(12) STANDARD PATENT (11) Application No. **AU 2010311223 B2**
(19) AUSTRALIAN PATENT OFFICE

(54) Title
Use of ferroquine in the treatment or prevention of malaria

(51) International Patent Classification(s)
A61P 33/06 (2006.01) ***A61K 33/26*** (2006.01)
A61K 31/4706 (2006.01)

(21) Application No: **2010311223** (22) Date of Filing: **2010.10.29**

(87) WIPO No: **WO11/051634**

(30) Priority Data

(31) Number	(32) Date	(33) Country
0905212	**2009.10.30**	**FR**

(43) Publication Date: **2011.05.05**
(44) Accepted Journal Date: **2015.10.29**

(71) Applicant(s)
Sanofi

(72) Inventor(s)
Fraisse, Laurent;Struxiano, Annie

(74) Agent / Attorney
AJ PARK, L 9 Nishi 2 Phillip Law St, Canberra, ACT, 2601

(56) Related Art
Annex 10. Treatment of Plasmodium Vivax, P. ovale and P. Malariae infections", Guidelines for the Treatment of Malaria, World Health Organisation, 1 January 2006, pages 225 - 239, ISBN: 9789241546942

(12) DEMANDE INTERNATIONALE PUBLIÉE EN VERTU DU TRAITÉ DE COOPÉRATION EN MATIÈRE DE BREVETS (PCT)

(19) Organisation Mondiale de la Propriété Intellectuelle
Bureau international

(43) Date de la publication internationale
5 mai 2011 (05.05.2011)

PCT

(10) Numéro de publication internationale
WO 2011/051634 A1

(51) Classification internationale des brevets :
A61P 33/06 (2006.01) *A61K 33/26* (2006.01)
A61K 31/4706 (2006.01)

(21) Numéro de la demande internationale :
PCT/FR2010/052331

(22) Date de dépôt international :
29 octobre 2010 (29.10.2010)

(25) Langue de dépôt : français

(26) Langue de publication : français

(30) Données relatives à la priorité :
0905212 30 octobre 2009 (30.10.2009) FR

(71) Déposant *(pour tous les États désignés sauf US)* : **SANOFI-AVENTIS** [FR/FR]; 174 avenue de France, F-75013 Paris (FR).

(72) Inventeurs; et

(75) Inventeurs/Déposants *(pour US seulement)* : **FRAISSE, Laurent** [FR/FR]; c/o sanofi-aventis, Département Brevets, 174 avenue de France, F-75013 Paris (FR). **STRUXIANO, Annie** [FR/FR]; c/o sanofi-aventis, Département Brevets, 174 avenue de France, F-75013 Paris (FR).

(74) Mandataire : **VIDAL, Karine**; c/o sanofi-aventis, Département Brevets, 174 avenue de France, F-75013 Paris (FR).

(81) États désignés *(sauf indication contraire, pour tout titre de protection nationale disponible)* : AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) États désignés *(sauf indication contraire, pour tout titre de protection régionale disponible)* : ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), eurasien (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), européen (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Déclarations en vertu de la règle 4.17 :

— *relative à la qualité d'inventeur (règle 4.17.iv))*

Publiée :

— *avec rapport de recherche internationale (Art. 21(3))*

WO 2011/051634 A1

(54) **Title** : USE OF FERROQUINE IN THE TREATMENT OR PREVENTION OF MALARIA

(54) **Titre** : UTILISATION DE LA FERROQUINE DANS LE TRAITEMENT OU LA PREVENTION DU PALUDISME

(57) **Abstract** : The present invention relates to the use of ferroquine, the N-demethylated metabolite or any of the pharmaceutically acceptable salts thereof for treating and/or preventing infections caused by a parasite belonging to the Plasmodium genus, the life cycle of which includes a phase of hepatic latency in the human host.

(57) **Abrégé** : La présente invention a trait à l'utilisation de la ferroquine, de son métabolite N- déméthylé, ou de l'un de leurs sels pharmaceutiquement acceptables, dans le traitement et/ou la prévention d'infections causées par un parasite du genre Plasmodium, dont le cycle de vie comprend une phase de latence hépatique chez l'hôte humain.

