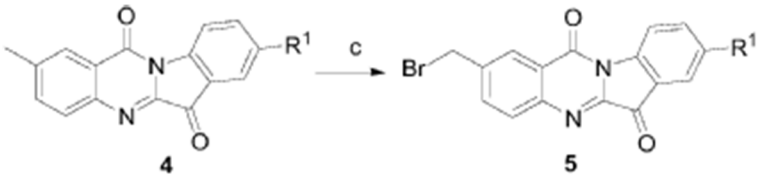
In another preferred embodiment, in Step (d), said inert solvent is constituted by DMF, preferably anhydrous DMF.

In another preferred embodiment, in Step (d), said reaction is carried out at approximately 10 - 40°C.

In another preferred embodiment, in Step (d), the duration of said reaction is 0.5 - 12 hours, with a range of 1 - 5 hours being preferable.

In another preferred embodiment, in Step (d), the molar ratio of the compound given in Formula 5, R^2H and triethylamine is 1 : (1 - 2) : (2 - 5).

In another preferred embodiment, said method further includes a Step (c) prior to Step (d):



(c) The compound given in Formula 4 is reacted with a bromination agent in an inert solvent to obtain the compound given in Formula 5; where the bromination agent used is preferably NBS.

In another preferred embodiment, the reaction in Step (c) is carried out in the presence of an initiator, and preferably carried out in the presence of AIBN.

In another preferred embodiment, Step (c) includes: mixing NBS and AIBN, followed by reacting with the compound given in Formula 4.

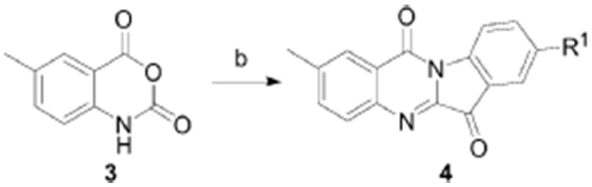
In another preferred embodiment, the inert solvent indicated in Step (c) is dichloromethane, with anhydrous dichloromethane being preferable.

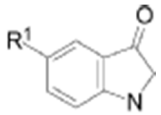
In another preferred embodiment, in Step (c), said reaction is carried out at approximately 75 – 85°C.

In another preferred embodiment, in Step (c), the duration of said reaction is 15 – 17 hours.

In another preferred embodiment, in Step (c), the molar ratio of the compound given in Formula 4, NBS and AIBN is 1 : 1 : (0.005 – 0.02).

In another preferred embodiment, said method further includes a Step (b) prior to Step (c):

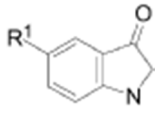


(b) The compound given in Formula 3 is reacted with  in an inert solvent in the presence of triethylamine to obtain the compound shown in Formula 4.

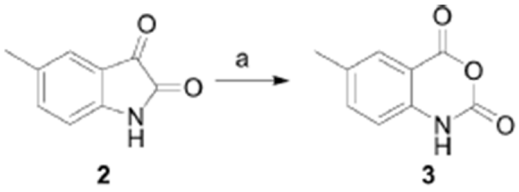
In another preferred embodiment, in Step (b), said inert solvent is constituted by toluene, preferably anhydrous toluene.

In another preferred embodiment, in Step (b), said reaction is carried out at approximately 100 – 120°C.

In another preferred embodiment, in Step (b), the duration of said reaction is 3.5 – 4.5 hours.

In another preferred embodiment, in Step (b), the molar ratio of the compound given in Formula 3,  and triethylamine is (0.2 – 0.5) : (0.2 – 0.5) : 1.

In another preferred embodiment, said method further includes a Step (a) prior to Step (b):



2 3

(a) The compound given in Formula 2 is reacted with an oxidizing agent in an inert solvent to obtain the compound given in Formula 3; where the oxidizing agent used is preferably meta-chloroperoxybenzoic acid.

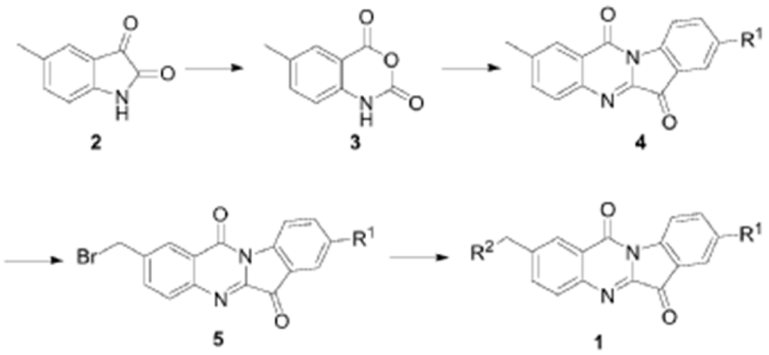
In another preferred embodiment, the inert solvent indicated in Step (a) is dichloromethane, with anhydrous dichloromethane being preferable.

In another preferred embodiment, in Step (a), said reaction is carried out at room temperature (with a range of 10 – 40°C being preferable).

In another preferred embodiment, in Step (a), the duration of said reaction is 1.5 – 2.5 hours.

In another preferred embodiment, in Step (a), the molar ratio of the compound given in Formula 2 and the oxidizing agent (0.5 – 1) : 1.

The fourth aspect of the present invention provides a method for the preparation of an N-benzyl tryptanthrin derivative as given in the second aspect of the present invention, where the synthesis route is as described below:



R^1 = H or F

The specific steps are as follows:

(1) Synthesis of Methylisatoic Anhydride (3)

Suspend 5-methylisatin in dry dichloromethane and add meta-chloroperoxybenzoic acid batchwise at 0°C, then stir for 1.5 - 2.5 hours at room temperature; once the reaction is confirmed as being complete via TLC, filtering off the white solid produced in the reaction and washing thrice with ethyl acetate to obtain 5-methylisatoic anhydride; note that the molar ratio of 5-methylisatin and meta-chloroperoxybenzoic acid is (0.5 - 1) : 1;

(2) Synthesis of 2-Methyltryptanthrin (4)

Suspend a mixture of methylisatoic anhydride, 5-fluoroisatin and triethylamine in dry toluene, then heat for 3.5 - 4.5 hours at a temperature of 100 - 120°C and distill off the solvent under reduced pressure; dissolve the resulting yellow solid in dichloromethane and add ethyl acetate, then filter the resulting yellow solid and wash with ethyl acetate three times to obtain 2-methyltryptanthrin; note that the molar ratio of methylisatoic anhydride, 5-fluoroisatin and triethylamine is (0.2 - 0.5) : (0.2 - 0.5) : 1;

(3) Synthesis of 2-bromomethyl-tryptanthrin (5)

Dissolve the 2-methyltryptanthrin obtained in Step (2) in dry dichloromethane at 75 - 85°C under the protection of nitrogen gas, then add in a mixture of NBS and AIBN batchwise. Heat the reaction solution to 75°C for 15 - 17 hours and confirm that the reaction is completed via

TLC. Once the reaction solution cools to room temperature, performing washing with a salt solution and drying over anhydrous sodium sulfate to obtain a concentrated yellow product; note that the molar ratio of 2-methyltryptanthrin, NBS and AIBN is 1 : 1 : (0.005 - 0.02);

(4) Synthesis of N-Benzyltryptanthrin (1)

Stir together 2-bromomethyl-tryptanthrin, an aliphatic amine and triethylamine at room temperature in dry DMF for 1.5 - 2.5 hours, and once the reaction is verified as being complete via TLC, adding 50 ml of water and performing three sequential extractions using 10 ml of dichloromethane; next, subject the entire volume of dichloromethane obtained to three washes with water and then dry over anhydrous sodium sulfate. Perform separation of the concentrated yellow solid obtained using silica gel to obtain a yellow colored N-benzyl tryptanthrin derivative; note that the molar ratio of 2-bromomethyl-tryptanthrin, the aliphatic amine and triethylamine is 1 : (1 - 2) : (2 - 5).

The fifth aspect of the present invention provides an application for the N-benzyl tryptanthrin derivative given in the first and second aspects of the present invention in the preparation of a drug designed to prevent and/or treat diseases which include a disorder of the IDO-mediated metabolism of tryptophan as a pathological feature.

The sixth aspect of the present invention provides an application for the compound given in Formula 1 as described in the first and second aspects of the present invention or a pharmaceutically acceptable salt thereof, to be used for:

(i) the preparation of an IDO inhibitor;

(ii) the preparation of a drug for the treatment of a tryptophan metabolism-related disease; and

(iii) non-therapeutic inhibition of IDO *in vitro*.

In another preferred embodiment, said tryptophan metabolism disorder-related disease is selected from the

following set: tumors, depression, anxiety, AIDS, autoimmune diseases, mental disorders, Lyme disease infections, streptococcal infections, neurodegenerative disorders (such as Alzheimer's disease, Huntington's disease and Parkinson's disease), depression, cancer (including T-cell leukemia and colon cancer), ocular diseases (such as cataracts and age-related yellowing) and autoimmune diseases.

The seventh aspect of the present invention provides a pharmaceutical composition, where said pharmaceutical composition includes: (i) a compound given in Formula 1 as described in the first and second aspects of the present invention or pharmaceutically acceptable salts thereof; as well as (ii) a pharmaceutically acceptable carrier.

In another preferred embodiment, said pharmaceutical composition is used for the treatment of diseases associated with a disorder in the tryptophan metabolism.

In another preferred embodiment, said tryptophan metabolism disorder-related disease is selected from the following set: tumors, depression, anxiety, AIDS, autoimmune diseases, mental disorders, Lyme disease infections, streptococcal infections, neurodegenerative disorders (such as Alzheimer's disease, Huntington's disease and Parkinson's disease), depression, cancer (including T-cell leukemia and colon cancer), ocular diseases (such as cataracts and age-related yellowing) and autoimmune diseases.

