D. No. 2227
E-104/23
E- 105/4/2023

200316337

To,
The Controller of Patents,
The Patent Office, Mumbai

Re: PRE-GRANT OPPOSITION UNDER SECTION 25 (1) AGAINST
Patent Application Number 202221034803 dated 17/06/2022

Mr. T. IYAR ..... **OPPONENT**
124, Anaikkaraipatty,
Madurai, Tamil Nadu – 625536, India

Vs

MAHARAJA KRISHNAKUMARSINHJI BHAVNAGAR UNIVERSITY
an Indian University of
MAHARAJA KRISHNAKUMARSINHJI BHAVNAGAR UNIVERSITY
Gaurishankar Lake Road, Bhavnagar- 364001, Gujarat, INDIA ..... **APPLICANT**

LIST OF DOCUMENTS

Sr. No.	Description	Page No.
1	Form 7A	2
2	Representation u/s 25(1)	3-8
3	Exhibit 1: Complete specification as filed by the Applicant as downloaded from InPass site including Form 1 & 2	9-35
3	Exhibit 2: Govt. Gujarat, The Maharaja KrishnakumarSinhji Bhavnagar University Act, 1978. (Gujarat Act No. 26 of 1978)	36-38
4	Exhibit 3: Birgul Ozden Kasimogullari et al, Fused Heterocycles: Synthesis of Some New Imidazo[1,2-a]-pyridine Derivatives, Molecules 2004, Vol-9, 894-901 (D1)	39-46
5	Exhibit 4: Sandeep Jain et al., Novel arylidene derivatives of quinoline based thiazolidinones: Synthesis, in vitro, in vivo and in silico study as antimalarials, Experimental Parasitology 185 (2018) 107e114	47-54

Iyar T.
Mr. **T. IYAR**
Opponent

To
The Controller of Patents,
The Patent Office, Mumbai

FORM – 7A

THE PATENTS ACT, 1970

(39 OF 1970)

&

THE PATENTS RULES, 2003

REPRESENTATION FOR OPPOSITION TO GRANT OF PATENT

(See section 25 (1) and rule 55)

I, Mr. T. IYAR, 124, Anaikkaraipatty, Madurai, Tamil Nadu – 625536, India, hereby give representation by way of opposition to the grant of patent in respect of Patent Application No. 202221034803 dated 17/06/2022 made by **MAHARAJA KRISHNAKUMARSINHJI BHAVNAGAR UNIVERSITY,** Bhavnagar, Gujarat. It is published under section 11A in the Official Journal of Indian Patent Office dated 22/07/2022.

The impugned Patent Application is opposed on the following grounds:-

Section 25(1)(b): **Lacks novelty;**

Section 25(1)(e): **lacks inventive step;**

Section 25(1)(f): **Not an invention within the meaning of the Indian Patent Act, 1970;**

Section 25(1)(g): **Clarity and Insufficiency**

My address for service in India is:

Mr. **T. IYAR**
124, Anaikkaraipatty,
Madurai, Tamil Nadu – 625536, India
Mob: 9163414641/42
Email: tiyer68@gmail.com

This 26th day of December 2022

Iyar T.
Mr. **T. IYAR**
Opponent

To
The Controller of Patents,
The Patent Office, Mumbai

REPRESENTATION UNDER SECTION 25(1) OF THE PATENTS ACT

I, Mr. T. IYAR, 124, Anaikkaraipatty, Madurai, Tamil Nadu – 625536, India, (hereinafter called 'Opponent') make the following representation under Section 25(1) of the Act in opposing the grant of patent on the application indicated in the cause title.

1. Locus Standi:

Locus standi is not a requirement for filing a pre-grant opposition. The purpose of filling the pre-grant opposition is to assist the Controller of Patents. However, the opponent is a pharmacist and doing research in the field of Medicinal Chemistry.