20103111223 21 Sep 2015

USE OF FERROQUINE IN THE TREATMENT OR PREVENTION OF MALARIA

The present invention relates to the use of active agents that are useful for preventing and/or treating infections by the parasite *Plasmodium vivax*. More generally described is use against a parasite of the genus *Plasmodium*, whose life cycle comprises a hepatic lag phase in the human host.

More specifically, the present invention relates to the use of ferroquine for this purpose. Also described is the use of the N-demethylated metabolite of ferroquine for this purpose.

Malaria is one of the primary infectious causes of mortality worldwide and annually affects more than 5 000 000 people, among which 3 000 000 die each year.

This plague mainly affects sub-Saharan Africa, south-east Asia and Latin America.

Four main species of Plasmodium responsible for the transmission of malaria are generally distinguished: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae*, the first two being the most widespread.

The parasites *P. falciparum* and *P. vivax* are distinguished from each other in terms of geographical coverage and their growth cycle in the human host.

P. vivax constitutes the plasmodium species that is the most widespread on all continents, except for sub-Saharan Africa where *P. falciparum* is predominant, despite the presence of *P. malariae* which may occasionally be the cause of up to a third of the cases of malaria in that area, and *P. ovale*, which is nevertheless rarer (Mendis K. *et al.*, The Neglected Burden of *Plasmodium vivax* Malaria, Am. J. Trop. Med. Hyg., 2001, **64** (1-2 suppl) : 97-106).

More specifically, the parasite *P. vivax* is predominantly located in south-east Asia and in the Pacific, where it is responsible for 49% of malaria

2010311223 21 Sep 2015

cases, but also, to a lesser extent, in the territories of east and south Africa. In point of fact, it is present in the Afro-Asiatic populations, especially in Kenya, in Tanzania and in the Indian Ocean islands, for instance Madagascar. Moreover, the prevalence of this species increases in South America and Central America, with 71% to 81% of the cases of malaria. It is especially found in Peru, Bolivia and French Guiana. 81% of cases of malaria are also attributable to *P. vivax* in the eastern Mediterranean regions and 100% in the ex-USSR countries.

The estimations concerning the geographical prevalence of this parasite vary according to the methodology used, but the one thing that is certain is that the importance of this parasite is largely underestimated.

As regards the growth of the parasite, the four abovementioned species are all transmitted *via* a female anophele mosquito bite. Once inside the human host, the parasite reaches the liver cells, undergoes an asexual replication phase therein and leads to the formation of vesicles, the schizonts. The vesicles thus formed are released into the hepatic sinusoids and thereafter enter the blood circulation and spread therein a flood of young pre-erythrocytic merozoites ready to infect the red blood cells. The young pre-erythrocytic merozoites enter the red blood cells and start the erythrocytic cycle. The successive divisions of the merozoites cause rupture of the parasite-infested red blood cells. These sudden and synchronous ruptures are the cause of bouts of fever. The merozoites released into the blood circulation infect new red blood cells or erythrocytes. This is the start of the erythrocytic asexual cycle or erythrocytic schizogony. After invasion of the erythrocytes by the merozoites, the growth of the parasite begins via the ring stage, and then evolves into the trophozoite form.

Moreover, it should be noted that for the species other than *P. falciparum*, certain pre-erythrocytic merozoites do not reach the blood directly but rather attack new hepatocytes.

These hepatic forms, known as hypnozoites, remain in the latent state for a time that is particular to the type of strain and dependant on its environment. They maintain parasitosis in the liver for 2 or 3 years in the

2010311223 21 Sep 2015

case of *P. ovale*, 3 to 5 years or more in the case of *P. vivax* and for the rest of the life in the case of *P. malariae*, before reactivating in successive waves, causing a strong fever, also known as a benign tertian fever, which is one of the forms of malaria.

With regard to this specificity, it is evident that active agents that are effective for treating infections caused by *P. falciparum* generally prove, on the other hand, to be insufficient in terms of efficacy with regard to infections induced by these other species, in so far as they are limited to removing the circulating forms of the parasite and do not in any way act on the quiescent forms stored in the human hepatocytes.

Now, the conventional antimalaria treatments available for treating all the parasitic infections of the genus *Plasmodium* are treatments whose efficacy has essentially been validated only on the species *P. falciparum*.

