

THIOTRIAZOLE COMPOUND AND ITS USE IN PARASITIC PROTOZOAL INFECTIONS

FIELD OF THE INVENTION

The present invention relates to a novel thiotriazole compound having pharmacological activity, processes for its preparation, pharmaceutical compositions and their use in the treatment of certain parasitic protozoal infections such as malaria, in particular infection by *Plasmodium falciparum*.

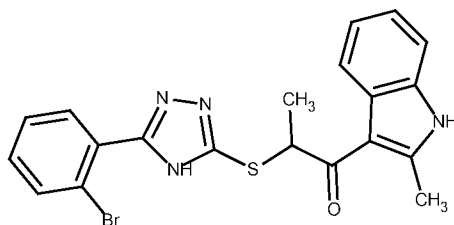
BACKGROUND OF THE INVENTION

Parasitic protozoal infections are responsible for a wide variety of diseases of medical and veterinary importance, including malaria in man and various coccidiosis in birds, fish and mammals. Many of the diseases are life-threatening to the host and cause considerable economic loss in animal husbandry, such as diseases caused by infection by species of *Eimeria*, *Theileria*, *Babesia*, *Cryptosporidium*, *Toxoplasma* (such as *Toxoplasma brucei*, African sleeping sickness and *Toxoplasma cruzi*, Chagas disease) and *Plasmodium* (such as *Plasmodium falciparum*), and the Mastigophora such as species of *Leishmania* (such as *Leishmania donovani*). Another parasitic organism of increasing concern is *Pneumocystis carinii*, which can cause an often fatal pneumonia in immunodeficient or immunocompromised hosts, including those infected with HIV.

Malaria is a mosquito-borne disease that, in humans, can be caused by five species of *Plasmodium* parasite, of which *Plasmodium falciparum* is the most virulent. In 2013, there were an estimated 128 million of people infected with malaria worldwide and malarial disease was responsible for an estimated 584,000 deaths (90% of them in sub-saharian Africa), young children and pregnant women being the most affected groups. In 2013, malaria killed an estimated 437,000 children under five years of age (WORLD HEALTH ORGANIZATION. (2014). *World malaria report*. Geneva, Switzerland, World Health Organization).

Resistance to classical treatments and emerging resistance to the current treatment of choice (artemisinins-based combination therapies) reveals the urgent need for new therapeutic agents with novel mechanisms of action (WORLD HEALTH ORGANIZATION. Joint assessment of the response to artemisinin resistance in the greater Mekong sub-region. November 2011–February 2012. Summary report.). In 2010, GSK released details of more than 13,500 chemical compounds that have already shown to inhibit *Plasmodium falciparum* parasite growth in the phenotypic screening approach. (Gamo, F.J. *et al.* (2010) Thousands of chemical starting points for antimalarial lead identification. *Nature* **465**, 305–310). Molecular structures and descriptions of these compounds were made publicly available in accessible databases under the name of TCAMS (Tres Cantos Antimalarial set) (<http://www.ebi.ac.uk/chemblntd>).

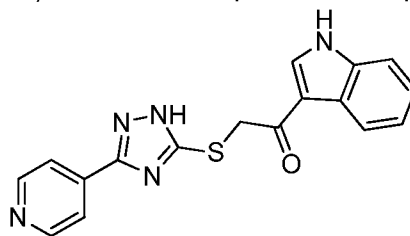
One such compound in the TCAMS compound collection is TCMDC-125114 (Compound 524404 ChEMBL database):



"Compound 524404"

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Another approach has been computer-aided drug design. Shah et al., (Journal of Chemical Modelling (2012), 52(3), 696-710) describe Compound 32 as part of their study.

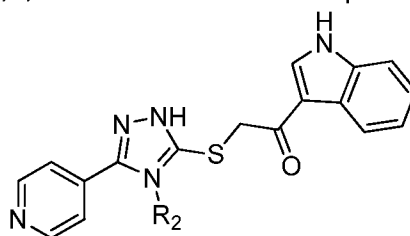


"Compound 32"

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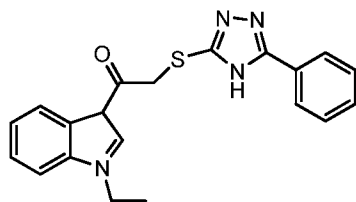
Compound 32 was found to be inactive in their assays (see p698, last paragraph of Shah et al., – "For example, compounds lacking R_2 substituents (27–34) or with shorter R_2 substituents (35–38) were inactive", and id., Table 2 on p699). The location of the R_2 substituent had it been present on Compound 32 is shown below (see Fig. 2 of Shah et al.,

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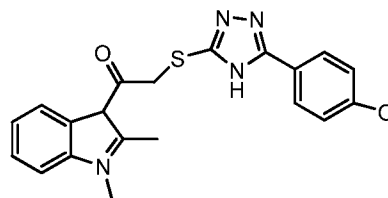


Four chemical library compounds with similar structures to those of CHEMBL524404 and Compound 32 have also been published, but do not have a known activity. Three are available from Vitas M Laboratory (website: <http://www.vitasmlab.com>) and the fourth is available from Enamine (website: <http://www.enamine.net>).

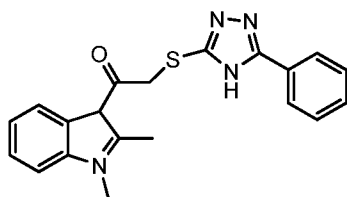
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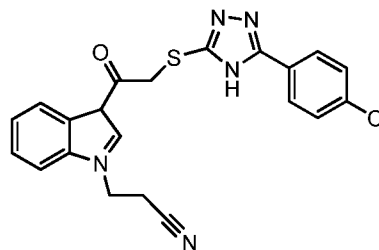
Vitas M Lab STK697314



Vitas M Lab STK697485



Vitas M Lab STK238706



Enamine Z25219839

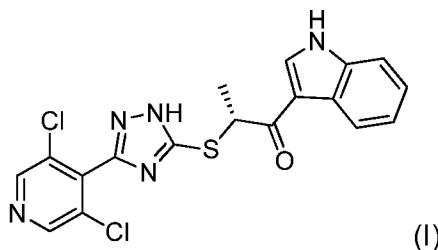
5 SUMMARY OF THE INVENTION

The present invention is directed to a novel thiotriazole compound for use in the chemotherapy of certain parasitic infections such as malaria, and in particular infection by *Plasmodium falciparum*, processes for its preparation and pharmaceutical compositions comprising such a compound.

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DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a compound of Formula (I):



(I)

15 (R)-2-((3-(3,5-dichloropyridin-4-yl)-1H-1,2,4-triazol-5-yl)thio)-1-(1H-indol-3-yl)propan-1-one or a salt thereof.

Also included in the present invention are pharmaceutically acceptable salts. In certain embodiments of the invention, pharmaceutically acceptable salts of a compound of Formula (I) may be preferred over the respective free base because such salts impart greater stability or solubility to the molecule thereby facilitating formulation into a dosage form. Therefore, the present invention also covers the pharmaceutically acceptable salts of a compound of Formula (I). As used herein, the term "pharmaceutically acceptable salt" refers to a salt that retains the desired biological activity of the subject compound and exhibits minimal undesired toxicological effects. For a review on suitable salts see Berge et al, J. Pharm. Sci., 1977, 66,

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1-19. The term "pharmaceutically acceptable salt" includes any pharmaceutically acceptable acid or basic addition salts. These pharmaceutically acceptable salts may be prepared *in situ* during the final isolation and purification of the compound, or by separately reacting the purified compound with a suitable acid or base, respectively. The salt may precipitate from solution and be collected by filtration or may be recovered by evaporation of the solvent.

Therefore, according to a further aspect, the invention provides a pharmaceutically acceptable salt of a compound of Formula (I) thereof.

The compound of Formula (I) contains a basic functional group and is therefore capable of forming pharmaceutically acceptable acid addition salts by treatment with a suitable acid. A pharmaceutically acceptable acid addition salt may be formed by reaction of a compound of Formula (I) with a suitable strong inorganic or organic acid (such as hydrobromic, hydrochloric, sulfuric, nitric, phosphoric, perchloric, p-toluenesulfonic, benzenesulfonic, methanesulfonic, ethanesulfonic, 2-hydroxyethanesulfonic, naphthalenesulfonic (e.g. 2-naphthalenesulfonic), optionally in a suitable solvent such as an organic solvent, to give the salt which is usually isolated for example by crystallisation and filtration. Pharmaceutically acceptable acid addition salts include a hydrobromide, hydrochloride, hydroiodide, sulfate, bisulfate, nitrate, phosphate, perchlorate, p-toluenesulfonate, benzenesulfonate, methanesulfonate, ethanesulfonate, 2-hydroxyethanesulfonate or naphthalenesulfonate (e.g. 2-naphthalenesulfonate) salt. In one embodiment, a pharmaceutically acceptable acid addition salt of a compound of Formula (I) is a salt of a strong acid, for example a hydrobromide, hydrochloride, hydroiodide, sulfate, nitrate, perchlorate, phosphate p-toluenesulfonic, benzenesulfonic or methanesulfonic salt.