2. Reason for filling the opposition:

I do not find the merit of the invention filed under the impugned application and in my opinion that the invention as filed does not deserve monopoly for the grounds herein below:

Preliminary observations:

The Applicant has filed the Expedited request (RQ no. E20222027375, 27/07/2022) claiming that the applicant is *an institute established by a Central, Provincial or State Act, which is owned or controlled by the Government* and also, the Applicant has submitted a document *(Govt. of Gujarat, Gujarat Act No. 26 of 1978, as modified up to 31st December, 2017)* as support for the such claim. Such request has been considered by the Indian Patent Office and the Examination was carried out in the expedited mode and the FER was issued on 29/08/2022. It is clear from the document (as support) is effective up to 2017 whereas the date of application is 17/06/2022. It means that the document filed at the time of RQ (dated 27/07/2022) is not valid. Hence, the RQ (E20222027375, 27/07/2022) cannot be taken on record. Therefore whatever the submission and the amendment has been carried out at the time of FER Response (from the Applicant's end) may be disregarded. In view of the above, I being an opponent consider the as filed claims which have been published on 22/07/2022 by the Indian Patent Office and the objection/ground as herein below is in view of as filed claims only.

3. Grounds:

Section 25(1)(b): **lacks novelty;**

Section 25(1)(e): **lacks inventive step**;

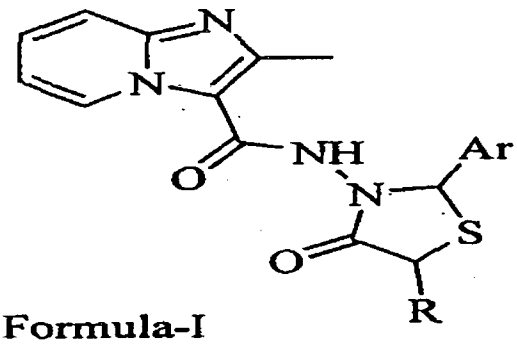
Section 25(1)(f): **Not an invention**;

Section 25(1)(g): **Clarity & Insufficiency**

Novelty:

As filed claims of the impugned application:

1. The Thiazolidin-3-yl-Imidazo-pyridine-3-carboxamide of formula I, or its pharmaceutically, metabolites thereof,



Formula-I

wherein R is H or -CH3, -CH2-COOH.

Aryl/heteroaryl ring is substituted by mono or di-substituents with nitro, halogen, N, N-dimethyl, cinnamyl, methyl and methoxy groups or derivatives, metabolites thereof.

2. The Thiazolidin-3-yl-Imidazo-pyridine-3-carboxamide of formula I as claimed in claim-l is,

   a. *N-(2-(3* -chloropheny l)-4-oxothiazolidin -3 -yl)-2-methylimidazo[1 ,*2-a*]pyridine-3-carboxamide;

   b. N-(2-(3-fluorophenyl)-4-oxothiazolidin-3-yl)-2-methylimidazo[1,2-a]pyridine-3-carboxamide;

   c. *N-(2-(2*-hydroxyphenyl)-4-oxothiazo lidin -3 -Y l)-2-methy limidazo[1 ,*2-a*]pyridine-3-carboxamide;

d. *N-(2-(* 4-hydroxyphenyl)-4-oxothiazolidin-3-yl)-2-methylimidazo[1 ,*2-a*]pyridine-3-carboxamide;

e. *N-(2-(* 4-hydroxy~ 3 -methoxyphenyl)-4-oxothiazolidin-3 -yl)-2-methylimidazo[1 ,2- *a*]pyridine-3-carboxamide;

f. 2-Methyl-N-(4-oxo-2-(o-tolyl)thiazolidin-3-yl)imidazo [1 ,*2-a*]pyridine-3-. carboxamide;

g. 2-Methyl-N-(4-oxo-2-(3,4,5-trimethoxyphenyl)thiazolidin-3-yl)imidazo[1 ,2- *a*]pyridine-3-carboxamide;

h. N-(2-(2-methoxyphenyl)~4-oxothiazolidin-3-yl)-2-methylimidazo[1 ,*2-0*]pyridine- 3-carboxamide;

i. *N-(2-(* 4-methoxyphenyl)-4-oxothiazolidin-3-yl)-2-methylimidazo[1,2-a]pyridine-3-carboxamide;

j. 2-Methyl-N-(2-(3-nitrophenyl)-4-oxothiazolidin-3-yl)imidazo[1,2-a]pyridine-3-carboxamide;