Thus, chloroquine was and remains the first-line treatment for *P. vivax* malaria since 1946. It is often recommended with a relay with 8-aminoquinoline, primaquine (World Health Organization, "Annex 10. Treatment of *Plasmodium vivax, P. ovale and P. malariae* infections" 01/01/06, Guidelines for the treatment of malaria, p. 225-239).

However, the phenomena of *P. vivax* resistance to chloroquine make this active agent less and less effective against this parasite.

As regards primaquine, it has major toxicity problems and causes an increased risk of haemolysis in the case of individuals deficient in glucose-6-phosphate dehydrogenase. These individuals suffering from glucose-6-phosphate dehydrogenase deficiency often originate from Africa, the Middle East, India, the Mediterranean basin or south-east Asia.

Other compounds, such as quinine, chloroquine, mefloquine and artemisinine derivatives have little or no effect on the hepatic form of the parasite.

Antifolates and atovaquone, which were initially used in combination for treating the parasite in its circulating phase, have also been acknowledged as being active on the hepatocytes. However, numerous cases of resistance to these active agents have appeared.

2010311223 21 Sep 2015

As regards ACT (Artemisinine-based Combination Therapy), very few studies have to date been devoted to its efficacy on *P. vivax*.

There are therefore no real effective treatments at the present time for treating and preventing infections by *P. vivax* and more generally for treating the hepatic latent hypnozoite forms, which are characteristic of relapses.

Consequently, there is a need at the present time for a preventive and curative treatment that is specific for infections caused by a parasite of the genus *Plasmodium*, whose life cycle includes a hepatic lag phase in the human host. More specifically, there is a need for a treatment method for infections of blood cells caused by *Plasmodium vivax*, particularly at the immature ring stage and mature trophozoite stage of parasitic growth. It is an object of the present invention to go at least some way to satisfying these specific desiderata; and/or to at least provide the public with a useful choice.

In this specification where reference has been made to patent specifications, other external documents, or other sources of information, this is generally for the purpose of providing a context for discussing the features of the invention. Unless specifically stated otherwise, reference to such external documents is not to be construed as an admission that such documents, or such sources of information, in any jurisdiction, are prior art, or form part of the common general knowledge in the art.

In the description in this specification reference may be made to subject matter that is not within the scope of the claims of the current application. That subject matter should be readily identifiable by a person skilled in the art and may assist in putting into practice the invention as defined in the claims of this application.

SUMMARY OF THE INVENTION

The present invention relates to the use of ferroquine or a pharmaceutically acceptable salt thereof, for treating infections of blood cells

2010311223 21 Sep 2015

caused by *Plasmodium vivax* at the stages of parasitic growth of the immature trophozoite "ring stage" and of the mature tropozoite.

The invention also relates to a method for treating infections of blood cells caused by *Plasmodium vivax* at the stages of parasitic growth of the immature trophozoite "ring stage" and of the mature tropozoite, the method comprising administering a therapeutically effective dose of ferroquine or a pharmaceutically acceptable salt thereof to an infected patient.

DESCRIPTION

Described is the use of ferroquine or its N-demethylated metabolite or a pharmaceutically acceptable salt thereof for the treatment and/or prevention of infections caused by a parasite of the genus *Plasmodium*, whose life cycle includes a hepatic lag phase in the human host.

The infections are caused in particular by *P. vivax*, *P. ovale* or *P. malariae* and more particularly by *P. vivax.*

Thus described is ferroquine or its N-demethylated metabolite or a pharmaceutically acceptable salt thereof for its use for the treatment and/or prevention of infections of blood and/or liver cells infected with a parasite of the genus *Plasmodium*, in particular *P. vivax*, *P. ovale* or *P. malariae*, and more particularly *P. vivax*.

The present invention is directed towards ferroquine or a pharmaceutically acceptable salt thereof for its use for treating and/or preventing infections of blood cells at the ring stage and mature trophozoite stage of parasitic growth. In one embodiment, the patient to be treated is deficient in glucose-6-phosphate dehydrogenase.

Also described is the use of the N-demethylated metabolite of ferroquine, or a pharmaceutically acceptable salt thereof, for treating and/or preventing infections of blood cells at the ring stage and mature tophozoite stage of parasite growth.