Suitable pharmaceutically acceptable salts of a compound of Formula (I) include mono- or di-basic salts with the appropriate base. A Pharmaceutically acceptable basic addition salt may be formed by reaction of a compound of Formula (I) with a suitable inorganic or organic base. Pharmaceutically acceptable basic addition salts include sodium, potassium, calcium, magnesium, ammonium, N-methylglucamine, ammonium and choline salts

The invention includes within its scope all possible stoichiometric and non-stoichiometric forms of the salts of a compound of Formula (I).

A compound of Formula (I) or a pharmaceutically acceptable salt thereof, may exist as a solid or liquid, both of which are included in the invention. In the solid state, a compound of Formula (I) or a pharmaceutically acceptable salt thereof, may exist as either amorphous material or in crystalline form, or as a mixture thereof. It will be appreciated that pharmaceutically acceptable solvates of a compound of Formula (I) or a pharmaceutically acceptable salt thereof, may be formed wherein solvent molecules are incorporated into the crystalline lattice during crystallisation. Solvates may involve non-aqueous solvents such as ethanol, isopropanol, dimethylsulfoxide (DMSO), acetic acid, ethanolamine, and ethyl

acetate, or they may involve water as the solvent that is incorporated into the crystalline lattice. Solvates wherein water is the solvent that is incorporated into the crystalline lattice are typically referred to as "hydrates."

- 5 Salts of a compound of Formula (I) may be prepared by contacting appropriate stoichiometric amounts of the free base/acid with the appropriate acid/base in a suitable solvent. The free base/acid of a compound of Formula (I) may for example be in solution with the appropriate acid/base added as a solid or both the free base/acid of a compound of Formula (I) and the appropriate acid/base may independently be in solution.

10 Suitable solvents for solubilising a compound of Formula (I) free base/acid include for example alcohols such as isopropanol; ketones such as acetone; acetonitrile or toluene. If the base is to be added as a solution in a solvent, the solvent used may include acetone, methanol or water.

15 The salts of a compound of Formula (I) may be isolated in solid form by conventional means from a solution thereof obtained as above. For example, a non-crystalline salt may be prepared by precipitation from solution, spray drying or freeze drying of solutions, evaporating a solution to a glass, or vacuum drying of oils, or solidification of melts obtained
20 from reaction of the free base and the acid. A compound of Formula (I) may also be prepared as an amorphous molecular dispersion of drug substance in a polymer matrix, such as hypromellose acetate succinate (HPMCAS) using a process such as spray-dried dispersion (SDD).

25 The salts of a compound of Formula (I) may be prepared by directly crystallising from a solvent in which the salt has limited solubility, or by triturating or otherwise crystallising a non-crystalline salt. For example, organic solvents such as acetone, acetonitrile, butanone, 1-butanol, ethanol, 1-propanol or tetrahydrofuran or mixtures of such solvents may be used. An improved yield of the salts may be obtained by the evaporation of some or all of the
30 solvent or by crystallisation at elevated temperature followed by controlled cooling, for example in stages. Careful control of the precipitation temperature and seeding may be used to improve the reproducibility of the production process and the particle size distribution and form of the product.

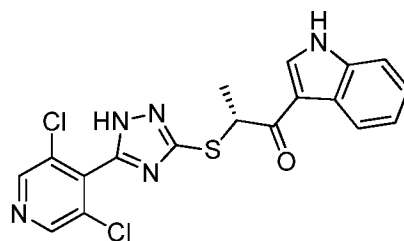
35 Salts and solvates of a compound of Formula (I) which are suitable for use in medicine are those wherein the counterion or associated solvent is pharmaceutically acceptable. However, salts and solvates having non-pharmaceutically acceptable counterions or associated solvents are within the scope of the present invention, for example, for use as intermediates in the preparation of a compound of Formula (I) or salts, solvates thereof and
40 their pharmaceutically acceptable salts and solvates.

It will be appreciated by those skilled in the art that certain protected derivatives of a compound of Formula (I), which may be made prior to a final deprotection stage, may not possess pharmacological activity as such, but may, in certain instances, be administered orally or parenterally and thereafter metabolised in the body to form compounds defined in the first aspect which are pharmacologically active. Such derivatives may therefore be described as “prodrugs”. All protected derivatives and prodrugs of compounds defined in the first aspect are included within the scope of the invention. Examples of suitable prodrugs for the compounds of the present invention are described in *Drugs of Today*, Volume 19, Number 9, 1983, pp 499 – 538 and in *Topics in Chemistry*, Chapter 31, pp 306 – 316 and in “Design of Prodrugs” by H. Bundgaard, Elsevier, 1985, Chapter 1 (the disclosures in which documents are incorporated herein by reference). It will further be appreciated by those skilled in the art, that certain moieties, known to those skilled in the art as “pro-moieties”, for example as described by H. Bundgaard in “Design of Prodrugs” (the disclosure in which document is incorporated herein by reference) may be placed on appropriate functionalities when such functionalities are present within the compound of Formula (I). Suitable prodrugs for compounds of Formula (I) or salts or solvates thereof include : amides, carbamates, azo-compounds, phosphamides, glycosides. Therefore, in one aspect of the present invention, there is provided prodrugs of a compound of Formula (I).

As described above, a compound of Formula (I) may be in the form of its free base or a pharmaceutically acceptable salt, solvate, or prodrug of a compound of Formula (I), which upon administration to the recipient is capable of providing (directly or indirectly) a compound of Formula (I), or an active metabolite or residue thereof. Such pharmaceutically acceptable salts, solvates, and prodrugs are recognizable to those skilled in the art, without undue experimentation. Nevertheless, reference is made to the teaching of Burger’s *Medicinal Chemistry and Drug Discovery*, 5th Edition, Vol 1: Principles and Practice, which is incorporated herein by reference to the extent of teaching such derivatives.

Furthermore, some of the crystalline forms of a compound of Formula (I) or salts and solvates thereof may exist in one or more polymorphic form, which are included in the present invention.

It will further be appreciated that a compound of Formula (I) may exist in different tautomeric forms. All possible tautomers are contemplated to be within the scope of the present invention. In a single crystal structure analysis of a compound of formula (I) the tautomeric form was found to be



(IA)

(R)-2-((5-(3,5-dichloropyridin-4-yl)-1H-1,2,4-triazol-3-yl)thio)-1-(2H-indol-3-yl)propan-1-one.

- 5 A compound of Formula (I) or a pharmaceutically acceptable salt thereof can be useful in the treatment of certain parasitic infections such as parasitic protozoal infections by the malarial parasite *Plasmodium falciparum*, species of *Eimeria*, *Pneumocytis carinii*, *Trypanosoma cruzi*, *Trypanosoma brucei* or *Leishmania donovani*. In particular, a compound of Formula (I) or a pharmaceutically acceptable salt thereof can be useful for treatment of infection by
- 10 *Plasmodium falciparum*. Accordingly, the invention is directed to methods of treating such conditions. Therefore, there is provided a compound of Formula (I) or a pharmaceutically acceptable salt thereof, for use in therapy.

15 In one aspect of the invention there is provided a compound of Formula (I) or a pharmaceutically acceptable salt thereof for use in the treatment of a parasitic protozoal infection.

In another aspect of the invention there is provided a compound of Formula (I) or a pharmaceutically acceptable salt thereof for use in the treatment of malaria.

20 In another aspect of the invention, there is provided a compound of Formula (I) or a pharmaceutically acceptable salt thereof for use in the treatment of an infection by *Plasmodium falciparum*.

25 In another aspect of the invention, there is provided the use of a compound of Formula (I) or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for the treatment of a parasitic protozoal infection.

30 In another aspect of the invention, there is provided the use of a compound of Formula (I) or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for the treatment of malaria.

In another aspect of the invention, there is provided the use of a compound of Formula (I) or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for the

35 treatment of an infection by *Plasmodium falciparum*.

In another aspect of the invention, there is provided a method for the treatment of a human or animal subject suffering from a parasitic protozoal infection, which method comprises administering to said human or animal subject an effective amount of a compound of Formula (I) or a pharmaceutically acceptable salt thereof.

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In another aspect of the invention, there is provided a method for the treatment of a human or animal subject suffering from malaria, which method comprises administering to said human or animal subject an effective amount of a compound of Formula (I) or a pharmaceutically acceptable salt thereof.

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In another aspect of the invention, there is provided a method for the treatment of a human or animal subject suffering from a parasitic protozoal infection by *Plasmodium falciparum*, which method comprises administering to said human or animal subject an effective amount of a compound of Formula (I) or a pharmaceutically acceptable salt thereof.

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The methods of treatment of the invention comprise administering a safe and effective amount of a compound according to Formula (I), and a pharmaceutically acceptable salt thereof to a patient in need thereof.