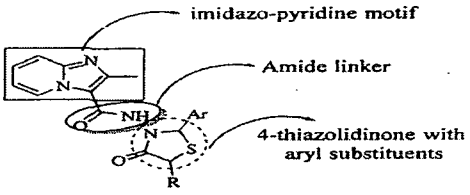
k. 2-*Methyl-N-(2-(* 4-nitrophenyl)-4-oxothiazolidin-3-yl)imidazo[1 ,*2-a*]pyridine-3-carboxamide;

1. 2-Methyl-N-(4-oxo-2-styrylthiazolidin-3-yl)imidazo[1 ,*2-a*]pyridine-3-carboxamide.

3. The one-pot synthesis of Thiazolidin-3-yl-Imidazo-pyridine-3-carboxamide of formula I as claimed in claim-l optimizing the in-process isolation of intermediate compounds of formulae IV, III and II in presence of a catalyst.
4. The one-pot synthesis of compound of formula 1 as claimed in claim 3, wherein the catalyst is ammonium persulphate (APS).
5. Thiazolidin-3-yl-Imidazo-pyridine-3-carboxamide of formula I, or its pharmaceutical salt as claimed in claim 1 for the treatment of malaria.
6. Thiazolidin-3-yl-Imidazo-pyridine-3-carboxamide of formula I, or its pharmaceutical salt as claimed in claim 6, for the treatment of *plasmodium falciparum.*

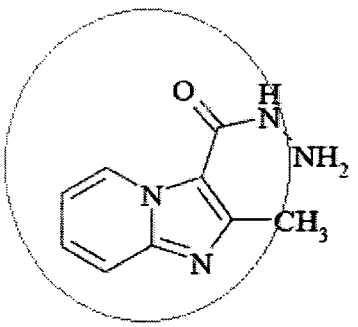
The claimed compound of Formula I as above is the combination of two moieties i.e. imidazo-pyridine and quinoline based 4-thiazolidinones using an amide linker.

I reproduced the phrase as mentioned in Page No. 9 of the impugned application:



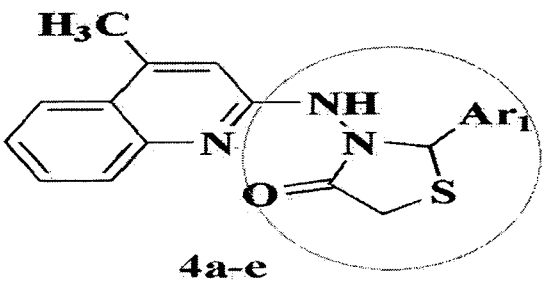
Formula-I
4-thiazolidinone embedded with imidazo-pyridine motif through amide linkage

Compound 2 of D1:



D2 is related to arylidene derivatives of quinoline based **thiazolidinones**: Synthesis, in vitro, in vivo and in silico study as **antimalarials**

Compound of 4a-e of D2:



4a-e

D1 discloses the imidazo-pyridine and D2 discloses quinoline based 4-thiazolidinones. Both the prior art teaches the amide linker. D2 is related to anti-malarial. The field of the invention is medicinal chemistry. Since the impugned invention is related to the field of the medicinal chemistry, combination of the moieties is the logical consideration for a skilled person. Hence, claim 1 is not novel.

Inventive step:

The above finding as stated in the Novelty ground can be applied for assessment of inventive step in the present context. D1 suggests imidazo-pyridine ring and D2 suggests quinoline based 4-thiazolidinones for antimalarial activity. Then it is obvious for a skilled person to combine the teaching of D1 & D2 in order to reach the compound as claimed in claim 1 of the impugned application.

No unexpected effect/surprising feature is observed in the impugned application as filed.

Non-patentable u/s 3(d):

Impugned application talked about the anti-malarial activity of the compound (Table 2, 1a-1l) in view of 'potency'. The 'potency' is different from the "therapeutic efficacy" as the compound patent as India is concerned. The requirement for Section 3(d) is that there must be a study & data thereof which could establish the claimed compound (Formula I) will produce an enhanced or superior efficacy (therapeutic) on molecular basis than what could be achieved with imidazo-pyridine or quinoline based 4-thiazolidinones individually. The impugned application is absolutely failed to disclose such study/data. Hence, Section 3(d) attracts the instant claims.