The eighth aspect of the present invention provides an IDO inhibitor, where said IDO inhibitor includes a compound given in Formula 1 as described in the first and second aspects of the present invention, or a pharmaceutically acceptable salt thereof.

The ninth aspect of the present invention provides a method for non-therapeutically inhibiting IDO activity *in vitro*, where said method includes the following process: a compound given in Formula 1 as described in the first and second aspects of the present invention or

a pharmaceutically acceptable salt thereof is brought into contact with an inhibition target in an amount which produces effective inhibition.

The tenth aspect of the present invention provides a method for the preparation of a pharmaceutical composition used to treat a disease associated with a disorder in the tryptophan metabolism, where, in said method: a compound given in Formula 1 as described in the first and second aspects of the present invention or a pharmaceutically acceptable salt thereof is mixed with a pharmaceutically acceptable carrier in a therapeutically effective amount to create a pharmaceutical composition.

The eleventh aspect of the present invention provides a method for treating a disease associated with a disorder in the tryptophan metabolism, where, in said method: a compound given in Formula 1 as described in the first and second aspects of the present invention or a pharmaceutically acceptable salt thereof is applied in a therapeutically effective amount to a treatment subject.

In another preferred embodiment, said tryptophan metabolism disorder-related disease is selected from the following set: tumors, depression, anxiety, AIDS, autoimmune diseases, mental disorders, Lyme disease infections, streptococcal infections, neurodegenerative disorders (such as Alzheimer's disease, Huntington's disease and Parkinson's disease), depression, cancer (including T-cell leukemia and colon cancer), ocular diseases (such as cataracts and age-related yellowing) and autoimmune diseases.

It should be noted that within the scope of the present invention, the aforementioned technical characteristics of the present invention and the technical characteristics described more specifically in the following text (e.g., the examples) can be combined with each other, thereby creating novel or preferred technical solutions. These are not listed exhaustively here due to space constraints.

Detailed Description

Following long-term and in-depth research performed by the inventors of the present invention, the inventors unexpectedly discovered a type of N-benzyl tryptanthrin derivative with the structure as shown in Formula 1 which exhibits a highly specific IDO inhibitory activity, where the IDO inhibitory activity (IC_{50}) is unexpectedly significantly improved (the IC_{50} value can fall by 1 - 3 orders of magnitude) in said compound versus certain other N-benzyl tryptanthrin derivatives having a highly similar structure, and said compound therefore has a good application potential. The present invention was completed by the inventors based on the aforementioned discovery.

Terms

Unless otherwise specified, all of the compounds pertaining to the present invention shall include all optical isomers or tautomeric forms thereof.

The term "C1 - C4 alkyl group" refers to a straight or branched chain alkyl group having 1 - 4 carbon atoms such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl or similar groups.

The term "C2 - C4 alkenyl group" refers to a straight or branched alkenyl group having 2 to 4 carbon atoms such as ethenyl, propenyl, butenyl or similar groups.

The term "C2 - C4 alkynyl group" refers to a straight or branched alkynyl group having 2 to 4 carbon atoms such as ethynyl, propynyl, butynyl or similar groups.

The term "5 - 7 membered heterocyclic ring" refers to a single ring containing 5 - 7 elements, where said ring does not have a completely conjugated π-electron system. In particular, in the context of the present invention, said ring may optionally contain 1 - 3 heteroatoms, where said heteroatoms include O and N. Representative examples of saturated rings include: a piperazinyl group, morpholino group, etc.

Unless otherwise specified, the term "substitution" refers to cases in which one or more hydrogen atoms present on a given group are substituted by a substituent

selected from the following set: C1 – C4 alkyl groups, C1 – C4 haloalkyl groups, amino-protecting groups (preferably t-butoxycarbonyl) and halogens.
The term "halogen" refers to F, Cl, Br and I.

N-Benzyl Tryptanthrin Derivatives

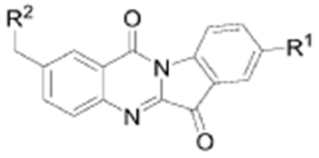
Tryptanthrin is a type of indole quinazoline alkaloid, bearing the chemical name indolo[2,1-b]quinazoline-6,12-dione. Tryptanthrin is a yellow needle-like crystal in its pure form and can be found primarily in plants belonging to the indigo family, such as *Strobilanthes cusia*, *Polygonum tinctorium* and *Isatis tinctoria*. Alternatively, it can be extracted from a fermentation broth containing certain microorganisms.

Although tryptanthrin can be extracted from indigo plants as well as the metabolites of microorganisms, the separation process is long and yield is low, making it difficult to satisfy research-related and clinical drug demands. Only by exploring rapid, high yield, simple and readily available synthetic pathways can more resources be provided for tryptanthrin applications, making further research, development and application possible.

In order to overcome the deficiencies of existing techniques, the present invention has made structural modifications to tryptanthrin in order to improve the solubility and pharmacological activity of tryptanthrin, with the objective of obtaining an active compound with application value. Research and pharmacological testing pertaining to the present invention show that N-benzyl tryptanthrin derivatives which are produced by introducing a water soluble group into the tryptanthrin molecule have the potential to be more efficient as an IDO inhibitor, exhibiting various pharmacological activities, such as anti-bacterial, anti-inflammatory and anti-tumor activities, and having a wide application potential. Furthermore, compared to conventional methods for synthesizing tryptanthrin derivatives, the synthesis method constituted by the present invention offers the advantages of being simple to operate while using mild

conditions and producing a high yield, making it more suitable for industrial production.

Specifically, the N-benzyl tryptanthrin derivative constituted by the present invention has the general structure as shown in Formula 1 below:



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where

R^1 corresponds to hydrogen or fluorine;

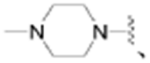
R^2 corresponds to $-NR^3R^4$;

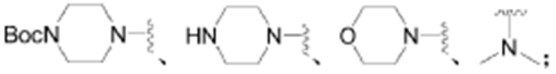
here, R^3 and R^4 are each independently selected from the following set: C1–C4 alkyl groups; or, R^3 and R^4 together with an adjacent nitrogen atom form a substituted or unsubstituted 5 – 6 membered saturated ring, where said 5 – 6 membered saturated ring includes at least one nitrogen atom as well as an optional 1 – 2 heteroatoms selected from the following set: O and N;

here, substitution refers to cases in which one or more hydrogen atoms present on a given group (preferably hydrogen atoms present on a nitrogen atom) are substituted by a substituent selected from the following set: C1 – C4 alkyl groups, t-butoxycarbonyl groups and halogens.

Preferably, R^2 should bear a nitrogen atom as well as a heteroatom selected from the following set: O and N.

Preferably, R^2 should be a substituted or unsubstituted group selected from the following set:





where "〰" indicates a connection site;

here, substitution refers to cases in which one or more hydrogen atoms present on a given group are substituted by a substituent selected from the following set: C1 – C4 alkyl groups and halogens.

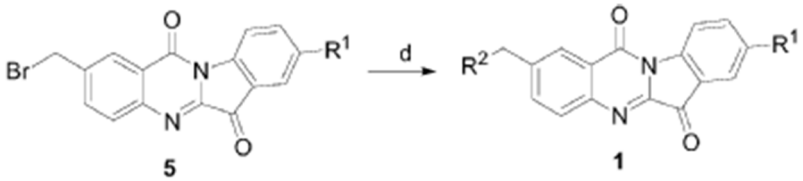
The compound described by the present invention, the tautomeric forms, structural analogs or pharmaceutically acceptable salts thereof, as well as compositions containing at least one instance of said compound, a structural analog thereof or a pharmaceutically acceptable salt thereof can all be used to inhibit IDO, as well as in applications for the treatment and/or prevention of diseases which include the IDO-mediated tryptophan metabolism as a pathological feature. Such diseases include, but are not limited to, tumors, cancer, ocular diseases, autoimmune diseases, mental disorders, depression and anxiety. Said applications include *in vitro* and *in vivo* applications, as well as applications in the preparation of drugs, IDO inhibitors and pharmaceutical compositions. In particular, the inventors of the present invention discovered that, in a particularly preferred embodiment, when R^1 corresponds to F, the compound exhibits an optimal IDO inhibitory activity.

Preparation of N-Benzyl Tryptanthrin Derivatives

In recent years, pharmaceutical chemists have made extensive efforts to research the synthesis of tryptanthrin and its derivatives, and the main method used to synthesize tryptanthrin has been the reaction of isatin with isatoic anhydride; said method is simple, provides a high yield and uses mild reaction conditions. Furthermore, it is possible to add additional functional groups to the starting materials isatin and isatoic anhydride to synthesize a variety of different functional tryptanthrins. Currently, the main method used to synthesize isatin involves reacting chloral hydrate, hydroxylamine and aniline in aqueous hydrochloric acid to produce oximes, after which, ring closure is induced under concentrated sulfuric acid to obtain isatin; said method is extremely well suited to the synthesis of halogens and alkyl-containing tryptanthrins and provides a high yield. However, it is difficult to use this method to produce tryptanthrin containing active groups, due to

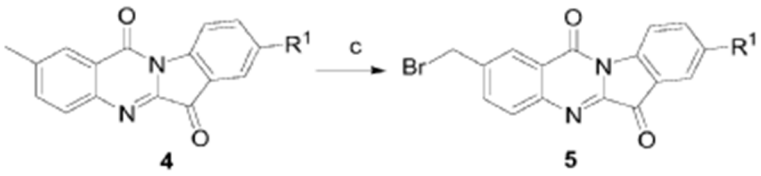
the fact that said active groups readily produce byproduct reactions during the process of synthesizing isatin.