2010311223 21 Sep 2015

Also described is ferroquine, or its N-demethylated metabolite or a pharmaceutically acceptable salt thereof for its use for preventing infections of blood cells at the ring stage and mature trophozoite stage of parasitic growth.Also described is ferroquine or its N-demethylated metabolite or a pharmaceutically acceptable salt thereof or its use for treating, preventing and eliminating the quiescent hypnozoite forms stored in human hepatocytes, especially derived from infections caused by a parasite of the genus *Plasmodium*, in particular *P. vivax*, *P. ovale* or *P. malariae*, and more particularly *P. vivax*.

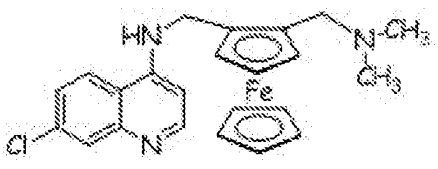
Also described is ferroquine or its N-demethylated metabolite or a pharmaceutically acceptable salt thereof, for its use for preventing relapses due to an infection caused by the parasite P. *vivax*.

Also described is ferroquine or its N-demethylated metabolite or a pharmaceutically acceptable salt thereof for its use for treating and/or preventing benign tertian fevers due to an infection caused by *P. vivax*..

Also described is ferroquine or its N-demethylated metabolite or a pharmaceutically acceptable salt thereof for its use for treating and/or preventing infections caused by *P. vivax* in the case of patients deficient in glucose-6-phosphate dehydrogenase.

This ferroquine and other related derivatives that differ from it as regards the substituents present on the quinoline ring are described in WO 96/35698.

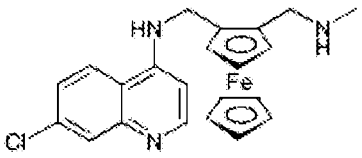
Structurally, it results from the insertion of a ferrocene group into a chloroquine molecule and corresponds to a compound of the following structure:



Ferroquine

2010311223 21 Sep 2015

Its (N)-demethylated metabolite corresponds to a compound of the following structure:



The parasitic strains specifically targeted in WO 96/35698 are the strains *P. falciparum*. Specifically, the development of such complexes is precisely based on the affinity of this parasite for the iron present in the red blood cells it infects.

Ferroquine is an antimalaria agent known as having higher activity than chloroquine in various parasites of the genus *Plasmodium* (Barends et al., "In vitro activity of ferroquine (SSR 97193) against P. falciparum isolates from the Thai-Burmese border" Malaria journal, Biomed central, vol. 6, no. 1, p. 81 ; Fouda et al., "On the medicinal chemistry of ferrocene" Database biosis Biosciences information service; Olliaro et al., "The global portfolio of new antimalarial medicines under development" Clinical pharmacology and therapeutics, vol. 85, no. 6, p. 584-595).

The *in vitro* activity of ferroquine on chloroquine-resistant strains of *P. falciparum* is indicated; complementary studies are, however, necessary (Barends et al., "In vitro activity of ferroquine (SSR 97193) against *P. falciparum* isolates from the Thai-Burmese border" Malaria journal, Biomed central, vol. 6, No. 1, p. 81).

The present patent application demonstrates that ferroquine proves to be particularly effective for treating infections by *P. vivax*. Unlike chloroquine, and entirely unexpectedly, it can not only eliminate the parasite in its circulating phase in blood cells, but it also does so with equal efficacy on the immature trophozoite forms ("ring stage") and mature trophozoite forms of P. *vivax*.

2010311223 21 Sep 2015

The active agents useful according to the present invention may be in free base form, but also in salt, hydrate or solvate form (the latter being defined as associations or combinations of ferroquine with, respectively, one or more water or solvent molecules).

As regards the salts that are suitable for use in the present invention, examples that may mentioned include the tartrate, L-tartrate, ditartrate, or dihydrochloride salts.

Advantageously, ferroquine is used in free base form.

Also described is a pharmaceutical composition comprising, as active principles, ferroquine or its N-demethylated metabolite or a pharmaceutically acceptable salt thereof, for its use for treating infections by *P. vivax*.

Such a pharmaceutical composition contains therapeutically effective doses of ferroquine or its (N)-demethylated metabolite, or a pharmaceutically acceptable salt thereof, hydrate thereof or solvate thereof of ferroquine and also at least one pharmaceutically acceptable excipient. The said excipients are chosen, according to the pharmaceutical form and the desired mode of administration, from the usual excipients known to those skilled in the art.