Insufficiency & clarity:

How to synthesis the compound Thiazolidin-3-yl-Imidazo-pyridine-3-carboxamide of formula I is not clear from the claim as there is no process steps.

RELIEF SOUGHT:

The opponent states that it has established and made out a case on each of the aforesaid grounds of opposition and pray to the Ld. Controller for the following relief(s):

1) Take on record the present representation;
2) Refusal of the application;
3) Forward copy of reply of applicant and evidence if any and any amendments filed;
4) Leave to file a replication to the reply of the applicant and evidence; and
5) Grant of hearing

Date: Dec. 26, 2022

Iyar.T.

Mr. T. IYAR
Opponent

To,
The Controller of Patents,
The Patent Office, Mumbai

FORM 2

THE PATENTS ACT, 1970

(39 OF 1970)

COMPLETE SPECIFICATION

(See section 10 and rule 13)

Thiazolidin-3-yl-Imidazo-pyridine-3-carboxamide as antimalarial agents

Applicant

MAHARAJA KRISHNAKUMARSINHJI BHAVNAGAR UNIVERSITY,

Gaurishankar Lake Road, Bhavnagar – 364002, Gujarat, India

FIELD OF THE INVENTION

The present invention relates to development of new heterocyclic hybrids consisting of Thiazolidin-3-yl-Imidazo-pyridine-3-carboxamide as per Formula-I. More specifically, the present invention relates to strategically designed Thiazolidin-3-yl-Imidazo-pyridine-3-carboxamide of Formula-I as antimalarial agent. Formulas (IV), (III), and (II) are condensed together, optimizing the in-process isolation of intermediate compounds. Further the present invention also relates to novel process for preparing the Thiazolidin-3-yl-Imidazo-pyridine-3-carboxamides thereof. Furthermore, the present invention relates to the chemical composition of heterocyclic hybrids of Formula-I, demonstrating molecular docking to evaluate higher inhibitory potency against *Plasmodium falciparum* thereof.

BACKGROUND OF THE INVENTION

Despite efforts to eradicate the malarial disease in the tropical century, infection remains a major global problem. According to the World Health Organization's most recent global malaria report 2021, there are an estimated 241 million malaria infections and 627,000 malaria deaths in 2020, that equates to 14 million more cases in 2020 than in 2019, and 69000 more deaths. *Plasmodium falciparum and Plasmodium vivax* are the two species spread through the bites of infected female Anopheles mosquitos, with the former being lethal. Malaria is particularly prevalent in Africa, where children under five account for 90% of all deaths. Malaria has a significant financial and socioeconomic impact in countries where it is endemic due to the illness's chronic and severe symptoms. Approximately 25% of the endemic nation's wages are spent on treating malaria, hence reducing the impact of this infection. The financial load on the African continent is projected to be $12 billion per year. In 2020, India had 1.7% of malaria infections and 1.2% of malaria deaths.

Mosquito control strategies (pesticide-treated nets and indoor residual spraying) had been extremely effective, but are currently ineffectual due to increased insecticide resistance. Similarly, first-line drug-based medications, especially Artemisinin combination therapy (ACTs), are at risk (Fernandez-Alvaro, Journal of medicinal chemistry 2016, 59:5587-5603) affected due to the emergence of resistance. Nonetheless, several natural medications, such as Marinoquinoline A-F and Aplidiopsamine A, are accessible to treat malarial illnesses. Hybrid heterocycles have also played an important role in the fight against malarial resistance. Quinine, artemether, lumefantrine, primaquine, doxycycline, atovaquone, and quinidine based compounds have shown significant antimalarial potential (Kumar, Sahil, *J. Enzyme Inhib. Med. Chem.* 2016, 31:173-186).