20 As used herein, "treatment" means: (1) the amelioration or prevention of the condition being treated or one or more of the biological manifestations of the condition being treated, (2) the interference with (a) one or more points in the biological cascade that leads to or is responsible for the condition being treated or (b) one or more of the biological manifestations of the condition being treated, or (3) the alleviation of one or more of the symptoms or effects
25 associated with the condition being treated. The skilled artisan will appreciate that "prevention" is not an absolute term. In medicine, "prevention" is understood to refer to the prophylactic administration of a drug to substantially diminish the likelihood or severity of a condition or biological manifestation thereof, or to delay the onset of such condition or biological manifestation thereof.

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As used herein, "safe and effective amount" means an amount of the compound sufficient to significantly induce a positive modification in the condition to be treated but low enough to avoid serious side effects (at a reasonable benefit/risk ratio) within the scope of sound medical judgment. A safe and effective amount of a compound of Formula (I) or a
35 pharmaceutically acceptable salt thereof will vary with the particular compound chosen (e.g. depending on the potency, efficacy, and half-life of the compound); the route of administration chosen; the nature of the infection and/or condition being treated; the severity of the infection and/or condition being treated; the age, size, weight, and physical condition of the patient being treated; the medical history of the patient to be treated; the duration of the
40 treatment; the nature of concurrent therapy; the desired therapeutic effect; and like factors, but can nevertheless be routinely determined by the skilled artisan.

As used herein, "patient" refers to a human or other animal.

A compound of Formula (I) or a pharmaceutically acceptable salt thereof may be administered by any suitable route of administration, including systemic administration. Systemic administration includes oral administration, parenteral administration, transdermal administration, rectal administration, and administration by inhalation. Parenteral administration refers to routes of administration other than enteral, transdermal, or by inhalation, and is typically by injection or infusion. Parenteral administration includes intravenous, intramuscular, and subcutaneous injection or infusion. Inhalation refers to administration into the patient's lungs whether inhaled through the mouth or through the nasal passages.

A compound of Formula (I) or a pharmaceutically acceptable salt thereof may be administered once only, or according to a dosing regimen wherein a number of doses are administered at varying intervals of time for a given period of time. For example, doses may be administered one, two, three, or four times per day. Doses may be administered until the desired therapeutic effect is achieved or indefinitely to maintain the desired therapeutic effect. The dosage will also vary according to the nature of the intended treatment, wherein "treatment" is as defined above, for example a greater dose of compound may be given for amelioration as compared with prevention of a condition being treated. Suitable dosing regimens for a compound of the invention depend on the pharmacokinetic properties of that compound, such as absorption, distribution, and half-life, which can be determined by the skilled artisan. In addition, suitable dosing regimens for compounds of the invention, including the duration such regimens are administered, depend on the route of administration of the compound, on the condition being treated, the severity of the condition being treated, the age and physical condition of the patient being treated, the medical history of the patient to be treated, the nature of any concurrent therapy, the desired therapeutic effect, and like factors within the knowledge and expertise of the skilled artisan. It will be further understood by such skilled artisans that suitable dosing regimens may require adjustment given an individual patient's response to the dosing regimen or over time as individual patient needs change. It will also be appreciated that if a compound of Formula (I) or a pharmaceutically acceptable salt thereof is administered in combination with one or more additional active therapeutic agents as discussed further hereinbelow, the dosing regimen of the compound of the invention may also vary according to the nature and amount of the one or more additional active therapeutic agents as necessary.

Typical daily dosages may vary depending upon the particular route of administration chosen. Typical daily dosages for oral administration are predicted to be in a range from about 25 to about 1000 mg/kg

The compounds of the invention may also be used in combination with other active therapeutic agents. The invention thus provides, in a further aspect, a combination

comprising a compound of Formula (I) or a pharmaceutically acceptable salt thereof together with a further active therapeutic agent. When a compound of Formula (I) or a pharmaceutically acceptable salt thereof is used in combination with a second active therapeutic agent which is active against the same disease state the dose of each compound may differ from that when the compound is used alone. Appropriate doses will be readily appreciated by those skilled in the art. It will be appreciated that the amount of a compound of the invention required for use in treatment will vary with the nature of the condition being treated and the age and the condition of the patient and will be ultimately at the discretion of the attendant physician or veterinarian.

A compound of Formula (I) or a pharmaceutically acceptable salt thereof may be used alone or in combination with one or more additional active therapeutic agents, such as other antiparasitic drugs, for example antimalarial drugs.

Such other active therapeutic agents include antimalarial drugs such as (e.g. chloroquine, mefloquine, primaquine, pyrimethamine, quinine, artemisinin, halofantrine, doxycycline, amodiaquine, atovaquone, tafenoquinedapsone, proguanil, sulfadoxine, cycloguanil) and fansidar.

The combinations referred to above may conveniently be presented for use in the form of a pharmaceutical formulation and thus pharmaceutical formulations comprising a combination as defined above together with a pharmaceutically acceptable carrier and/or excipient comprise a further aspect of the invention. The individual components of such combinations may be administered either sequentially or simultaneously in separate or combined pharmaceutical formulations by any convenient route.

When administration is sequential, either a compound of Formula (I) or a pharmaceutically acceptable salt thereof, or the one or more additional active therapeutic agent(s) may be administered first. When administration is simultaneous, the combination may be administered either in the same or different pharmaceutical composition. When combined in the same formulation it will be appreciated that a compound of Formula (I) or a pharmaceutically acceptable salt thereof, and the one or more additional active therapeutic agent(s) must be stable and compatible with each other and the other components of the formulation. When formulated separately a compound of Formula (I) or a pharmaceutically acceptable salt thereof, and the one or more additional active therapeutic agent(s) may be provided in any convenient formulation, conveniently in such manner as are known for such compounds in the art.

Compositions

A compound of Formula (I) or a pharmaceutically acceptable salt thereof will normally, but not necessarily, be formulated into pharmaceutical compositions prior to administration to a patient. In one aspect, the invention is directed to pharmaceutical compositions comprising a

compound of Formula (I) or a pharmaceutically acceptable salt thereof. In another aspect the invention is directed to a pharmaceutical composition comprising (a) a compound of Formula (I) or a pharmaceutically acceptable salt thereof and (b) one or more pharmaceutically acceptable carriers and/or excipients. In another aspect, the invention provides a pharmaceutical composition comprising a compound of Formula (I) or a pharmaceutically acceptable salt thereof. In a further aspect, the invention provides a pharmaceutical composition comprising (a) a compound of Formula (I) or a pharmaceutically acceptable salt thereof and (b) one or more pharmaceutically acceptable carriers..

- 10 The carrier and/or excipient must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

The pharmaceutical compositions of the invention may be prepared and packaged in bulk form wherein a safe and effective amount of a compound of the invention can be extracted and then given to the patient such as with powders or syrups. Alternatively, the pharmaceutical compositions of the invention may be prepared and packaged in unit dosage form wherein each physically discrete unit contains a safe and effective amount of a compound of the invention. When prepared in unit dosage form, the pharmaceutical compositions of the invention typically contain from about 0.1 to 1000mg, in another aspect 0.1 mg to about 500 mg of a compound of the invention.

The pharmaceutical compositions of the invention typically contain one compound of Formula (I) or a pharmaceutically acceptable salt thereof. However, in certain embodiments, the pharmaceutical compositions of the invention contain more than one compound of Formula (I) or a pharmaceutically acceptable salt thereof. For example, in certain embodiments the pharmaceutical compositions of the invention contain two compounds of the invention. In addition, the pharmaceutical compositions of the invention may optionally further comprise one or more additional active therapeutic compounds. The pharmaceutical compositions of the invention typically contain more than one pharmaceutically acceptable excipient. However, in certain embodiments, the pharmaceutical compositions of the invention contain one pharmaceutically acceptable excipient.

A compound of Formula (I) or a pharmaceutically acceptable salt thereof, and the pharmaceutically acceptable excipient or excipients will typically be formulated into a dosage form adapted for administration to the patient by the desired route of administration. For example, dosage forms include those adapted for (1) oral administration such as tablets, capsules, caplets, pills, troches, powders, syrups, elixirs, suspensions, solutions, emulsions, sachets, and cachets; (2) parenteral administration such as sterile solutions, suspensions, and powders for reconstitution; (3) transdermal administration such as transdermal patches; (4) rectal administration such as suppositories; and (5) inhalation such as aerosols and solutions.

Suitable pharmaceutically acceptable excipients will vary depending upon the particular dosage form chosen. In addition, suitable pharmaceutically acceptable excipients may be chosen for a particular function that they may serve in the composition. For example, certain pharmaceutically acceptable excipients may be chosen for their ability to facilitate the production of uniform dosage forms. Certain pharmaceutically acceptable excipients may be chosen for their ability to facilitate the production of stable dosage forms. Certain pharmaceutically acceptable excipients may be chosen for their ability to facilitate the carriage or transport of a compound of Formula (I) or a pharmaceutically acceptable salt thereof from one organ, or portion of the body, to another organ, or portion of the body, once administered to the patient. Certain pharmaceutically acceptable excipients may be chosen for their ability to enhance patient compliance.