Following lengthy research efforts, the inventors of the present invention have successfully developed a synthesis method which can be used to prepare a tryptanthrin having active groups. In particular, said method includes the following step:



(d) The compound given in Formula 5 (2-bromomethyl-tryptanthrin) is reacted with R^2H in an inert solvent in the presence of triethylamine to obtain the compound shown in Formula 1.

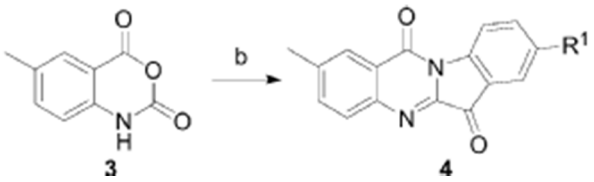
In another preferred embodiment, said method further includes the following step:

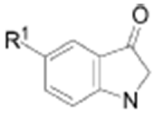


(c) The compound given in Formula 4 (2-methyltryptanthrin) is reacted with a bromination agent in an inert solvent to obtain the compound given in Formula 5; where the bromination agent used is preferably NBS.

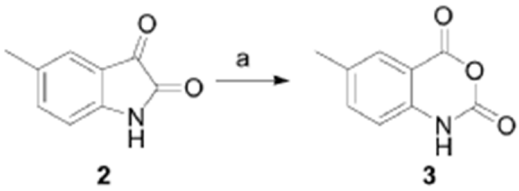
In a preferred embodiment of the present invention, the bromination reaction is carried out in the presence of an initiator such as AIBN. A preferred embodiment includes: mixing NBS and AIBN, followed by a reacting with the compound given in Formula 4.

In another preferred embodiment, said method further includes the following step:



(b) The compound given in Formula 3 is reacted with  in an inert solvent in the presence of triethylamine to obtain the compound shown in Formula 4.

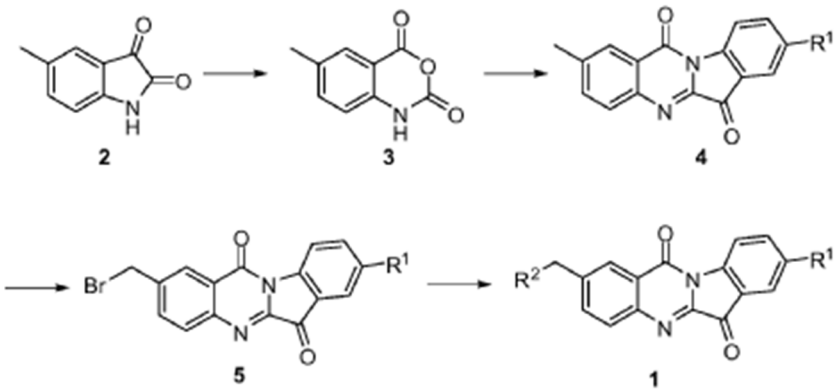
In another preferred embodiment, said method further includes the following step:



(a) The compound given in Formula 2 is reacted with an oxidizing agent in an inert solvent to obtain the compound given in Formula 3; where the oxidizing agent used is preferably meta-chloroperoxybenzoic acid.

The reaction times, reaction temperatures, etc., given in the aforementioned steps can be adjusted based on actual conditions; for example, methods common to the field (e.g., a TLC assay) can be used to confirm the end of the reaction. The solvents used in each step are not particularly restricted in any way, and inert solvents which do not react with the reactants which are common to the field can be used. It should be understood that once the aforementioned reaction scheme is disclosed in the present application, the specific conditions in each step can be established by a person skilled in the art given their existing knowledge within the field.

Among the methods for the preparation of N-benzyl tryptanthrin derivatives constituted by the present invention, an optimal synthesis route is given below:



(1) Synthesis of Methylisatoic Anhydride (3)

Suspend 5-methylisatin in dry dichloromethane and add meta-chloroperoxybenzoic acid batchwise at 0°C, then stir for 1.5 - 2.5 hours at room temperature; once the reaction is confirmed as being complete via TLC, filtering off the white solid produced in the reaction and washing thrice with ethyl acetate to obtain 5-methylisatoic anhydride; note that the molar ratio of 5-methylisatin and meta-chloroperoxybenzoic acid is (0.5 - 1) : 1;

(2) Synthesis of 2-Methyltryptanthrin (4)

Suspend a mixture of methylisatoic anhydride, 5-fluoroisatin and triethylamine in dry toluene, then heat for 3.5 - 4.5 hours at a temperature of 100 - 120°C and distill off the solvent under reduced pressure; dissolve the resulting yellow solid in dichloromethane and add ethyl acetate, then filter the resulting yellow solid and wash with ethyl acetate three times to obtain 2-methyltryptanthrin; note that the molar ratio of methylisatoic anhydride, 5-fluoroisatin and triethylamine is (0.2 - 0.5) : (0.2 - 0.5) : 1;

(3) Synthesis of 2-bromomethyl-tryptanthrin (5)

Dissolve the 2-methyltryptanthrin obtained in Step (2) in dry dichloromethane at 75 - 85°C under the protection of nitrogen gas, then add in a mixture of NBS and AIBN batchwise. Heat the reaction solution to 75°C for 15 - 17 hours and confirm that the reaction is completed via TLC. Once the reaction solution cools to room temperature, performing washing with a salt solution and drying over anhydrous sodium sulfate to obtain a

concentrated yellow product; note that the molar ratio of 2-methyltryptanthrin, NBS and AIBN is 1 : 1 : (0.005 - 0.02);

(4) Synthesis of N-Benzyltryptanthrin (1)

Stir together 2-bromomethyl-tryptanthrin, an aliphatic amine and triethylamine at room temperature in dry DMF for 1.5 - 2.5 hours, and once the reaction is verified as being complete via TLC, adding 50 ml of water and performing three sequential extractions using 10 ml of dichloromethane; next, subject the entire volume of dichloromethane obtained to three washes with water and then dry over anhydrous sodium sulfate. Perform separation of the concentrated yellow solid obtained using silica gel to obtain a yellow colored N-benzyl tryptanthrin derivative. Here, the molar ratio of 2-bromomethyl-tryptanthrin, the aliphatic amine and triethylamine is 1 : (1 - 2) : (2 - 5).

In the above formulas, the definitions for each group are as given above.

IDO Inhibitors and Their Applications

Due to the fact that the N-benzyl tryptanthrin derivative constituted by the present invention exhibits a highly specific IDO inhibitory activity, it can be used in the preparation of drugs used to treat and/or prevent diseases which include a disorder of the IDO-mediated metabolism of tryptophan as a pathological feature (such as tumors).

Said disease involving a disorder of the tryptophan metabolism includes any diseases which include a disorder of the IDO-mediated metabolism of tryptophan as a pathological feature which are known or unknown within the field, and preferably, said tryptophan metabolism disorder-related disease is selected from the following set: tumors, depression, anxiety, AIDS, autoimmune diseases, mental disorders, Lyme disease infections, streptococcal infections, neurodegenerative disorders (such as Alzheimer's disease, Huntington's disease and Parkinson's disease), depression, cancer (including T-

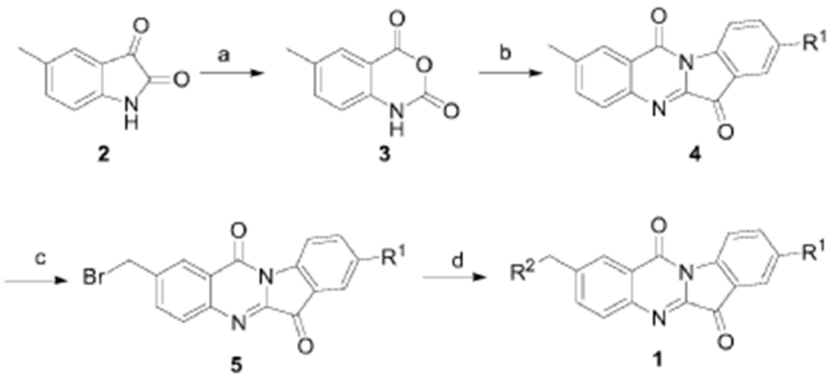
cell leukemia and colon cancer), ocular diseases (such as cataracts and age-related yellowing) and autoimmune diseases.

Compared with existing techniques, the present invention offers the following primary advantages:

(1) The present invention provides a class of IDO inhibitors with a novel structure which, compared to compounds having similar structures, exhibit a surprising improvement in activity. Said compounds not only produce an IDO inhibitory activity, but they can also serve as further modified pharmaceutical intermediates at the same time, and have potential application value in the development of novel cancer treatment drugs.

(2) The present invention also provides a method for the synthesis of N-benzyl tryptanthrin derivatives, and said synthesis route offers the advantages of being simple to operate, employing mild reaction conditions, reducing solvent use, reducing pollution, etc., and is suitable for mass production.

In the following section the present invention will be further described by way of specific embodiment examples. It should be understood that these examples are merely illustrative of the present invention and are not intended to limit the scope of the present invention. For experimental methods included in the following embodiment examples which do not specify specific conditions, conventional conditions or conditions recommended by the manufacturer are used. Unless otherwise indicated, percentages and partial quantities are calculated by weight. Unless otherwise specified, the reagents and starting materials given in the following embodiment examples correspond to commercially available products. A specific synthesis route is given below:



Reaction conditions for each step:

Step a. m-CPBA(2eq.), CH_2Cl_2, RT;

Step b. Et_3N, toluene, reflux, 75%;

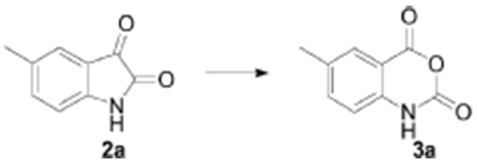
Step c. NBS, AIBN, CH_2Cl_2, reflux;

Step d. K_2CO_3, KI, DMF, amine.