The appropriate unit administration forms include oral forms such as tablets, soft or hard gel capsules, powders, granules and oral solutions or suspensions, sublingual, buccal, intratracheal, intraocular, intranasal and inhalation administration forms, topical, transdermal, subcutaneous, intramuscular or intravenous administration forms, rectal administration forms and implants. The compounds according to the invention may be used in creams, gels, ointments or lotions for topical application.

The preferred routes of administration are the oral, rectal and injectable routes.

For example, when a solid composition in tablet form is prepared, the active ingredients are mixed with one or more pharmaceutical excipients, such as gelatin, starch, lactose, magnesium stearate, talc, silica, gum arabic,

2010311223 21 Sep 2015

mannitol, microcrystalline cellulose, hydroxypropylmethylcellulose, croscarmellose, magnesium stearate, hypromellose or the like. The tablets may be coated with sucrose, a cellulose derivative or other suitable coating materials. The tablets may be made via various techniques, such as direct tableting, dry granulation, wet granulation or hot melting.

A preparation in the form of gel capsules may also be obtained by mixing the active ingredients with a diluent and pouring the mixture obtained into soft or hard gel capsules.

Aqueous suspensions, isotonic saline solutions or sterile and injectable solutions that contain pharmacologically compatible dispersants and/or wetting agents, for example propylene glycol or butylene glycol, are used for parenteral administration.

There may be particular cases in which higher or lower doses are appropriate; such doses do not depart from the scope of the invention. According to the usual practice, the dose that is appropriate to each patient is determined by the doctor according to the mode of administration and the weight and response of the said patient.

By way of example, a unit form of administration of ferroquine in tablet form may comprise the following components:

Ferroquine 50 mg
Mannitol 224 mg
Sodium croscaramellose 6 mg
Corn starch 15 mg
Hydroxypropylmethylcellulose 2 mg
Magnesium stearate 3 mg

Also described is a method for treating and/or preventing malaria that includes the administration, to a patient infected with *P. vivax*, of a therapeutically effective dose of ferroquine or its (N)-demethylated metabolite, or a pharmaceutically acceptable salt thereof, a hydrate or a solvate thereof.

2010311223 21 Sep 2015

The test below is given purely as an illustration and does not in any way limit the scope of the present invention.

in vitro* evaluation of the efficacy of ferroquine *versus* other reference antimalaria agents, on blood isolates infected with *P. vivax

The test that follows is performed on 110 isolates of *P. vivax*, collected in the north-west of Thailand (Tak province) on consenting patients, infected with *P. vivax* and in the acute phase of the infection, who came for consultation at the Shoklo Malaria Research Unit (SMRU).

The samples are collected 5 hours after their arrival and placed in 5 ml heparinized tubes, at room temperature.

The parasitaemia of the collected isolates is about 4432 parasites/µl.

99 isolates are finally successfully cultured and, among these, only those containing more than 80% of trophozoites on microscopic observation are retained.

The platelets and leukocytes are removed from these isolates, by analogy with the method described in Kanlaya *et al.*, Malaria Journal, 2009, 8:115.

About sixty samples are finally retained and ten isolates per plate or per experiment are tested in duplicate.

The activity of the various active agents is tested in predosed wells, by analogy with the method described in Barends *et al.*, Malaria Journal 2007,6:81 and Brice *et al.*, Antimicrobial agents and chemotherapy, Jan 2003, pp 170-173.

Figure 1 showing a dose/response diagram illustrates the EC50 results (effective concentration 50) obtained for ferroquine and reference antimalaria agents, chloroquine, mefloquine and piperaquine.

Figure 2 showing a dose/response diagram illustrates the IC50 results (inhibitory concentration 50) obtained for ferroquine and chloroquine.

2010311223 21 Sep 2015

As indicated in the abovementioned Figure 1, the lowest EC50, of about 6 nM, is that of ferroquine, thus demonstrating its increased efficacy relative to the standard antimalaria agents.