Suitable pharmaceutically acceptable excipients include the following types of excipients: binders, disintegrants, lubricants, glidants, granulating agents, coating agents, wetting agents, solvents, co-solvents, suspending agents, emulsifiers, sweeteners, flavouring agents, flavour masking agents, coloring agents, anticaking agents, humectants, chelating agents, plasticizers, viscosity increasing agents, antioxidants, preservatives, stabilizers, surfactants, and buffering agents. The skilled artisan will appreciate that certain pharmaceutically acceptable excipients may serve more than one function and may serve alternative functions depending on how much of the excipient is present in the formulation and what other ingredients are present in the formulation.

Skilled artisans possess the knowledge and skill in the art to enable them to select suitable pharmaceutically acceptable excipients in appropriate amounts for use in the invention. In addition, there are a number of resources that are available to the skilled artisan which describe pharmaceutically acceptable excipients and may be useful in selecting suitable pharmaceutically acceptable excipients. Examples include Remington's Pharmaceutical Sciences (Mack Publishing Company), The Handbook of Pharmaceutical Additives (Gower Publishing Limited), and The Handbook of Pharmaceutical Excipients (the American Pharmaceutical Association and the Pharmaceutical Press).

The pharmaceutical compositions of the invention are prepared using techniques and methods known to those skilled in the art. Some of the methods commonly used in the art are described in Remington's Pharmaceutical Sciences (Mack Publishing Company).

In one aspect, the invention is directed to a solid or liquid oral dosage form such as a liquid, tablet, lozenge or a capsule, comprising a safe and effective amount of a compound of the invention and a carrier. The carrier may be in the form of a diluent or filler. Suitable diluents and fillers in general include lactose, sucrose, dextrose, mannitol, sorbitol, starch (e.g. corn starch, potato starch, and pre-gelatinized starch), cellulose and its derivatives (e.g. microcrystalline cellulose), calcium sulfate, and dibasic calcium phosphate. A liquid dosage form will generally consist of a suspension or solution of the compound or pharmaceutically

acceptable derivative in a liquid carrier for example, ethanol, olive oil, glycerine, glucose (syrup) or water (e.g. with an added flavouring, suspending, or colouring agent). Where the composition is in the form of a tablet or lozenge, any pharmaceutical carrier routinely used for preparing solid formulations may be used. Examples of such carriers include magnesium stearate, terra alba, talc, gelatin, acacia, stearic acid, starch, lactose and sucrose. Where the composition is in the form of a capsule, any routine encapsulation is suitable, for example using the aforementioned carriers or a semi solid e.g. mono di-glycerides of capric acid, Gelucire™ and Labrasol™, or a hard capsule shell e.g. gelatin. Where the composition is in the form of a soft shell capsule e.g. gelatin, any pharmaceutical carrier routinely used for preparing dispersions or suspensions may be considered, for example aqueous gums or oils, and may be incorporated in a soft capsule shell.

An oral solid dosage form may further comprise an excipient in the form of a binder. Suitable binders include starch (e.g. corn starch, potato starch, and pre-gelatinized starch), gelatin, acacia, sodium alginate, alginic acid, tragacanth, guar gum, povidone, and cellulose and its derivatives (e.g. microcrystalline cellulose). The oral solid dosage form may further comprise an excipient in the form of a disintegrant. Suitable disintegrants include croscopovidone, sodium starch glycolate, croscarmellose, alginic acid, and sodium carboxymethyl cellulose. The oral solid dosage form may further comprise an excipient in the form of a lubricant. Suitable lubricants include stearic acid, magnesium stearate, calcium stearate, and talc.

There is further provided by the present invention a process of preparing a pharmaceutical composition, which process comprises mixing the compound of Formula (I) or a pharmaceutically acceptable salt or solvate thereof, together with a pharmaceutically acceptable carrier and/or excipient.

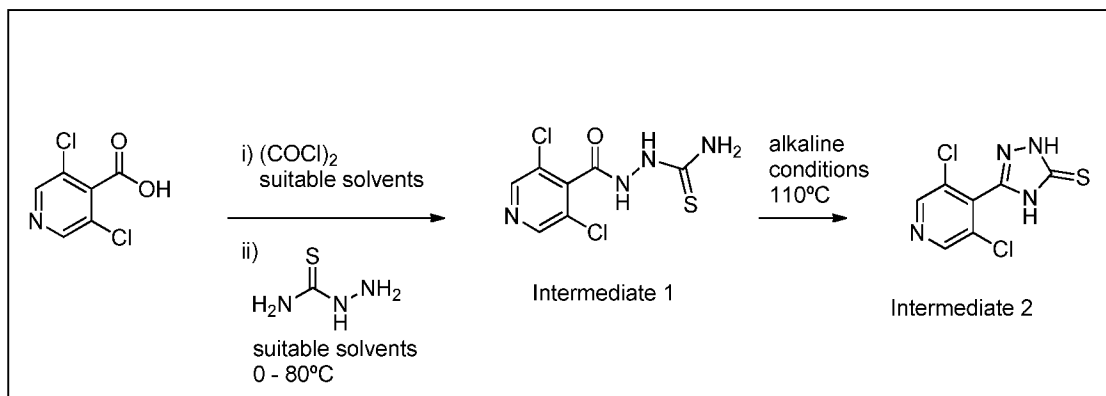
Preparations for oral administration may be suitably formulated to give controlled/extended release of the active compound.

PROCESSES

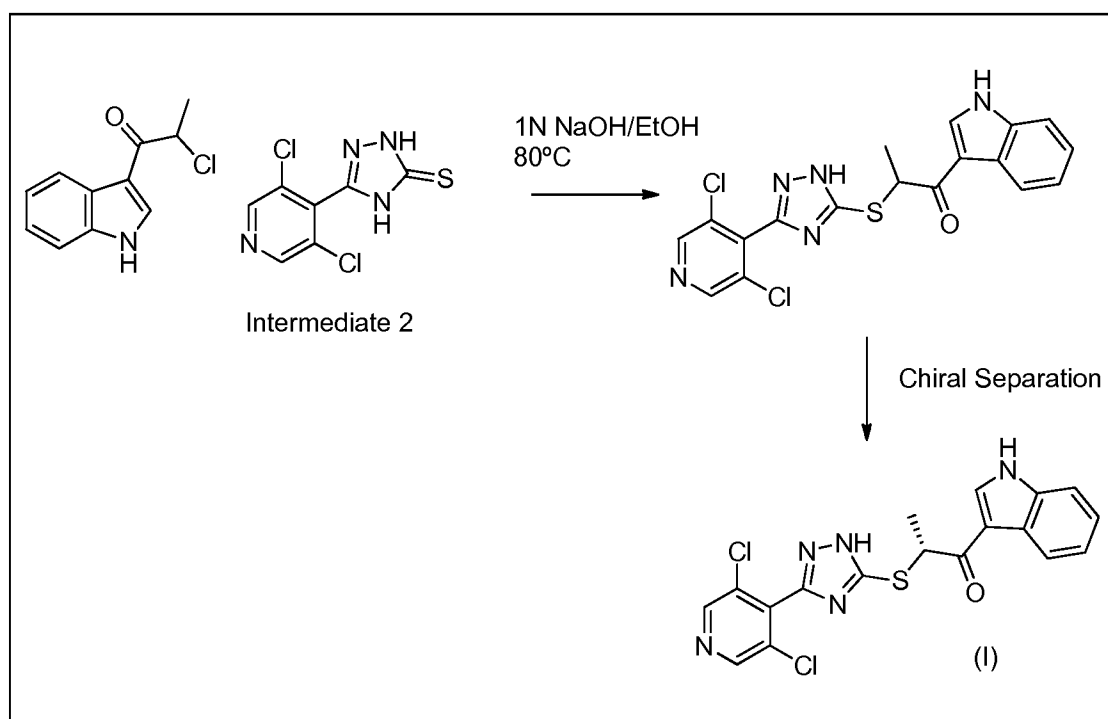
A general procedure for acid chloride formation and amide formation is described in the literature (*J. Chem. Res. 2008 (22), 530-533*) using appropriate commercially available acids and anilines as starting materials.

The compound of formula (I) may be synthesised by asymmetric or non-asymmetric routes.