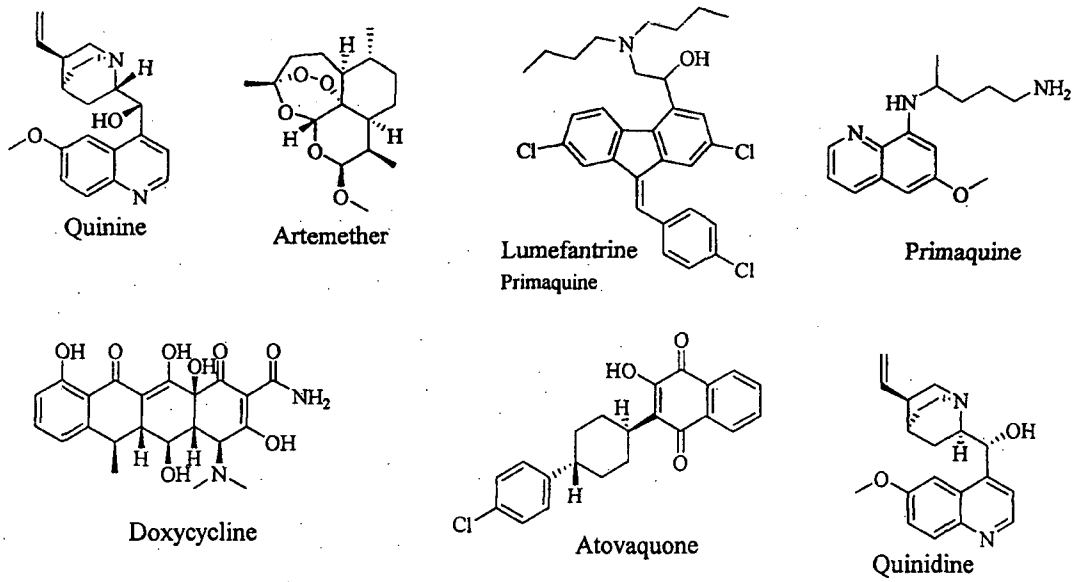
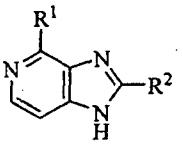


Fig. 1: Structures of commercially available medications used for the treatment of malaria

Nesrin Cesur et al., have synthesized some 2-aryl-3-substituted 4-thiazolidinones and screened for antifungal activity of the compounds (Nesrin Cesur et al, *Arch. Pharm.* (Weinheim) 1994, 327, 271-272.

André Horatscheck et al., have synthesized 2-(3,4-difluorophenyl)-4-(hexahydropyrrolo[1,2

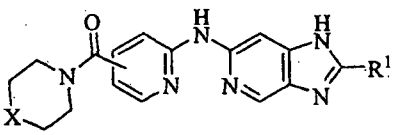
a]pyrazin-2(1*H*)-yl)-1*H*-imidazo[4,5-*c*]pyridines (Formula-2) (André Horatscheck et al., *J. Med. Chem.* 2020, *63* (21), 13013–13030). These reactions were carried out through multi-step reactions and characterized through various spectroscopic techniques. All synthesized compounds were screened for antimalarial activity.



Formula-2

R^1 and R^2 = Different substituents

Claire Le Manach et al., synthesized substituted 1*H*-imidazo[4,5-*c*]pyridin-6yl)amino)pyridin 4-yl)(piperidin-1-yl)methanones (Formula-3) (Claire Le Manach et al. *J. Med. Chem.* 2018, *61*(20), 9371-9385). The reaction was carried out via multi-step and characterized through various analytical tools. The *in-vitro* screening of antimalarial activity was carried out against *P. falciparum*.

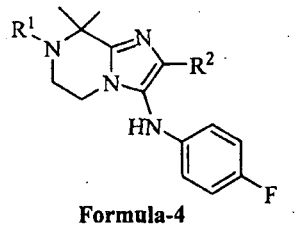


Formula-3

R^1 = *Various derivatives*

X = NR, CR'R", O, SO_2

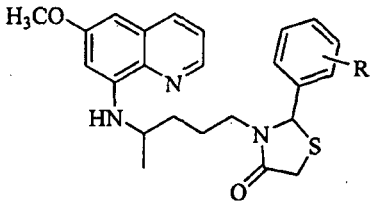
Tao Wu et al., have synthesized some novel *N*,2-bis(4-fluorophenyl)-5,6,7,8-tetrahydroimidazo[1,2-a] pyrazin-3-amines (Formula-4) (Tao Wu et al. *J. Med. Chem.* 2011, *54*, 5116-5130) via multi-step reactions and characterized through various analytical techniques. All the synthesized substituents were evaluated for their *in-vivo* antimalarial activity.