Embodiment Example 1 - Synthesis of 2-((dimethylamino) methyl)-8-fluoro-indolo[2,1-b]quinazoline-6,12-dione (1a)

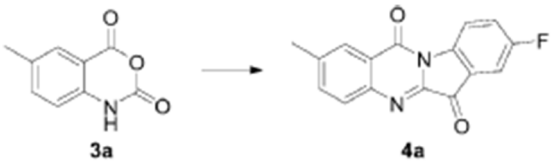
The specific steps are as follows:

Step (1): Synthesis of 6-methyl-1H-benzo[d][1,3]oxazine-1,4-dione (3a)



Compound 2a (500 mg, 3 mmol) was suspended in 10 ml of dry dichloromethane, after which meta-chloroperoxybenzoic acid (1.3 g, 6 mmol, 75%) was added batchwise at 0°C. TLC indicated that the reaction was complete after stirring the mixture for 2 hours at room temperature, after which, the white solid produced in the reaction was filtered off and three washes were performed with 10 ml ethyl acetate to obtain Compound 3a (350 mg, 65%).

Step (2): Synthesis of 8-fluoro-2-methyl-indolo[2,1-b]quinazoline-6,12-dione (4a)



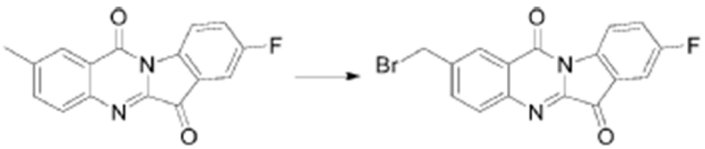
3a 4a

A mixture of Compound 3a (1 g, 5.6 mmol), 5-fluoroisatin (0.93 g, 5.6 mmol) and triethylamine (1.5 ml, 11.2 mmol) was suspended in dry toluene (10 ml) and heated at 110°C for 4 hours. The solvent was distilled off under reduced pressure and the yellow solid thus obtained was dissolved in 2 ml of dichloromethane, after which 2 ml of ethyl acetate was added and the yellow solid was filtered and washed three times with 2 ml ethyl acetate to yield a yellow solid compound, i.e. Compound 4a (1.1 g, 75%).

Characterization Data:

^{1}H NMR (400 MHz, DMSO) δ 8.50 (dd, *J* = 8.8, 4.2 Hz, 1H), 8.14 (s, 1H), 7.86 (d, *J* = 8.2 Hz, 1H), 7.82 – 7.76 (m, 1H), 7.76 – 7.67 (m, 1H), 7.44 – 7.37 (m, 1H), 2.53 (s, 3H).

Step (3): Synthesis of 2-(bromomethyl)8-fluoro-indole[2,1-b]quinazoline-6,12-dione (5a)



4a 5a

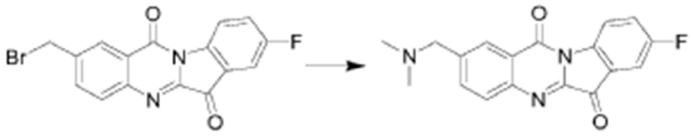
A mixture of NBS (381 mg, 2.14 mmol) and AIBN (29 mg, 0.18 mmol) was added in three batches to a dichloromethane solution (3.6 ml) containing Compound 4a (500 mg, 1.78 mmol) at a temperature of 80°C under the protection of nitrogen gas. The reaction solution was heated to 80°C for 16 hours. Once TLC confirmed that the reaction was complete, the reaction solution was cooled to room temperature, washed with a salt solution and dried over anhydrous sodium sulfate, to obtain a concentrated yellow product (5a).

Characterization Data:

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (dd, *J* = 8.8, 4.0 Hz, 1H), 8.45 (d, *J* = 1.9 Hz, 1H), 8.04 (d, *J* = 8.3 Hz, 1H), 7.90

(dd, *J* = 8.3, 2.1 Hz, 1H), 7.61 (dd, *J* = 6.5, 2.6 Hz, 1H), 7.52 (td, *J* = 8.6, 2.7 Hz, 1H), 4.65 (s, 2H).

Step (4): Synthesis of 2-((dimethylamino)methyl)-8-fluoroindolo[2,1-b]quinazoline-6,12-dione (1a)



5a 1a

Compound 5a (500 mg, 1.39 mmol), dimethylamine hydrochloride (227 mg, 2.78 mmol), potassium iodide (10 mg) and triethylamine (0.5 ml) were stirred in a 5 ml solution of DMF at room temperature for 2 hours. Once TLC confirmed that the reaction was complete, 50 ml of water was added and three consecutive extractions were performed using 10 ml of ethyl acetate, after which the organic phase was washed three times with water and drying was performed over anhydrous sodium sulfate. The concentrated yellow solid obtained was subjected to separation using a silica gel column to obtain a yellow compound (1a).

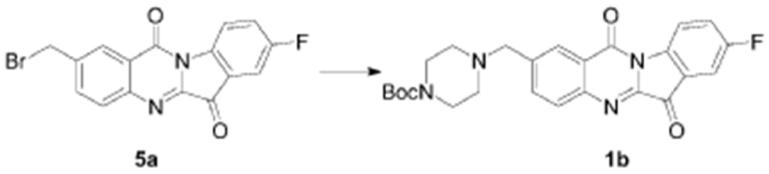
Characterization Data:

^{1}HNMR (400 MHz, $CDCl_3$) δ 8.63 (dd, *J* = 8.8, 4.1 Hz, 1H), 8.33 (d, *J* = 1.5 Hz, 1H), 7.98 (d, *J* = 8.3 Hz, 1H), 7.87 (dd, *J* = 8.3, 1.8 Hz, 1H), 7.58 (dd, *J* = 6.5, 2.6 Hz, 1H), 7.48 (td, *J* = 8.7, 2.7 Hz, 1H), 3.61 (s, 2H), 2.30 (s, 6H).

Embodiment Example 2 - Synthesis of 4-((8-fluoro-6,12-dioxo-6,12-dihydroxy-indolo[2,1-b]quinolin-2-yl) methyl)piperazine-1-carboxylic acid tert-butyl ester (1b)

Steps (1) - (3) are as specified in Embodiment Example 1.

Step (4): Synthesis of 4-((8-fluoro-6,12-dioxo-6,12-dihydroxy-indolo[2,1-b]quinolin-2-yl)methyl) piperazine-1-carboxylic acid tert-butyl ester (1b)

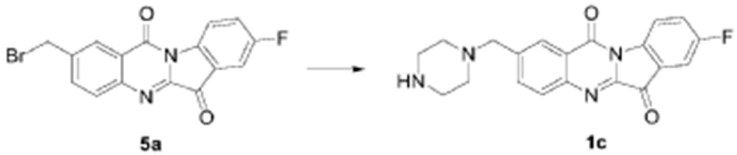


Compound 5a (500 mg, 1.39 mmol), N-Bocpiperazine (546 mg, 2.78 mmol), potassium iodide (10 mg) and triethylamine (0.5 ml) were stirred in a 5 ml solution of DMF at room temperature for 2 hours. Once TLC confirmed that the reaction was complete, 50 ml of water was added and three consecutive extractions were performed using 10 ml of ethyl acetate, after which the organic phase was washed three times with water and drying was performed over anhydrous sodium sulfate. The concentrated yellow solid obtained was subjected to separation using a silica gel column to obtain a yellow compound (1b).

Characterization Data:

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (dd, J = 8.8, 4.0 Hz, 1H), 8.39 (d, J = 1.5 Hz, 1H), 8.01 (d, J = 8.3 Hz, 1H), 7.90 (dd, J = 8.3, 1.8 Hz, 1H), 7.60 (dd, J = 6.5, 2.6 Hz, 1H), 7.51 (td, J = 8.6, 2.7 Hz, 1H), 3.71 (s, 2H), 3.47 (m, 4H), 2.46 (m, 4H), 1.48 (s, 9H).

Embodiment Example 3 - Synthesis of 8-fluoro-2-(piperazin-1-ylmethyl)indolo[2,1-b]quinazoline-6,12-dione (1c)



Steps (1) - (3) are as specified in Embodiment Example 1.

Step (4): Synthesis of 8-fluoro-2-(piperazin-1-ylmethyl)indolo[2,1-b]quinazoline-6,12-dione (1c)

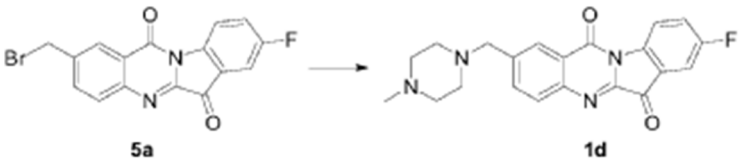
Compound 5a (500 mg, 1.39 mmol), N-Bocpiperazine (546 mg, 2.78 mmol), potassium iodide (10 mg) and triethylamine (0.5 ml) were stirred in a 5 ml solution of DMF at room temperature for 2 hours. Once TLC confirmed that the reaction was complete, 50 ml of water was added and three consecutive extractions were performed using 10 ml of

ethyl acetate, after which the organic phase was washed three times with water and drying was performed over anhydrous sodium sulfate to obtain a concentrated yellow solid. The yellow solid was dissolved in 3 ml of dichloromethane and a further 1 ml of trifluoroacetic acid was added at room temperature. The reaction solution was stirred for one hour at room temperature, and once TLC (CH_2Cl_2/MeOH = 10/1, Rf 0.2) confirmed that the reaction was complete, the solvent was removed under reduced pressure, 10 ml of a protective $NaHCO_3$ solution was added and ethyl acetate (10 ml x 3) was used to perform extraction, after which, the organic phase was washed with a salt solution and drying was performed to obtain a concentrated solid which was subject to separation using a silica gel column to obtain a yellow solid compound (1c).