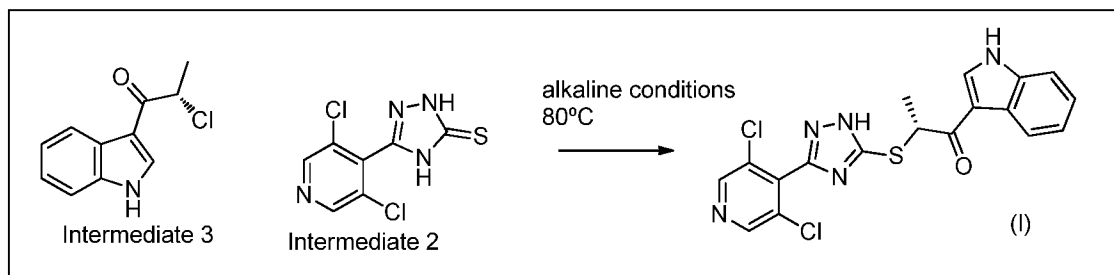
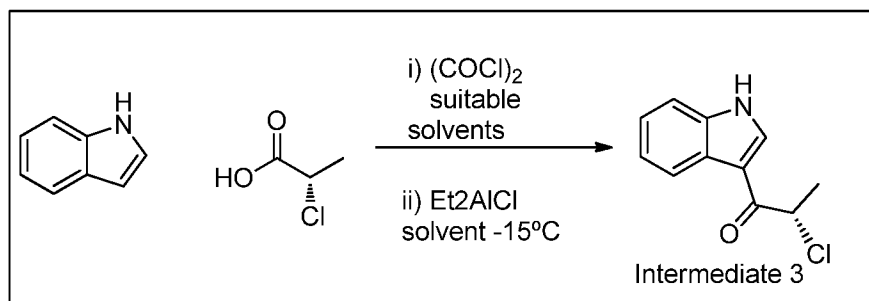
In both types of synthesis 3-(3,5-dichloropyridin-4-yl)-1H-1,2,4-triazole-5(4H)-thione (Intermediate 2) may be made using the following process.



In a non-asymmetric synthesis 2-chloro-1-(1H-indol-3-yl)propan-1-one and 3-(3,5-dichloropyridin-4-yl)-1H-1,2,4-triazole-5(4H)-thione (Intermediate 2) may be reacted together before chiral separation of the compound of Formula (I).



In an asymmetric synthesis (S)-2-chloro-1-(1H-indol-3-yl)propan-1-one (Intermediate 3) may be made by the addition of (2S)-2-chloropropanoyl chloride to indole, which is then added to 3-(3,5-dichloropyridin-4-yl)-1H-1,2,4-triazole-5(4H)-thione (Intermediate 2) to make the compound of Formula (I).



It will be readily apparent to those skilled in the art that the compound of Formula (I) may be prepared using methods analogous to those outlined above, or by reference to the experimental procedures detailed in the Examples provided herein. Further details for the preparation of the compound of Formula (I) are found in the Examples.

With appropriate manipulation and protection, the synthesis of a compound of Formula (I) is accomplished by methods analogous to those above. In any particular case, particular protecting groups may be required. Suitable protecting groups can be found, but are not restricted to, those found in T W Greene and P G M Wuts 'Protective Groups in Organic Synthesis', 3rd Ed (1999), J Wiley and Sons.

EXPERIMENTAL

Abbreviations

In describing the invention, chemical elements are identified in accordance with the Periodic Table of the Elements. Abbreviations and symbols utilised herein are in accordance with the common usage of such abbreviations and symbols by those skilled in the chemical arts. The following abbreviations are used herein:

Anh	anhydrous
Aq.	Aqueous
AUC	area under the concentration-time curve
AWCI	Antimalarial whole cell Screening
cat.	Catalytic
conc.	concentrated
DCM	Dichloromethane
DMF	Dimethylformamide
DMSO- <i>d</i> 6	Deuterated dimethylsulfoxide

	ED90	Dose that eradicates 90 per cent of parasitemia
	ES MS	Electrospray mass spectrometry
	EtOAc	Ethyl acetate
	FaSSIF	Fasted state simulated intestinal fluid
5	FeSSIF	Fed State simulated intestinal fluid
	g	grams
	h	hours
	HCl	hydrochloridic acid
	HPLC	High performance liquid chromatography
10	HTS	High throughput screening
	iPrOH	2-Propanol
	L	litre
	M	Molar
	uM	micromolar
15	ug	micrograms
	MeOH	Methanol
	min(s)	Minutes
	mL	millilitres
	mmol	millimoles
20	mg	milligrams
	MHz	megahertz
	MW	Microwave
	N	Normal
	NaHCO ₃	sodium bicarbonate
25	NaCl	sodium chloride
	HPLC	high performance liquid chromatography
	¹ H NMR	proton nuclear magnetic resonance spectroscopy
	PBS	phosphate-buffered solution
	P.falciparum	Plasmodium falciparum
30	Pf	Plasmodium falciparum
	rt	room temperature
	rpm	revolution per minute
	sat.	saturated
	SGF	simulated gastric fluid
35	THF	tetrahydrofuran
	UPLC	ultra performance liquid chromatography
	°C	degrees centigrade
	[³ H]hypoxanthine	tritiated hypoxanthine
40	δppm	chemical shift in parts per million

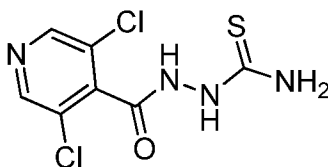
Compound Preparation

Examples

The following Examples illustrate the invention. These Examples are not intended to limit the scope of the invention, but rather to provide guidance to the skilled artisan to prepare and use the compounds, compositions, and methods of the invention. While particular
5 embodiments of the invention are described, the skilled artisan will appreciate that various changes and modifications can be made without departing from the spirit and scope of the invention.

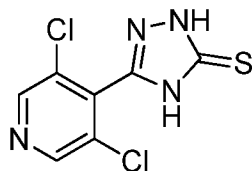
Where materials were commercially available, this is indicated in parentheses after the compound name, in capitals. For example, in the preparation of Intermediate 1, oxalyl
10 chloride was purchased from ALDRICH, so it is stated oxalyl chloride (ALDRICH)".

Intermediate 1 2-(3,5-dichloroisonicotinoyl)hydrazinecarbothioamide



i) To a suspension of 3,5-dichloroisonicotinic acid (MANCHESTER, 50 g, 260.42 mmol) in
20 DCM (500 ml) was added oxalyl chloride (ALDRICH, 24.24 ml, 286.462 mmol) and 30 drops of N,N-Dimethylformamide (DMF). The reaction mixture was stirred at rt for 3h. UPLC with NHMe₂ in CH₃CN showed reaction was completed. The solvent was evaporated in vacuo to yield the desired product as a greenish solid (3,5-dichloropyridine-4-carbonyl chloride) and was used for the next step without any further purification.

25 ii) A solution of Thiosemicarbazide, 99% (ALDRICH, 23.735 g, 260.42 mmol) and pyridine (21.02 mL, 260.42 mmol) in N,N-Dimethylformamide (DMF) (330 mL) was cooled to 0°C (ice bath) and a suspension of 3,5-dichloropyridine-4-carbonyl chloride in N,N-Dimethylformamide (DMF) (120 mL) was added at 0°C dropwise via cannula. Reaction was heated to 80°C for 3h. UPLC revealed reaction was completed. It was concentrated under reduced pressure in
30 the Genevac until dryness to give the crude material as a yellow solid. This solid was poured in portions into 250 ml of cold water. The resultant white suspension was centrifuged and the white solid was separated, dried under air stream to obtain the desired compound 2-(3,5-dichloroisonicotinoyl)hydrazinecarbothioamide (69.93 g, yield= 84 %,purity= 83% by UPLC and NMR). ¹H NMR (400 MHz, DMSO-*d*₆) δppm: 10.95 (s, 1 H), 10.00 (s, 1H), 8.86 (m, 1H),
35 8.78 (s, 2 H), 7.92 (m, 1H) [ES+ MS] m/z 265 (M+H)

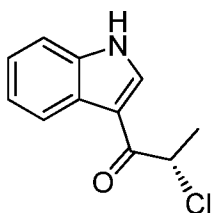
Intermediate 2 3-(3,5-dichloropyridin-4-yl)-1H-1,2,4-triazole-5(4H)-thione**METHOD A**

5 A mixture of 2-(3,5-dichloroisonicotinoyl)hydrazinecarbothioamide (Intermediate 1, 135.48 g, 426.61 mmol) in 2 l of aqueous solution 1M of NaOH was heated in 3.5 L Parr at 110 °C (T_{setted} = 120 °C, T_{measured} in the bath=110 °C) for 40 hours. Then the solution was transferred in a 4 L flask washing the reactor with water and at 0 °C and under stirring was
10 acidified with 200 ml of conc. HCl. Aqueous suspension was spin-dried with GeneVac and then the aqueous solution was removed affording to 47.71 g of 3-(3,5-dichloropyridin-4-yl)-1H-1,2,4-triazole-5(4H)-thione as grey solid (purity =97 % by UPLC and NMR). The product was used in the next step without further purifications. ¹H NMR (400 MHz, DMSO-*d*₆) δppm: 14.11 (s, 1H). 13.95 (s, 1H) 8.91 (s, 2H). [ES+ MS] m/z 247 (M⁺)

METHOD B

A suspension of 2-(3,5-dichloroisonicotinoyl)hydrazinecarbothioamide (Intermediate 1, 3.0 g, 11.32 mmol) in 1N sodium hydroxide (16.97 ml, 16.97 mmol) was placed in a reaction vessel
20 that was sealed and heated to 150°C for 60 min using a microwave Synthos 3000 device. Reaction mixture was carefully acidified by 6N HCl addition at 0°C until neutra pH, then concentrated up to three-quarters under reduced pressure. The solid was filtered and washed with water and DCM to obtain 3-(3,5-dichloropyridin-4-yl)-1H-1,2,4-triazole-5(4H)-thione as a pale yellow solid (1.63 g). Mother liqueour was concentrated up to three-quarters
25 under reduced pressure and new solid was filtered and washed with water and DCM to obtain additional 3-(3,5-dichloropyridin-4-yl)-1H-1,2,4-triazole-5(4H)-thione as a pale yellow solid (534 mg) as a yellow solid.