Formula-4

R^1, R^2 = Various substituents

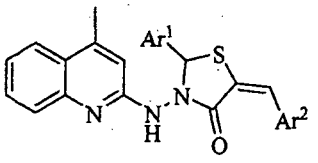
Anna Caroline C. Aguiar et al., have synthesized 3-(4-((6-methoxyquinolin-8-yl)amino)pentyl)-2-phenylthiazolidin-4-ones (Formula-5) (Anna Caroline C. Aguiar et al. *Malar. J.* 2017, *16* (110), 1–11) via one-pot synthesis. The synthesized compounds were evaluated for their *in-vitro* and *in-vivo* antimalarial activity against *P. vivax.*



Formula-5

R = Various substituents

Sandeep Jain et al. have synthesized aryl-3-(4-methylquinolin-2-ylamino)-2-phenylthiazolidin-4-ones (Formula-6) (Sandeep Jain et al. *Exp. Parasitol.* 2018, *185*, 107–114) and characterized with various spectroscopic techniques. The synthesized derivatives were evaluated for *in-vitro*, *in-vivo* and *in-silico* study as antimalarials.



Formula-6

Ar^1, Ar^2 = Various substituents

Researchers synthesized imidazo-pyridine (formulas 2, 3 and 4) and quinoline based 4-thiazolidinones (formulae 5 and 6) and tested them for antimalarial activity, which inspired us to develop hybrids of imidazo-pyridine and 4-thiazolidinones.

Still there is a need to offer a compound showing higher potency as antimalarial agents. The inventors have approached to strategically design heterocyclic hybrids Thiazolidin-3-yl-Imidazo-pyridine-3-carboxamide to develop as antimalarial agents. The said compounds are based on the fusion of two different pharmacophores i.e. imidazo-pyridine and 4-thiazolidinones which is promising to demonstrate the improved molecular docking to offer a better inhibitory potency against malaria specifically the *P. falciparum* thereof

OBJECTIVES OF THE INVENTION

The main objective of the present invention is to design and synthesize novel nitrogen and sulfur-containing heterocyclic hybrids, Thiazolidin-3-yl-Imidazo-pyridine-3-carboxamide of formula I.

Another objective of the invention is to disclose a novel process for preparing nitrogen and sulfur-containing heterocyclic hybrids, Thiazolidin-3-yl-Imidazo-pyridine-3-carboxamide of formula I optimizing the in-process isolation of intermediate compounds.

Yet another objective of the invention is to treat malaria using a method of treatment involving novel nitrogen and sulfur-containing heterocyclic hybrids, Thiazolidin-3-yl-Imidazo-pyridine-3-carboxamide of formula I.

Yet another objective of the invention is treating *P. falciparum* using a method of treatment involving novel nitrogen and sulfur-containing heterocyclic hybrids, Thiazolidin-3-yl-Imidazo-pyridine-3-carboxamide of formula I.

BRIEF DESCRIPTION OF THE DRAWINGS

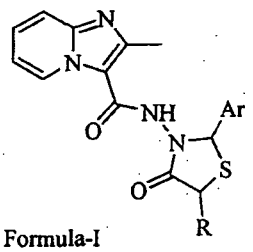
Fig. 1 Graphical Biological Results of Thiazolidin-3-yl-Imidazo-pyridine-3-carboxamide (Give the graphical results at the end of specification)

SUMMARY OF THE INVENTION

The quinine, artemether, lumefantrine, primaquine, doxycycline, atovaquone, and quinidine used to treat malaria. The present invention relates to a novel hybrid heterocyclic of Thiazolidin-3-yl-Imidazo-pyridine-3-carboxamide compounds amalgamating two separate hetero moieties of imdazo-pyridine and 4-thiazolidine, wherein both hydrides are connected with amide linker strategically designed to yield the novel compound showing higher potency as antimalarial agent.