As indicated in the abovementioned Figure 2, ferroquine is tested for its activity on the mature trophozoite stages and the young immature trophozoites ("ring stage") of *P. vivax*. Chloroquine is 10 to 20 times less active on the mature trophozoite stages than on the ring stages, as also described in Russell, B. *et al.* 2008, Antimicrob. Agents Chemother. 52:1040-5. On the other hand, and entirely unexpectedly, ferroquine is active on both stages of the parasite tested, with comparable activities of 14 and 21 nM for the ring and mature trophozoite stages, respectively. This demonstrates potential activity of ferroquine on various stages of growth of *P. vivax*, higher than that of chloroquine and thus a therapeutic advance over chloroquine.

The term "comprising" as used in this specification means "consisting at least in part of". When interpreting each statement in this specification that includes the term "comprising", features other than that or those prefaced by the term may also be present. Related terms such as "comprise" and "comprises" are to be interpreted in the same manner.

2010311223 01 Oct 2015

CLAIMS

1. The use of ferroquine or a pharmaceutically acceptable salt thereof, for treating infections of blood cells caused by *Plasmodium vivax* at the stages of parasitic growth of the immature trophozoite "ring stage" and of the mature tropozoite.

2. The use as claimed in claim 1, wherein the treatment is the treatment of infections in patients deficient in glucose-6-phosphate dehydrogenase.

3. A method for treating infections of blood cells caused by *Plasmodium vivax* at the stages of parasitic growth of the immature trophozoite "ring stage" and of the mature tropozoite, the method comprising administering a therapeutically effective dose of ferroquine or a pharmaceutically acceptable salt thereof to an infected patient.

4. The method of claim 3, wherein the treatment is the treatment of infections in patients deficient in glucose-6-phosphate dehydrogenase.

5. A use as claimed in claim 1 or claim 2, substantially as herein described with reference to any example thereof and with or without reference to the accompanying drawings.

6. A method as claimed in claim 3 or claim 4, substantially as herein described with reference to any example thereof and with or without reference to the accompanying drawings.

FIGURE 1

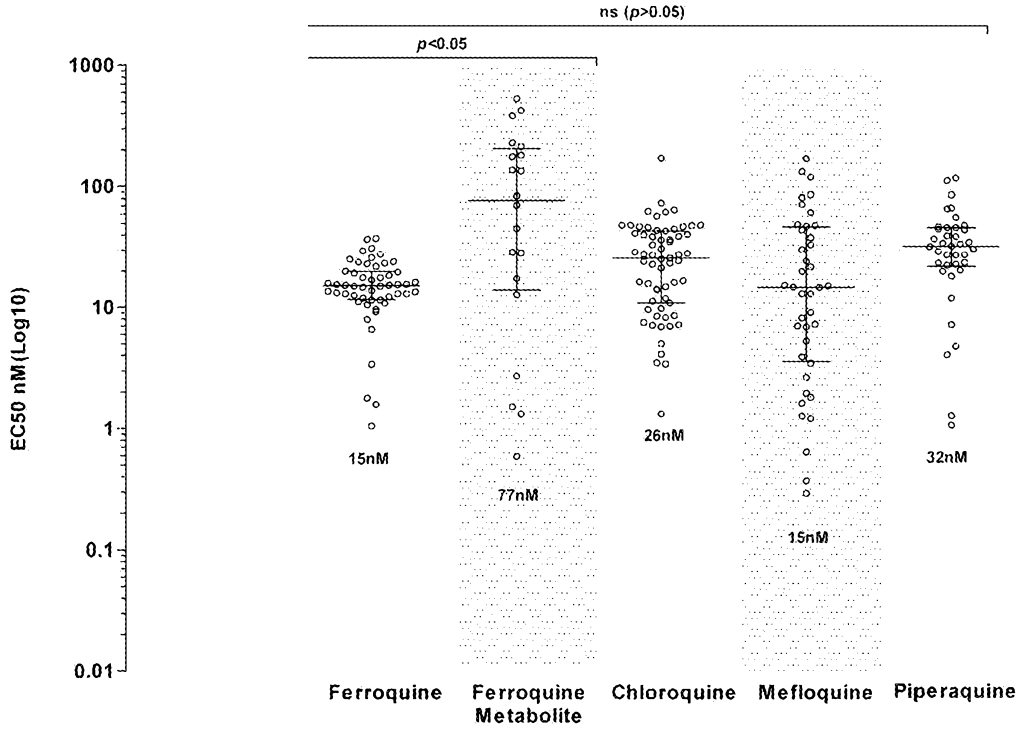


FIGURE 2