¹H NMR (400 MHz, DMSO-*d*₆) δppm: 14.20-14.02 (br.s., 2H), 8.97 (s, 2H).

Intermediate 3 (S)-2-chloro-1-(1H-indol-3-yl)propan-1-one

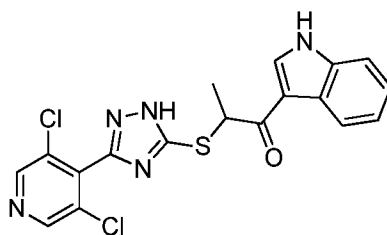
- i) Oxalyl Chloride (ALDRICH, 3.57 ml, 42.2 mmol) and DMF (ALDRICH, 25 drops) were added to a stirring solution of (2S)-2-chloropropanoic acid (ALDRICH, 4.17 g., 89.6 mmol.) in DCM (65 ml). The solution was stirred 2 hours at room temperature.

Reaction was then slightly concentrated with rotavapor ($P = 350$ mmBar, $T_{\text{bath}} = 28^\circ\text{C}$) affording to the crude solution of intermediate ((2S)-2-chloropropanoyl chloride) that was directly used in the following step without further purifications (Step A).

- ii) To a solution of indole (ALDRICH, 10.0 g, 85.3 mmol) in DCM (65 ml) at -10°C was added dropwise over 10 minutes Diethylaluminum chloride solution (1.8 M in toluene) (ALDRICH, 29.4 mmol, 16.4 ml) and the mixture was stirred at the same temperature for further 5 minutes. After cooling the solution at -10°C , the concentrated solution of (2S)-2-chloropropanoyl chloride (step i) in DCM. was added dropwise over 15 minutes. Reaction mixture was allowed to stir for 1.5 h during this time the reaction temperature gradually warmed to rt. The reaction was carefully quenched at 0°C with MeOH (ethane evolution) and a solution of NaHCO_3 . Then the product was extracted from the aqueous phase with EtOAc (three times with 300 ml). The collected organic phases were dried over Na_2SO_4 , evaporated at rotavapor affording to 17.2 g of (S)-2-chloro-1-(1H-indol-3-yl)propan-1-one (grey solid, 94% of purity by UPLC) that was used in the next step without further purifications.

^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm: 11.00 (s, 1 H), 8.5 (d, 1H), 8.20 (m, 1H), 7.51 (m, 1H), 7.25 (m, 2H), 5.57 (q, 1H), 1.65 (d, 3H). [ES+ MS] m/z 207 (M^+)

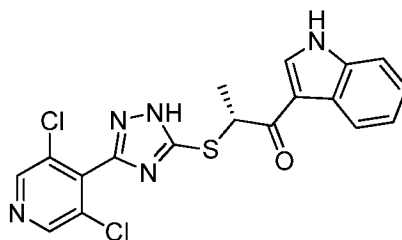
Example 1 2-((3-(3,5-dichloropyridin-4-yl)-1H-1,2,4-triazol-5-yl)thio)-1-(1H-indol-3-yl)propan-1-one



3-(3,5-dichloropyridin-4-yl)-1H-1,2,4-triazole-5(4H)-thione (Intermediate 2, 589 mg, 2.384 mmol) was suspended in 4mL NaOH 1N, and then 2-chloro-1-(1H-indol-3-yl)propan-1-one (SANTILABS, 450 mg, 2.167 mmol) was added dissolved in 15mL EtOH at rt. Reaction mixture was then heated at 80°C for 3h. Ethanol was removed from reaction mixture, and resulting aqueous solution was extracted with EtOAc (2x10mL). Although part of the product were found in AcOEt, but most of the impurities were removed with the EtOAc and target product remained mainly in aqueous layer, with a small impurity. Aqueous layer was then extracted then with DCM (7mL). Aqueous solution was then acidified by adding 1N HCl and then it was extracted with EtOAc (3 x 10mL). Organic layer was dried over anhydrous sodium sulphate, filtered and concentrated to obtain 2-((3-(3,5-dichloropyridin-4-yl)-1H-1,2,4-triazol-5-yl)thio)-1-(1H-indol-3-yl)propan-1-one (730 mg, 81%).

Racemic mixture of Example 1 was separated by semi preparative chiral HPLC to obtain Example 2 (see Method C).

Example 2 (R)-2-((3-(3,5-dichloropyridin-4-yl)-1H-1,2,4-triazol-5-yl)thio)-1-(1H-indol-3-yl)propan-1-one

**METHOD C**

2-((3-(3,5-dichloropyridin-4-yl)-1H-1,2,4-triazol-5-yl)thio)-1-(1H-indol-3-yl)propan-1-one

5 (Example 1, 600mg, 1.43mmol) was separated by semi-preparative chiral HPLC (Column Chiralpack IC 20x250nm, Isocratic gradient Heptane:iPrOH 90:10, time 30mins., Wavelength 254nm, Flow 18mL/min) to obtain (*R*)-2-((3-(3,5-dichloropyridin-4-yl)-1H-1,2,4-triazol-5-yl)thio)-1-(1H-indol-3-yl)propan-1-one. (first eluted compound) (e.e>99%;

10 ¹H NMR (400 MHz, DMSO-*d*₆) δppm: 14.81 (s, 1 H), 12.19 (s, 1 H), 8.89 (s, 2H), 8.49 (d, 1H), 8.25-8.23 (m, 1H), 7.56-7.54 (m, 1H), 7.29 (m 2H), 5.37 (q, 1H), 4.43 (br.s., 1H), 3.84 (m, 1H), 1.70 (d, 3H) [ES+ MS] m/z 418 (M+H) (e.e>99%, absolute configuration confirmed by *ab initio* VCD analysis)

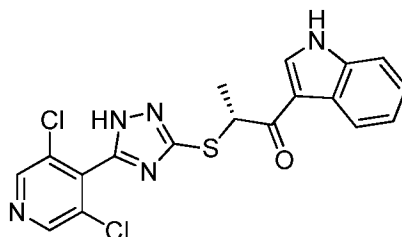
METHOD D

15 To a solution of (2*S*)-2-chloro-1-(1H-indol-3-yl)propan-1-one (Intermediate 3, 26.0 g., 96.4 mmol) in DMF (280mL) was added 3-(3,5-dichloropyridin-4-yl)-4,5-dihydro-1H-1,2,4-triazole-5-thione (Intermediate 2, 26.7 g., 91.8 mmol) and K₂CO₃ (ALDRICH, 33.3 g, 241 mmol.). The reaction mixture was stirred at room temperature for 1.5 hours, then more 3-(3,5-dichloropyridin-4-yl)-4,5-dihydro-1H-1,2,4-triazole-5-thione (Intermediate 2, 1.6 g., 4.6 mmol) were added and the mixture was stirred at rt for further 1.5 hours. Then 200 mL of water were added and some impurities were extracted with 120mL of DCM. Then the pH of the aqueous solution was adjusted to pH=7 adding solid NaHCO₃ and the product was extracted from the aqueous solution with EtOAc (9 times with 550 mL). The collected organic phases were dried with Na₂SO₄ and then the solvent was removed at rotavapor affording to the crude product that was purified by flash chromatography (Isolera, SNAP 1500g SiO₂, eluent = EtOAc in Cyclohexane, from 30% to 60%). The collected fractions containing the product was concentrated at rotavapor until 80 mL of solvent: a white solid was formed and the mother liquor was concentrated giving 10.2 g of (*R*)-2-((3-(3,5-dichloropyridin-4-yl)-1H-1,2,4-triazol-5-yl)thio)-1-(1H-indol-3-yl)propan-1-one (e.r =87/13) as yellowish solid.