Characterization Data:

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (dd, *J* = 8.8, 4.0 Hz, 1H), 8.37 (s, 1H), 8.00 (d, *J* = 8.3 Hz, 1H), 7.89 (dd, *J* = 8.3, 1.6 Hz, 1H), 7.59 (dd, *J* = 6.5, 2.6 Hz, 1H), 7.50 (td, *J* = 8.7, 2.7 Hz, 1H), 3.70 (s, 2H), 3.51 – 3.44 (m, 4H), 2.46 (s, 4H).

Embodiment Example 4 – Synthesis of 8-fluoro-2-((4-methyl-piperazin-1-yl)methyl)indolo[2,1-b] quinazoline-6,12-dione (1d)



Steps (1) – (3) are as specified in Embodiment Example 1.

Step (4): Synthesis of 8-fluoro-2-((4-methyl-piperazin-1-yl) methyl)indolo[2,1-b]quinazoline-6,12-dione (1d)

Compound 5a (220 mg, 0.61 mmol), N-methylpiperazine (122 mg, 1.22 mmol), potassium iodide (10 mg) and triethylamine (0.5 ml) were stirred in a 5 ml solution of DMF at room temperature for 2 hours. Once TLC confirmed that the reaction was complete, 50 ml of water was added

and three consecutive extractions were performed using 10 ml of ethyl acetate, after which the organic phase was washed three times with water and drying was performed over anhydrous sodium sulfate. The concentrated yellow solid obtained was subjected to separation using a silica gel column to obtain a yellow compound (1d).

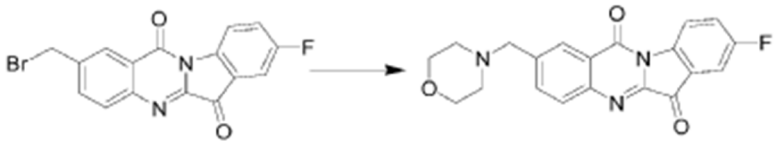
Characterization Data:

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (dd, *J* = 8.8, 4.0 Hz, 1H), 8.38 (d, *J* = 1.5 Hz, 1H), 7.99 (d, *J* = 8.3 Hz, 1H), 7.87 (dd, *J* = 8.3, 1.8 Hz, 1H), 7.59 (dd, *J* = 6.5, 2.6 Hz, 1H), 7.50 (td, *J* = 8.7, 2.7 Hz, 1H), 3.50 (s, 2H), 2.59 (s, 8H), 2.38 (s, 3H).

Embodiment Example 5 – Synthesis of 8-fluoro-2-((morpholino-1-yl)methyl)indolo[2,1-b]quinazoline-6,12-dione (1e)

Steps (1) – (3) are as specified in Embodiment Example 1.

Step (4): Synthesis of 8-fluoro-2-((morpholino-1-yl) methyl)indolo[2,1-b]quinazoline-6,12-dione (1e)



5a 1e

Compound 5a (500 mg, 1.39 mmol), morpholine (242 mg, 2.78 mmol), potassium iodide (10 mg) and triethylamine (0.5 ml) were stirred in a 5 ml solution of DMF at room temperature for 2 hours. Once TLC (EtOAc, Rf 0.5) confirmed that the reaction was complete, 50 ml of water was added and three consecutive extractions were performed using 10 ml of ethyl acetate, after which, the organic phase was washed three times with water and drying was performed over anhydrous sodium sulfate to obtain a concentrated yellow solid which was subjected to separation using a silica gel column (EtOAc) to obtain a yellow compound (1e).

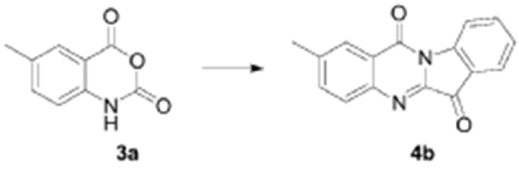
Characterization Data:

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (dd, *J* = 8.8, 4.1 Hz, 1H), 8.39 (s, 1H), 8.01 (d, *J* = 8.3 Hz, 1H), 7.90 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.60 (dd, *J* = 6.5, 2.7 Hz, 1H), 7.51 (td, *J* = 8.6, 2.7 Hz, 1H), 3.79 – 3.73 (m, 4H), 3.70 (s, 2H), 2.52 (s, 4H).

Embodiment Example 6 – Synthesis of 2-((4-methyl-piperazin-1-yl)methyl)indolo[2,1-b]quinazoline-6,12-dione (1f)

Step (1) is as specified in Embodiment Example 1.

Step (2): Synthesis of 2-methyl-indole[2,1-b]quinazoline-6,12-dione (4b)

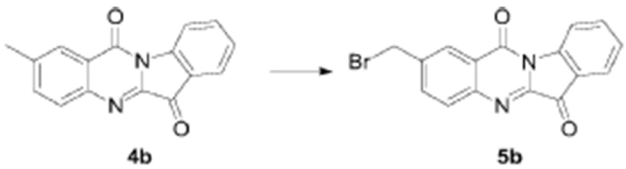


A mixture of Compound 3a (1.7 g, 9.6 mmol), isatin (1.4 g, 9.6 mmol) and triethylamine (2.7 ml, 19.2 mmol) was suspended in dry toluene (18 ml) and heated at 110°C for 4 hours. Once the reaction solution had cooled to room temperature, the resulting yellow solid was filtered and washed three times will 2 ml of ethyl acetate to obtain a yellow solid compound (4b) (0.5 g, 20%).

Characterization Data:

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (d, *J* = 8.1 Hz, 1H), 8.25 (s, 1H), 7.94 (dd, *J* = 7.8, 5.0 Hz, 2H), 7.85 – 7.75 (m, 1H), 7.71 – 7.64 (m, 1H), 7.44 (t, *J* = 7.5 Hz, 1H), 2.58 (s, 3H).

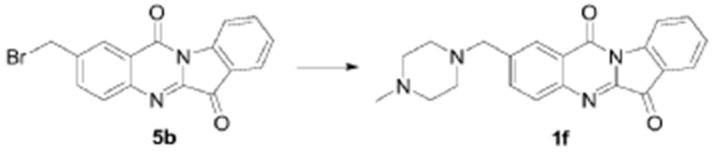
Step (3): Synthesis of 2-methyl-indole[2,1-b]quinazoline-6,12-dione (5b)



A mixture of NBS (381 mg, 2.14 mmol) and AIBN (29 mg, 0.18 mmol) was added in three batches to a dichloromethane solution (5 ml) containing Compound 4b (262 mg, 1.0 mmol) at a temperature of 80°C under the protection of nitrogen gas. Once the reaction solution

had cooled to room temperature, washing was performed with a salt solution and drying was performed over anhydrous sodium sulfate to obtain a concentrated yellow product (5b) (245 mg, 91%).

Step (4): Synthesis of 2-((4-methyl-piperazin-1-yl) methyl)indolo[2,1-b]quinazoline-6,12-dione (1f)



Compound 5b (220 mg, 0.61 mmol), N-methylpiperazine (116 mg, 1.616 mmol), potassium iodide (10 mg) and triethylamine (0.5 ml) were stirred in a 5 ml solution of DMF at room temperature for 2 hours. Once TLC confirmed that the reaction was complete, 50 ml of water was added and three consecutive extractions were performed using 10 ml of ethyl acetate, after which the organic phase was washed three times with water and drying was performed over anhydrous sodium sulfate. The concentrated yellow solid obtained was subjected to separation using a silica gel column to obtain a yellow compound (1f) (120 mg, 55%).

Characterization Data:

^{1}H NMR (400 MHz, CDCl3) δ 8.66 (d, *J* = 8.1 Hz, 1H), 8.39 (s, 1H), 8.01 (d, *J* = 8.3 Hz, 1H), 7.94 (d, *J* = 7.5 Hz, 1H), 7.89 (dd, *J* = 8.2, 1.7 Hz, 1H), 7.82 (t, *J* = 7.8 Hz, 1H), 7.45 (t, *J* = 7.5 Hz, 1H), 3.71 (s, 2H), 2.71 – 2.41 (m, 8H), 2.33 (s, 3H).

Embodiment Example 7 – Detection of IDO Inhibitory Activity

During the construction of a plasmid containing a human IDO gene, expressed in *Escherichia coli*, extraction and purification were performed according to the method reported by Littlejohn, et al. (Takikawa O, Kuroiwa T, Yamazaki F, et al. J. Biol. Chem. 1988, 263, 2041 – 2048). The IDO inhibitory activity with respect to each compound was detected using the methods described in the literature. 50 mM potassium phosphate buffer (pH 6.5),

40 mM vitamin C, 400 μg/ml catalase, 20μM methylene blue and IDO enzyme were mixed together in a 96-well plate. The substrate L-tryptophan and the sample to be tested were then added to the aforementioned mixture. The reaction was carried out for 60 minutes at 37°C, after which the reaction was terminated by adding 30% (w/v) trichloroacetic acid. The 96-well plate was heated to 65°C for 15 minutes to complete the conversion from formylkynurenine to kynurenine, and centrifugation was performed with 6000 g for 5 minutes. 100 μl of supernatant was removed from each well and transferred to a new 96-well plate, after which an acetic acid solution of 2% (w/v) p-(dimethylamino)benzaldehyde was added, and the yellow color produced as a result of the resulting reaction with kynurenine was observable at 490 nm in a microplate reader; the experimental results are shown in Table 1.

Embodiment Example 8 - Determining Whether or Not an Inhibitor is Reversible

A series of different enzymatic concentrations were tested, given a fixed concentration of inhibitor, and the resulting reaction rates were measured. A graph of reaction rate versus enzyme concentration (ν - [E]) was plotted and it was possible to determine whether or not the inhibitor was reversible based on the characteristics of the resulting curve.