35 9.7 g of (*R*)-2-((3-(3,5-dichloropyridin-4-yl)-1H-1,2,4-triazol-5-yl)thio)-1-(1H-indol-3-yl)propan-1-one (e.r =87/13) were solubilized in a flask at rt in 28 mL of iPrOH. Then, the flask was let at 5°C one night in the fridge. Then the organic solution was removed, the solid was washed with 10mL of DCM affording 5.96 g of (*R*)-2-((3-(3,5-dichloropyridin-4-yl)-1H-1,2,4-triazol-5-yl)thio)-1-(1H-indol-3-yl)propan-1-one (e.r =99/1)

^1H NMR (400 MHz, DMSO- d_6) δ ppm: 14.81 (s, 1 H), 12.19 (s, 1H), 8.89 (s, 2H), 8.49 (d, 1H), 8.25-8.23 (m, 1H), 7.56-7.54 (m, 1H), 7.29 (m 2H), 5.37 (q, 1H), 4.43 (br.s., 1H), 3.84 (m, 1H), 1.70 (d, 3H) [ES+ MS] m/z 418 (M+H)

- 5 **Example 3** (*R*)-2-((3-(3,5-dichloropyridin-4-yl)-1H-1,2,4-triazol-3-yl)thio)-1-(1H-indol-3-yl)propan-1-one



10 A compound made in a similar manner to Example 1 (158.6 mg) was suspended in acetonitrile (1 ml), seeded with ~5-10 mg of a previous batch of the compound and left to stir at RT. After 1 hour MeCN (0.6ml) was added. Several days later approximately 1/3 of the slurry was decanted into a new vial (Vial 2). Water was added (2 drops) and the vial placed in
15 the Thermix to temperature cycle. Once a small amount of solid was present in Vial 2 the solution was decanted into a new vial (Vial 3) with a lid having two pieced holes to allow slow evaporation. Once the solution had evaporated down to a solid, it was analysed by Raman spectroscopy and found to be concordant with Form 1 (see Example 4A).

20 The crystal and molecular structures of the solid were determined from three-dimensional X-ray diffraction data collected at 150(2) K. The study confirmed the atomic connectivity, the absolute configuration of the chiral centre and the tautomer present.

Crystal data and refinement summary: $\text{C}_{18}\text{H}_{13}\text{Cl}_2\text{N}_5\text{OS}$; $M = 418.29$; colourless lath; $0.44 \times 0.14 \times 0.07$ mm; orthorhombic; space group $P2_12_12_1$ (#19); $a = 7.07404(8)$ Å, $b = 15.02549(18)$ Å, $c = 16.9998(2)$ Å, $\alpha = \beta = \gamma = 90^\circ$, $V = 1806.92(4)$ Å³; $Z = 4$; $D_{\text{calc}} = 1.538$ Mg m⁻³; $\lambda = 1.54178$ Å; $\theta_{\text{max}} = 66.87^\circ$; reflections collected = 12966; independent reflections = 3200; $R_{\text{int}} = 0.0300$; coverage = 99.7 %; restraints = 0; parameters = 254; $S = 1.049$; $R_1 [I > 2\sigma(I)] = 0.0217$; wR_2 (all data) = 0.0553; absolute structure parameter = -0.013(10); and
30 largest difference peak and hole = 0.196 and -0.147 eÅ⁻³.

Example 4**Equipment and Acquisition Details for PXRD, DSC and TGA**

Powder X-Ray Diffraction (PXRD). PXRD diffractograms were acquired using PANalytical X'Pert Pro diffractometer on Si zero-background wafers. All diffractograms were collected using a Cu K α (45 kV/40 mA) radiation and a step size of 0.02° 2 θ and X'celeratorTM RTMS (Real Time Multi-Strip) detector. Nickel filter was used to reduce unwanted radiation, unless noted otherwise. Configuration on the incidental beam side: fixed divergence slit (¼ deg), 0.04 rad soller slits, anti-scatter slit (¼ deg), and 10 mm beam mask. Configuration on the diffracted beam side: fixed divergence slit (¼ deg) and 0.04 rad soller slit. Peak positions were determined using Highscore software and the margin of error in each peak position expressed in terms of 2 theta angles (2 θ) is approximately $\pm 0.1^\circ$ 2 θ .

Differential scanning calorimetry (DSC). DSC was conducted with a TA Instruments Q100 differential scanning calorimeter equipped with an autosampler and a refrigerated cooling system under 40 mL/min N₂ purge. DSC thermograms were obtained at 15°C/min in crimped Al pans.

Thermogravimetric Analysis (TGA). TGA thermograms were obtained with a TA Instruments Q500 thermogravimetric analyzer under 40 mL/min N₂ purge at 15°C/min in Pt or Al pans.

Example 4A Characterisation of Form 1 of a compound of Formula (I)

The PXRD spectrum of a solid form of a compound of Formula (I) is shown in Figure 1.

Characteristic PXRD angles and d-spacings for the compound of Formula (I) are recorded in Table 1.

Pos. [°2Th.]	d-spacing [Å]
11.815	7.49046
12.8985	6.86358
13.6672	6.47921
14.6427	6.0497
15.7447	5.62864
16.1365	5.49287
16.6842	5.31376
17.2005	5.1554
19.5789	4.53417
20.0377	4.43138
20.7943	4.27182
21.6683	4.10145
23.1954	3.83478
24.0057	3.70714

24.2524	3.66998
24.9854	3.56395
25.5156	3.49108
26.034	3.42273
26.7886	3.32801
27.047	3.2968
27.5734	3.23504
28.026	3.18382
28.9656	3.08265
29.5683	3.02117
30.2072	2.95871
31.3538	2.85308
31.7757	2.81615
32.7158	2.73735
34.1644	2.62453
34.4904	2.60046
35.5224	2.52724
35.981	2.49608
37.436	2.40234
38.4379	2.342
38.8149	2.32012
39.4484	2.28431

Table 1: PXRD diffraction angles and d-spacings for compound of Formula (I)

Differential Scanning Calorimetry and Thermogravimetric Analysis for Form 1:

The DSC heating curve showed a melt endotherm with an onset temperature of 196.26 °C.

- 5 The TGA heating curve showed negligible weight loss until approximately 230 °C.

Exmple 4B Characterisation of Form 2 of a compound of Formula (I)

The PXRD spectrum of a solid form of a compound of Formula (I) is shown in Figure 2.

10

Characteristic PXRD angles and d-spacings for a compound of Formula (I) are recorded in Table 2.

Pos. [°2Th.]	d-spacing [Å]
9.7961	9.02913
11.4885	7.70256
12.6019	7.02444
15.1496	5.84838

16.2761	5.44607
16.5621	5.35266
17.2669	5.13572
19.5172	4.54838
19.9081	4.45995
20.6204	4.30746
21.3953	4.15317
21.9294	4.0532
22.2932	3.98788
22.9466	3.87579
24.2054	3.67701
24.6831	3.60691
25.0281	3.55797
25.3139	3.51844
26.2061	3.40065
27.0411	3.2975
27.7503	3.21482
28.8809	3.09149
29.2872	3.04952
30.0177	2.97695
31.1555	2.87079
32.3462	2.76777
32.8216	2.72876
33.6776	2.66134
34.185	2.62299
35.2821	2.5439
35.9675	2.49698
37.4525	2.40132
38.389	2.34487
39.0847	2.30472

Differential Scanning Calorimetry and Thermogravimetric Analysis of Form 2:

The DSC heating curve showed a melt endotherm with an onset temperature of 202.75°C.

The TGA heating curve showed negligible weight loss until approximately 220 °C.

5

Example 4C Characterisation of Form 3 of a compound of Formula (I)

The PXRD spectrum of a solid form of a compound of Formula (I) is shown in Figure 3.

- 10 Characteristic PXRD angles and d-spacings for a compound of Formula (I) are recorded in Table 3.

Pos. [°2Th.]	d-spacing [Å]
6.2202	14.20956
11.4888	7.7024
13.5619	6.52929
15.396	5.75534
15.7657	5.62121
16.6668	5.31928
17.214	5.15138
18.6955	4.74638
19.9163	4.45812
20.3599	4.36198
21.072	4.21615
22.1716	4.00948
22.8429	3.89314
23.0814	3.85346
23.718	3.75144
24.4201	3.64516
24.9917	3.56307
26.1373	3.40944
26.8487	3.3207
27.0518	3.29623
27.6985	3.22072
29.735	3.00461
31.6944	2.82319
32.3192	2.77003
32.941	2.71914
33.6369	2.66447
34.2064	2.6214
34.8984	2.57099
37.1549	2.41987
39.1197	2.30274

Differential Scanning Calorimetry and Thermogravimetric Analysis of Form 3:

The DSC heating curve showed a melt endotherm with an onset temperature of 203.58°C

The TGA heating curve showed negligible weight loss until approximately 220 °C.

Biological Assays

A compound of this invention may be tested in one of several biological assays to determine the concentration of compound which is required to have a given pharmacological effect. The assays are described below.