Reaction Conditions: 50 mM potassium phosphate buffer (pH 6.5), 40 mM vitamin C, 400 μg/ml catalase, 20 μM methylene blue and 300 mM of the substrate L-tryptophan or simultaneously 100 mM of inhibitor were added to a 500 μl the reaction system, after which the mixture was maintained at 37°C for 5 minutes, after which varying volumes of an IDO enzyme were added to the aforementioned mixture and the resulting reaction was allowed to proceed for 30 minutes at 37°C, and 200 μL of 30% (w/v) trichloroacetic acid was added to terminate the reaction; the reaction system was heated to 65°C for 15 minutes to complete the conversion of formylkynurenine to

kynurenine, after which centrifugation was performed for 10 minutes at 12,000 rpm, the supernatant was removed and mixed with an equal volume of 2% (w/v) p-dimethylaminobenzaldehyde acetic acid solution, and measurements were performed using a plate reader at a wavelength of 490 nm. ν – [E] was plotted and the experimental results are shown in Table 1.

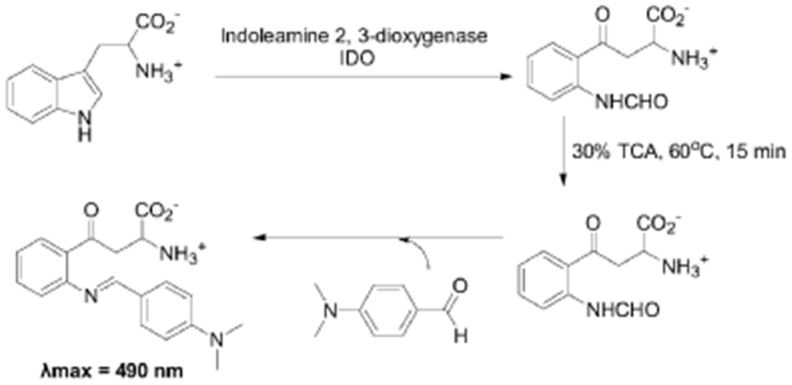
Embodiment Example 9 – Determining Inhibitor Type and Ki Value Measurement

50 mM potassium phosphate buffer (pH 6.5), 40 mM vitamin C, 400 μg/ml catalase, 20 μM methylene blue and 100, 250 or 300 mM of the substrate L-tryptophan were added to a 500 μl the reaction system and varying concentrations of the compound were added to each reaction system for a given single substrate concentration, after which the mixture was maintained at 37°C for 5 minutes, after which 10 μl of IDO (approximately 20 nM) was added to the aforementioned mixture and the resulting reaction was allowed to proceed for 30 minutes at 37°C, and 200 μL of 30% (w/v) trichloroacetic acid was added to terminate the reaction; the reaction system was heated to 65°C in a water bath for 15 minutes to complete the conversion of formylkynurenine to kynurenine, after which centrifugation was performed for 10 minutes at 12,000 rpm, the supernatant was removed and mixed with an equal volume of 2% (w/v) p-dimethylaminobenzaldehyde acetic acid solution, and measurements were performed using a plate reader at a wavelength of 490 nm. A Dixon plot (1/v – [I]) was used to determine the type of inhibitor constituted by the compound and a plot of S/v – [I] was created to obtain an inhibitor Ki value; the experimental results are shown in Table 1.

Embodiment Example 10 – Measurement of Median Effective Inhibition Concentration IC_{50}

50 mM potassium phosphate buffer (pH 6.5), 40 mM vitamin C, 400 μg/ml catalase, 20μM methylene blue, 150 mM of the substrate L-tryptophan and inhibitor were mixed together. Mixtures containing 100, 200, 400, 600, 800, 1000 or 1200

μM of inhibitor were heated to 37°C for 5 minutes and an IDO enzyme was then added to the above mixtures. The reaction was allowed to proceed at 37°C for 30 minutes, after which 200 μL of 30% (w/v) trichloroacetic acid was added to terminate the reaction; the reaction system was heated to 65°C for 15 minutes to complete the conversion of formylkynurenine to kynurenine, after which centrifugation was performed for 10 minutes at 12,000 rpm, 200 μL of the supernatant was removed and mixed with an equal volume of 2% (w/v) p-dimethylaminobenzaldehyde acetic acid solution, and the yellow color produced as a result of the resulting reaction with kynurenine was observable at 490 nm in a microplate reader; the results thus obtained were fed into IC_{50} calculation software to obtain inhibitor IC_{50} values; and the experimental results are shown in Table 1.



Embodiment Example 11 – Measurement of Median Effective Inhibition Concentration IC_{50} (Cells)

The liposome Lipofectamin 2000 was used to transfect HEK 293 cells with the pcDNA3.1-hIDO plasmid. When measuring the inhibition activity at the cellular level, the HEK293 cell culture medium corresponded to high glucose DMEM, containing 50 U/mL penicillin, 50 U/mL streptomycin and 10% FBS, and culturing was performed at 37°C under 5% CO_2. Twenty four hours following cell transfection with the plasmid, the test drug was added and incubation was performed, after which the supernatant was transferred to another 96-well plate and 10 μL 30% (w/v) trichloroacetic acid was added and the mixture was heated

to 65°C for 15 minutes to complete the conversion of formylkynurenine to kynurenine, after which centrifugation was performed for 10 minutes at 12,000 rpm, an equal volume of 2% (w/v) p-dimethylaminobenzaldehyde acetic acid solution was mixed in, and the absorbance was measured at 490 nm using a plate reader; the experimental results are shown in Table 1.

Measurements of the IDO inhibitory activity of the compounds obtained in Embodiment Examples 1 – 6 were performed using the methods given in Embodiment Examples 7 – 11 above, and the IDO inhibitor 1-methyl-tryptophan (1-MT, commercially available), which is currently commonly used in both *in vivo* and *in vitro* experiments, was used as a control; the results are shown in Table 1.

Table 1. IDO inhibitory activity of N-benzyl tryptanthrin derivatives (I) synthesized in the above embodiment examples

Compound	Inhibition Type	K_i (μM) (*in vitro*)	IC_{50} (μM) (Cell)	IC_{50} (μM)
	Competitive	34	380	18.4
Embodiment Example 1	Anticompetitive	0.31	0.11	1.01×10^{-3}
Embodiment Example 2	Anticompetitive	7.21	1.88	0.64
Embodiment Example 3	Noncompetitive	4.12	0.68	7.38×10^{-2}
Embodiment Example 4	Anticompetitive	2.64	0.50	2.24×10^{-2}
Embodiment Example 5	Anticompetitive	0.47	0.40	1.21×10^{-3}
Embodiment Example 6	Anticompetitive	5.97	2.52	0.97

Example 12 – T-Cell Proliferation Assay

12.1. Isolation of Spleen Lymphocytes (Performed in accordance with the lymphocyte separation medium instructions):

1. Two mice were sacrificed by cervical dislocation and spleens were removed on a sterile table and placed into a 6 cm plate containing an RP1640 culture medium for later use.

2. A 100 μm mesh was placed in a 50 mL centrifuge tube and each spleen was cut into smaller pieces using scissors, milled on top of the sieve and a small amount of RP1640 was added for preservation purposes.

3. A lymphocyte separation medium equivalent to twice the volume of the resulting slurry was then added.

4. Centrifugation was performed at 800 g for 20 minutes, after which three distinct layers were visible, with the middle layer showing a slight yellowish tint.

5. Cells in the middle layer were removed and an RP1640 culture medium was added to perform a reverse wash. Cells were then centrifuged at 250 g for 10 minutes at room temperature, and the cells were collected.

6. The culture medium was aspirated off and cells were resuspended in the RP1640 medium, after which a cell count was performed.

12.2. LLC (Lewis Lung Carcinoma) Cell Treatment:

1. A medium (high glucose DMEM, 10% FBS) was aspirated off and washing was performed 1 – 2 times using PBS;

2. 0.25% trypsin was added to initiate digestion;

3. The trypsin was aspirated off and a culture medium was added, after which cells were pipetted off and transferred to a 1.5 mL centrifuge tube;

4. Centrifugation followed by aspiration of the supernatant was performed, and cells were resuspended in 1 mL of the DMEM culture medium;

5. Mitomycin C (final concentration: 25 μg/mL) was added, the mixture was pipetted up and down until homogeneous, and the mixture was placed in a 37°C bath for 30 minutes;

6. Washing was performed 3 times with RP1640, the cells were counted and the sample was set aside.

12.3. Experimental Procedure

1. Treated LLC cells (2 × 10^4 cells/well; stimulator cells) and spleen lymphocytes (10^5 cells/well; reaction cells) were added to a 96-well plate, after which RP1640 (10% FBS was added to bring the volume to 200 μL;

2. Grouping was performed and 50 μM IDO inhibitor was added for the administration group, after which samples were placed in a 37°C, 95% RH, 5% CO_2 incubator and cultured for 72 hours;

3. A WST-1 reagent kit was used to measure T-cell proliferation, and absorbance values at a wavelength of 450 nm were measured using a plate reader;

4. Calculation of the T Lymphocyte Proliferation Rate:

T lymphocyte proliferation rate (%) = [administration group well (T lymphocytes + LLC cells + IDO inhibitor) OD value

- control well (T lymphocytes + LLC cells) OD value] / control well (T lymphocytes + LLC cells) OD value × 100%

12.4. Experimental Results

Table 2. Stimulation of T-cell proliferation by N-benzyl tryptanthrin derivatives synthesized in the aforementioned embodiment examples

Embodiment Example 1	Embodiment Example 2	Embodiment Example 3	Embodiment Example 4	Embodiment Example 5	Embodiment Example 6	T-Cell Proliferation Rate
4.88%	38.48	40.64	41.24	50.27	39.40	13.84

Embodiment Example 13 - The Anti-Tumor Effect of 8-fluoro-2-((4-methyl-piperazin-1-yl)methyl)indolo[2,1-b]quinazoline-6,12-dione (Embodiment Example 4)

1. Establishment of LLC Lewis Tumor Model: Healthy female C57BL/6 mice weighing 20 ± 1 g and purchased from the Shanghai Slyke Experimental Animal Center were raised in an SPF grade laboratory. Sterile saline was used to suspend Lewis lung carcinoma cells at a concentration of 1 x 10^7 / mL. Cells were subcutaneously inoculated in the underarm region of mice under sterile operational conditions, with 0.2 mL being inoculated in each animal.