5

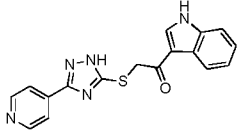
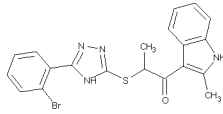
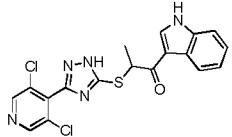
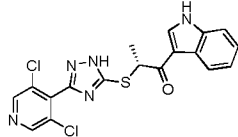
In vitro potency

P. falciparum growth inhibition assay.

The sensitivity of *P. falciparum* infected erythrocytes to the compound is determined in triplicate using the [³H]hypoxanthine incorporation method with an inoculum of 0.5% parasitemia (ring stage) and 2% hematocrit. The parasites were grown in RPMI 1640, 25 mM HEPES and supplemented with 5% Albumax. Plates are incubated at 37°C, 5% CO₂, 5% O₂, 90% N₂. After 24h of incubation, [3H]hypoxanthine is added and plates are incubated for another 24 h. After that period, plates are harvested on a glass fiber filter using a TOMTEC Cell harvester 96. Filters are dried and melt on scintillator sheets and the bound radioactivity is quantified by use of a Wallac Microbeta Trilux (Model 1450 LS- Perkin Elmer). IC50s are determined using Grit 5 program (Grit program; Erithacus Software, Horley, Surrey, United Kingdom).

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Results

	Compound 32 	Compound 524404 	Example 1 	Example 2 
PfIC50(μM)	> 5	0.06-0.044	0.052	0.019-0.053

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In vivo efficacy

P. falciparum *In Vivo* efficacy assay.

Antimalarial *in vivo* efficacy was determined using the *P. falciparum* mouse model following the procedure described in: Jimenez-Díaz, M.B., Mulet, T., Viera, S., Gómez, V., Garuti, H., Ibañez, J., Alvarez-Doval, A., Shlutz, D.L., Martinez, A., Improved Murine Model Of Malaria Using Plasmodium falciparum (Competent Strains and Non-Myelodepleted NOD-*scid* IL2R_{null}

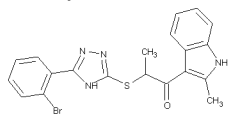
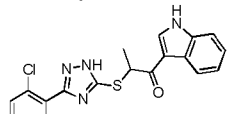
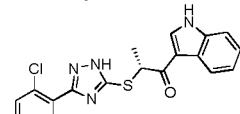
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Mice Engrafted with Human Erythrocytes) *Antimicrob. Agents Chemother* **2009**, 53 (10), 4533-4536

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Results

	Compound 524404	Example 1	Example 2
			
ED ₉₀ (mg/kg) / AUC _{ED₉₀} (μg.h.ml-1day-1)	7.3 / <0.05	No data	2.2 / 0.17

Solubility assessment in biorelevant media

- 5 Compound's equilibrium solubility was measured at 4h room temperature in four biologically relevant fluids: Fasted State-(Simulated Intestinal Fluid (FaSSIF), Fed State Simulated Intestinal Fluid (FeSSIF), Simulated Gastric Fluid (SGF) and Phosphate Buffered Saline (PBS).

10 **Solvents and buffers**

Organic solvents of HPLC grade were used. Ultra pure water (Milli-Q grade) was used. Buffers were prepared with ultra pure water and filtered using 0.45 μ nylon filters.

I. Procedure.15 **Determination of equilibrium solubility (assuming chemical stability in the desired solvent is not a problem).**

- a) 1 mg of solid compound was weighted in one 4 mL glass vial and 1 mL of the corresponding medium freshly prepared (SGF, FaSSIF, FeSSIF or PBS) was added. All these samples were prepared by duplicate.
- 20 b) The samples were stirred (roller mixer) for 4 hr at room temperature. If required, additional solid compound (0.1 mg) was added to maintain excess of it (saturated solutions).
- c) After 4 hours, the samples were centrifuged (10000 rpm, 10 min.) and the supernatants were transfer to an HPLC vial and analysed by LC-MS (previous dilution with mobile phase when needed).
- 25 d) The pH of the final solution in each sample was measured with a pH-meter (WTW pH330i and a pH-electrode Sentix 41).

LC-MS assay for analytical quantification

- 30 All supernatants were analysed by LC-MS. Quantification of those samples was carried out against calibration curves obtained from 1 mg/mL DMSO (Aldrich *cat. ref.*: 27685-5) stock solutions, by dilution with the mobile phase used in the chromatography. Depending on the solubility range, U.V. (1 μg/mL to 100 μg/mL) or MS (1 μg/mL to 1 ng/mL) detector were used in the quantification.

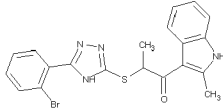
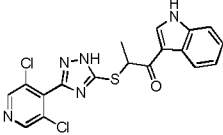
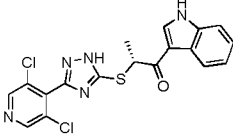
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Analysis of data

The analysis of all LC-MS data was performed with MassLynx 3.4 software and Analyst 1.4.2. Statistical and graphic analysis of data was performed using Microsoft Excel. The concentration (μM) and solubility ($\mu\text{g/ml}$) for each compound was calculated using the peak areas from the sample and those from the calibration curve.

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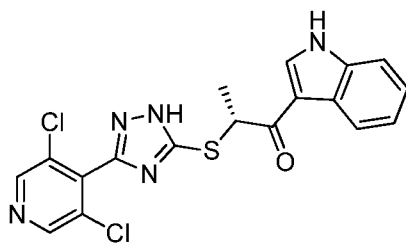
Results

	Compound 524404	Example 1	Example 2
			
FaSSIF ($\mu\text{g/mL}$)	37	168	216
FeSSIF ($\mu\text{g/mL}$)	181	No data	578-675
SGF ($\mu\text{g/mL}$)	<0.1	No data	15-76
PBS ($\mu\text{g/mL}$)	<0.1	No data	169-289

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Claims

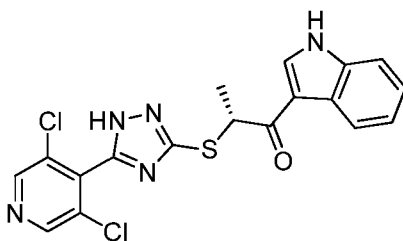
1. A compound of Formula (I)



(I)

(*R*)-2-((3-(3,5-dichloropyridin-4-yl)-1H-1,2,4-triazol-5-yl)thio)-1-(1H-indol-3-yl)propan-1-one or a tautomeric form thereof.

2. A tautomeric form of a compound of Formula 1 according to claim 1, which is



(IA)

(*R*)-2-((3-(3,5-dichloropyridin-4-yl)-1H-1,2,4-triazol-3-yl)thio)-1-(1H-indol-3-yl)propan-1-one.

3. A pharmaceutically acceptable salt of a compound of Formula (I) as defined in claims 1 or 2.

4. A pharmaceutical composition comprising (a) a compound of Formula (I) as defined in claims 1 or 2 or a pharmaceutically acceptable salt thereof and (b) one or more pharmaceutically acceptable carriers.

5. A compound of Formula (I) as defined in claims 1 or 2 or a pharmaceutically acceptable salt thereof, for use in therapy.

6. A compound of Formula (I) as defined in claims 1 or 2 or a pharmaceutically acceptable salt thereof, for use in the treatment of a parasitic protozoal infection.

7. The use of a compound of Formula (I) as defined in claims 1 or 2 or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for the treatment of a parasitic protozoal infection.

8. A combination of (a) a compound of Formula (I) as defined in claims 1 or 2 or a pharmaceutically acceptable salt thereof and (b) at least one anti-malarial agent.

9. A method for the treatment of a human or animal subject suffering from a parasitic protozoal infection, comprising administering to said human or animal subject an effective amount of a compound of Formula (I) as defined in claims 1 or 2 or a pharmaceutically acceptable salt thereof.

10. A compound for use as claimed in claim 5, a compound for use as claimed in claim 6, a compound for use in a combination for use as claimed in 7 or a compound for use as claimed in claim 8, wherein the parasitic protozoal infection is malaria.

11. A compound for use as claimed in claim 5, a compound for use as claimed in claim 6, a compound for use in a combination for use as claimed in 7 or a compound for use as claimed in claim 8, wherein the parasitic protozoal infection is *Plasmodium falciparum*.

12. A mixture comprising a compound of Formula (I), (*R*)-2-((3-(3,5-dichloropyridin-4-yl)-1H-1,2,4-triazol-5-yl)thio)-1-(1H-indol-3-yl)propan-1-one, as defined in claim 1 or a salt thereof, and its enantiomer (*S*)-2-((3-(3,5-dichloropyridin-4-yl)-1H-1,2,4-triazol-5-yl)thio)-1-(1H-indol-3-yl)propan-1-one or a salt thereof.

13. A mixture comprising a compound of Formula (IA), (*R*)-2-((3-(3,5-dichloropyridin-4-yl)-1H-1,2,4-triazol-3-yl)thio)-1-(1H-indol-3-yl)propan-1-one, as defined in claim 2 or a salt thereof, and its enantiomer (*S*)-2-((3-(3,5-dichloropyridin-4-yl)-1H-1,2,4-triazol-3-yl)thio)-1-(1H-indol-3-yl)propan-1-one or a salt thereof.