2. Experimental Grouping and Prophylactic Administration: Mice were randomly divided into five groups of 10 animals. The control group was given an equivalent volume of 0.5% sodium carboxymethyl cellulose via gavage; 1-methyl-tryptophan (1-MT) was administered at concentrations of 150 mg/kg in the high dosage group and 75 mg/kg in the low dosage group via gavage; 8-fluoro-2-((4-methyl-piperazin-1-yl)methyl)indolo[2,1-b]quinazoline-6,12-dione was administered at concentrations of 150 mg/kg in the High Dosage Group and 75 mg/kg in the Low Dosage Group via gavage. Tumor inoculation was performed following 7 days of continuous administration, after which administration was continued for 21 days and tumor growth was observed. Tumor length along the major axis (a) and minor axis (b) was measured starting on the day of tumor inoculation and every other day thereafter, with tumor volume = $ab^2/2$. On the day after administration was discontinued, mice were scarified via cervical dislocation and the tumors were removed, weighed and preserved in liquid nitrogen.

3. Treatment Results for Prophylactic Administration: Following prophylactic administration lasting one week followed by continued administration after inoculation, mice which were administered with 8-fluoro-2-((4-methyl-piperazin-1-yl)methyl)indolo[2,1-b]quinazoline-6,12-dione showed significantly inhibited Lewis lung carcinoma tumor growth *in vivo*; no significant tumor formation was observed in the high dosage group 20 days after administration was started, while tumor formation was noted just 11 days following the start of administration in the control group; mice in the 1-MT group showed tumor formation 13 days after the start of administration. On a tumor volume basis, the tumor inhibition rates observed for the 8-fluoro-2-((4-methyl-piperazin-1-yl)methyl)indolo[2,1-b] quinazoline-6,12-dione and 1-MT high dosage groups were 62.15% and 32.35% respectively, while tumor inhibition rates were 46.62% and 27.00% in the low dosage groups. On a tumor weight basis, the tumor

inhibition rates observed for the 8-fluoro-2-((4-methyl-piperazin-1-yl)methyl)indolo[2,1-b]quinazoline-6,12-dione and 1-MT high dosage groups were 57.8% and 28.2% respectively. Animals administered with 8-fluoro-2-((4-methyl-piperazin-1-yl) methyl) indolo [2,1-b] quinazoline-6,12-dione exhibited normal weight, a shiny coat and a more flexible response.

4. Experimental Grouping and Post-Inoculation Administration: Mice were randomly divided into four groups of 10 animals 48 hours after tumor inoculation was performed. An initial dose was administered once the tumor diameter had reached 5 mm. Animals in the control group were administered with an equal volume of 0.5% CMC (sodium carboxymethyl cellulose) via gavage; animals in the 1-MT group were administered with a dose of 200 mg/kg, via gavage; animals in the 8-fluoro-2-((4-methyl-piperazin-1-yl)methyl)indolo[2,1-b]quinazoline-6,12-dione group were administered a dose of 200 mg/kg, via gavage; animals in the paclitaxel group were administered with a dose of 100 mg/kg via intravenous injection, once per week; animals in the 8-fluoro-2-((4-methyl-piperazin-1-yl)methyl)indolo[2,1-b]quinolin oxazoline-6,12-dione + paclitaxel group were administered with 200 mg/kg 8-fluoro-2-((4-methyl-piperazin-1-yl)methyl)indolo[2,1-b]quinazoline-6,12-dione and 100 mg/kg paclitaxel (caudal vein intravenous injection) continuously for two weeks. The tumor length along the major axis (a) and minor axis (b) was measured starting on the day of administration and every other day thereafter, with tumor volume = $ab^2/2$. On the day after administration was discontinued, mice were scarified via cervical dislocation, tumors were removed, weighed and preserved in liquid nitrogen.

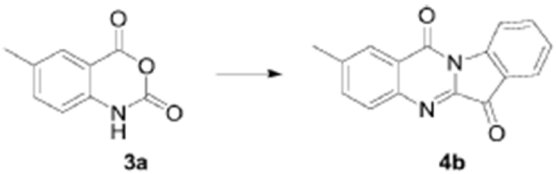
5. Treatment Results for Post-Inoculation Administration: After animals were subjected to two weeks of continuous administration of 200 mg/kg of 8-fluoro-2-((4-methyl-piperazin-1-yl)methyl)indolo[2,1-b]quinazoline-6,12-dione or 1-MT via gavage, *in vivo*

Lewis lung carcinoma tumor growth showed significant inhibition compared to the control group, and inhibition of tumor growth was more pronounced in the 8-fluoro-2-((4-methyl-piperazin-1-yl)methyl)indolo[2,1-b]quinazoline-6,12-dione group versus the 1-MT group. On a tumor volume basis, the tumor inhibition rates observed for the 8-fluoro-2-((4-methyl-piperazin-1-yl)methyl)indolo[2,1-b]quinazoline-6,12-dione, 1-MT, paclitaxel and 8-fluoro-2-((4-methyl-piperazin-1-yl)methyl)indolo[2,1-b]quinazoline-6,12-dione + paclitaxel groups were 23.38%, 15.68%, 51.45% and 72.48%, respectively. As a chemotherapy drug commonly used in clinical practice, paclitaxel generally produced inhibition of tumor growth, but the drug also produced stronger toxic side effects; during the course of the experiment, mice were observed as losing weight, losing their fur and showing less movement in general. On the other hand, although administration of only 8-fluoro-2-((4-methyl-piperazin-1-yl)methyl)indolo[2,1-b]quinazoline-6,12-dione did not result in complete disappearance of the tumor, a more significant inhibition of the tumor was observed and mice thus treated exhibited a normal weight, a shiny coat and a more flexible response.

Comparative Example - The Preparation of 2-methyl-indole[2,1-b]quinazoline-6,12-dione and Activity Testing

Step (1) is as specified in Embodiment Example 1.

Step (2): Synthesis of 2-methyl-indole[2,1-b]quinazoline-6,12-dione (4b)



A mixture of Compound 3a (1.7 g, 9.6 mmol), isatin (1.4 g, 9.6 mmol) and triethylamine (2.7 ml, 19.2 mmol) was suspended in dry toluene (18 ml) and heated at 110°C for 4 hours. Once the reaction solution had cooled to room temperature, the resulting yellow solid was filtered and

washed three times with 2 ml of ethyl acetate to obtain a yellow solid compound (4b) (0.5 g, 20%).

Characterization Data:

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (d, *J* = 8.1 Hz, 1H), 8.25 (s, 1H), 7.94 (dd, *J* = 7.8, 5.0 Hz, 2H), 7.85 – 7.75 (m, 1H), 7.71 – 7.64 (m, 1H), 7.44 (t, *J* = 7.5 Hz, 1H), 2.58 (s, 3H).

Using the measurement method described in Embodiment Example 9 – 11 to measure the activity of the compound obtained in the Comparative Example yielded an IDO inhibitory activity *in vitro* IC_{50} of 997.25 μM, while the Ki and *in vivo* IC_{50} were not measured.

As the experimental data in Table 1 demonstrates, the compound pertaining to the present application exhibits an exceptionally good IDO inhibitory activity relative to currently available conventional IDO inhibitors, as well as compounds with similar structures. More specifically, compared with the existing conventional IDO inhibitor 1-MT, the *in vitro* and intracellular IC_{50} values obtained for the compound constituted by the present application are lower by 1 – 3 orders of magnitude, indicating that the compound constituted by the present application produces an excellent IDO inhibitory activity.

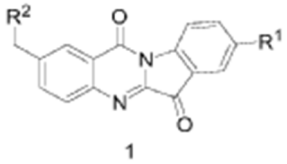
It is worth noting that there is a very significant improvement in the compound constituted by the present application versus other compounds with highly similar structures (such as the compound given in the Comparative Example) in terms of inhibitory activity. The inhibitory activity is still greatly increased even compared to certain IDO inhibitors which similarly feature active groups in their structure. Given the link between the compound structure and activity typically observed in this field, the difference observed between the compound constituted by the present application and similar structures is a highly exceptional phenomenon. This indicates that the compound constituted by the present application constitutes an extremely unique type of

structure, and the biological activity thereof is surprising.

All publications mentioned in the present invention are incorporated into the references as if each individual publication was cited as a reference. It should also be understood that after reading the instructions provided in the present invention, a person skilled in the art should be able to make various changes or modifications to the invention, and these equivalent forms also fall within the scope of the claims which are included in this application.

Claims

1. An N-benzyl tryptanthrin derivative, which is characterized in that said derivative has the general structure as shown in Formula 1 below:



1

where

R^1 corresponds to hydrogen or fluorine;

R^2 corresponds to $-NR^3R^4$;

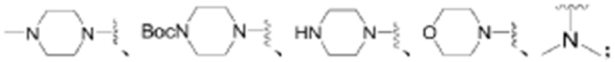
R^3 and R^4 are each independently selected from the following set: H, substituted or unsubstituted C1 – C4 alkyl groups, substituted or unsubstituted C2 – C4 alkenyl groups, substituted or unsubstituted C2 – C4 alkynyl groups, and substituted or unsubstituted C3 – C6 cycloalkyl groups;

or R^3 and R^4 together with an adjacent nitrogen atom form a substituted or unsubstituted 5 – 7 membered heterocyclic ring, where said 5 – 6 membered saturated ring includes 1 – 2 nitrogen atoms as well as 0 – 2 heteroatoms selected from the following set: O and S;

here, substitution refers to cases in which one or more hydrogen atoms present on a given group (preferably hydrogen atoms present on a nitrogen atom) are substituted by a substituent selected from the following set: C1 – C4 alkyl groups, C1 – C4 haloalkyl groups, amino-protecting groups (preferably t-butoxycarbonyl) and halogens.

2. The N-benzyl tryptanthrin derivative as claimed in claim 1, characterized in that R^3 and R^4 are each independently selected from the following set: C1-C4 alkyl groups; or, R^3 and R^4 together with an adjacent nitrogen atom form a substituted or unsubstituted 5 – 6 membered saturated ring, where said 5 – 6 membered saturated ring includes 1 or 2 nitrogen atoms as well as an optional 1 heteroatom selected from the following set: O.

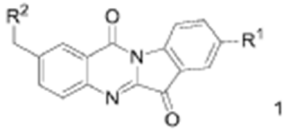
3. The N-benzyl tryptanthrin derivative as claimed in claim 1, characterized in that R^2 is a substituted or unsubstituted group selected from the following set:



where "〰" indicates a connection site;

here, substitution refers to cases in which one or more hydrogen atoms present on a given group are substituted by a substituent selected from the following set: C1 – C4 alkyl groups and halogens.

4. An N-benzyl tryptanthrin derivative, characterized in that the general structure of said derivative is as given below:



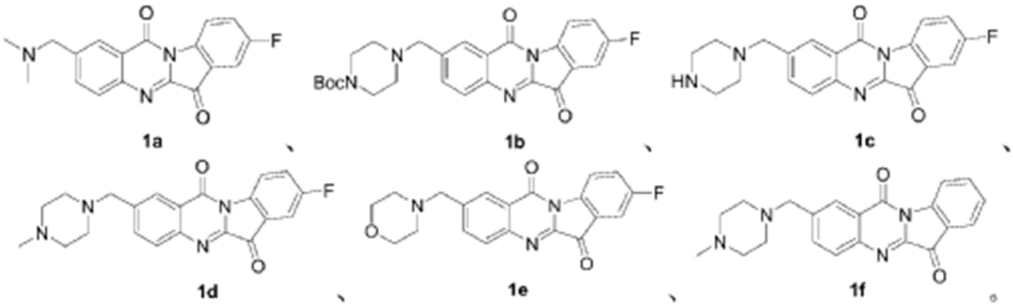
1

R^1 = H or F

R^2 =

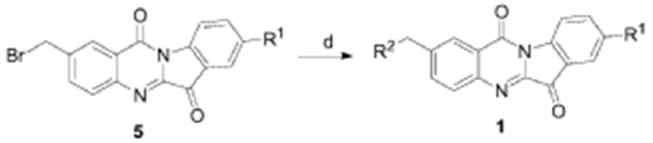
where the substituent R^2 corresponds to a cyclic imine or dialkyl-substituted amine and R^1 corresponds to hydrogen or fluorine.

5. The N-benzyl tryptanthrin derivative as claimed in claim 1 or 4, characterized in that said derivative is a compound selected from the following set:



1a 1b 1c 1d 1e 1f

6. A method for the preparation of the N-benzyl tryptanthrin derivative as claimed in claims 1 or 4, characterized in that it includes the following steps:



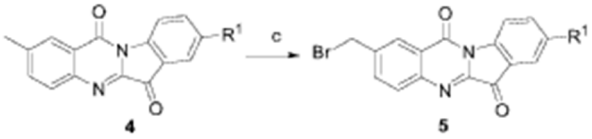
5 1

(d) The compound given in Formula 5 (2-bromomethyl-tryptanthrin) is reacted with R^2H in an inert solvent in

the presence of triethylamine to obtain the compound shown in Formula 1,

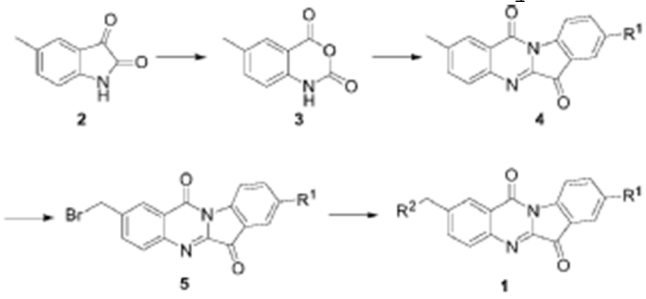
where R^1 and R^2 are as defined above.

7. The method as claimed in claim 6, characterized in that said method further includes a Step (c) prior to Step (d):



(c) The compound given in Formula 4 is reacted with a bromination agent in an inert solvent to obtain the compound given in Formula 5; where the bromination agent used is preferably NBS.

8. The method for the preparation of an N-benzyl tryptanthrin derivative as claimed in claim 4, characterized in that the synthesis route is as follows:



R^1 = H or F

The specific steps are as follows:

(1) Synthesis of Methylisatoic Anhydride (3)

Suspend 5-methylisatin in dry dichloromethane and add meta-chloroperoxybenzoic acid batchwise at 0°C, then stir for 1.5 - 2.5 hours at room temperature; once the reaction is confirmed as being complete via TLC, filter off the white solid produced in the reaction and wash thrice with ethyl acetate to obtain 5-methylisatoic anhydride; the molar ratio of 5-methylisatin and meta-chloroperoxybenzoic acid is (0.5 - 1) : 1;

(2) Synthesis of 2-Methyltryptanthrin (4)

Suspend a mixture of methylisatoic anhydride, 5-fluoroisatin and triethylamine in dry toluene, then heat for 3.5 - 4.5 hours at a temperature of 100 - 120°C and

distill off the solvent under reduced pressure; dissolve the resulting yellow solid in dichloromethane and add ethyl acetate, then filter the resulting yellow solid and wash with ethyl acetate three times to obtain 2-methyltryptanthrin; wherein the molar ratio of methylisatoic anhydride, 5-fluoroisatin and triethylamine is (0.2 – 0.5) : (0.2 – 0.5) : 1;

(3) Synthesis of 2-bromomethyl-tryptanthrin (5)

Dissolve the 2-methyltryptanthrin obtained in Step (2) in dry dichloromethane at 75 – 85°C under the protection of nitrogen gas, then add in a mixture of NBS and AIBN batchwise; heat the reaction solution at 75°C for 15 – 17 hours and confirm that the reaction is completed via TLC; once the reaction solution cools to room temperature, perform washing with a salt solution and drying over anhydrous sodium sulfate; the organic phase was concentrated to obtain a yellow product wherein the molar ratio of 2-methyltryptanthrin, NBS and AIBN is 1 : 1 : (0.005 – 0.02); and

(4) Synthesis of N-Benzyltryptanthrin (1)

Stir together 2-bromomethyl-tryptanthrin, an aliphatic amine and triethylamine at room temperature in dry DMF for 1.5 – 2.5 hours, and once the reaction is verified as being complete via TLC, add 50 ml of water and perform three sequential extractions using 10 ml of dichloromethane; next, subject the entire volume of dichloromethane obtained to three washes with water and then dry over anhydrous sodium sulfate; perform separation of the concentrated yellow solid obtained using silica gel to obtain a yellow-colored N-benzyl tryptanthrin derivative; wherein the molar ratio of 2-bromomethyl-tryptanthrin, the aliphatic amine and triethylamine is 1 : (1 – 2) : (2 – 5).

9. An application for the N-benzyl tryptanthrin derivative as claimed in claim 1 or 4 in the preparation of a drug designed to prevent and/or treat diseases which include a disorder of the IDO-mediated metabolism of tryptophan as a pathological feature.

10. The application for the compound given in Formula 1 as claimed in claim 1 or 4 or a pharmaceutically acceptable salt thereof, characterized in that it is used for:

(i) the preparation of an IDO inhibitor;

(ii) the preparation of a drug for the treatment of a tryptophan metabolism-related disease; and

(iii) non-therapeutic inhibition of IDO *in vitro*.

11. A pharmaceutical composition, characterized in that it includes: (i) the compound given in Formula 1 as claimed in claim 1 or 4 or a pharmaceutically acceptable salt thereof; as well as (ii) a pharmaceutically acceptable carrier.

12. An IDO inhibitor, characterized in that it includes the compound given in Formula 1 as claimed in claim 1 or 4 or a pharmaceutically acceptable salt thereof.

13. A method for non-therapeutically inhibiting IDO activity *in vitro*, characterized in that said method includes the following step: a compound given in Formula 1 as described in claim 1 or 4 or a pharmaceutically acceptable salt thereof is brought into contact with an inhibition target in an amount which produces effective inhibition.

14. A method for the preparation of a pharmaceutical composition used to treat a disease associated with a disorder in the tryptophan metabolism, characterized in that in said method: a compound given in Formula 1 as claimed in claim 1 or 4 or a pharmaceutically acceptable salt thereof is mixed with a pharmaceutically acceptable carrier in a therapeutically effective amount to create a pharmaceutical composition.

15. The method for treating a disease associated with a disorder in the tryptophan metabolism, characterized in that in said method: a compound given in Formula 1 as claimed in claim 1 or 4 or a pharmaceutically acceptable salt thereof is applied in a therapeutically effective amount to a treatment subject.