The main embodiment of the present invention is to design and synthesize a novel nitrogen and sulfur-containing heterocyclic hybrids of 2-methyl-*N*-(4-oxo-2-arylthiazolidin-3-yl)imidazo[1,2-*a*]pyridine-3-carboxamide (Formula-I).

Another embodiment of the invention is to disclose a novel process for preparing nitrogen and sulfur-containing heterocyclic hybrids of the Formula-I.

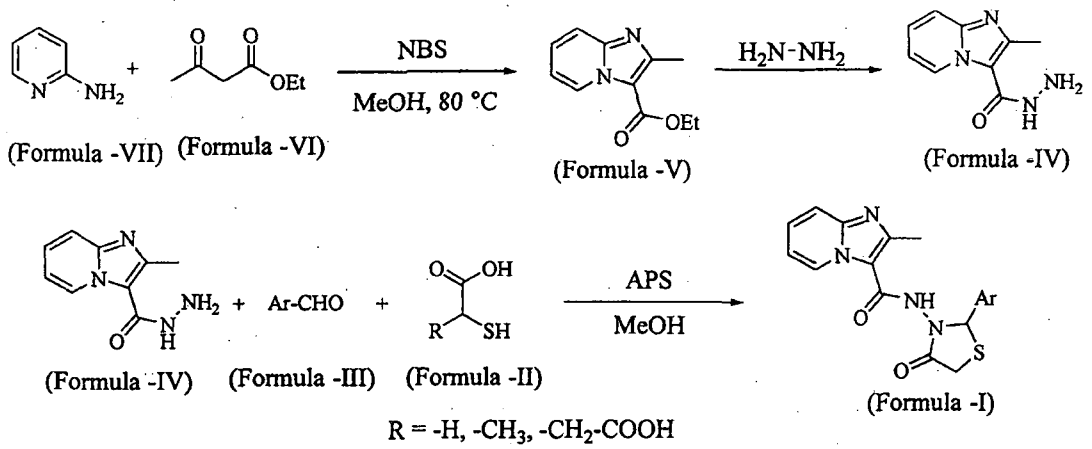


Formula-I

wherein,

R = H, $-CH_3$ and $-CH_2$-COOH and Ar is aryl/heteroaryl ring is substituted by mono or di-substituents with nitroaryl, halogen, *N,N*-dimethyl, cinnamyl, methyl and methoxy and like with various electron-withdrawing and electron-donating groups.

Yet another embodiment of the present invention provides a new process for the preparation of stable imidazo-pyridine clubbed with 4-thiazolidinone derivatives of Formula-I or pharmaceutically acceptable salts thereof following step as depicted in Scheme 1:



Scheme-1

In another embodiment, the invention relates to the use a novel imidazo-pyridine bearing 4-thiazolidinone moiety, to target multiple pathways associated with antimalarial diseases.

DETAILED DESCRIPTION OF THE INVENTION

This invention provides the design and development of novel antimalarial compounds demonstrating different structural configuration and optimizing their activity as well compared to the currently available drugs in the market i.e. chloroquine, hydroxychloroquine, quinine sulphate, primaquine, and mefloquine. These drugs are based on quinoline-containing heterocyclic compounds. Currently, malarial parasites have developed resistance to these drugs. In addition, the inventors have strategically designed and developed compound of formula (I), which contains a completely novel approach in designing of structure of a synthetic hybrid of two distinct pharmacophores through an amide linker, which will play a key role in the attachment of two heterocyclic moieties. Furthermore, we have developed a one-pot synthesis in the present invention optimizing the in-process isolation of intermediate compounds wherein which formulas (IV), (III), and (II) are condensed together, reducing the expense of intermediate isolation and also eliminating the yield loss during the process. The

present invention relates to one-pot synthesis optimizing the in-process the isolation of intermediate steps to yield compound of Formula (I).

The present invention provides a strategically designed novel approach in structural diversity of heterocyclic hybrids consisting of Thiazolidin-3-yl-Imidazo-pyridine-3-carboxamide formula (I) to combat the resistance of malarial species.

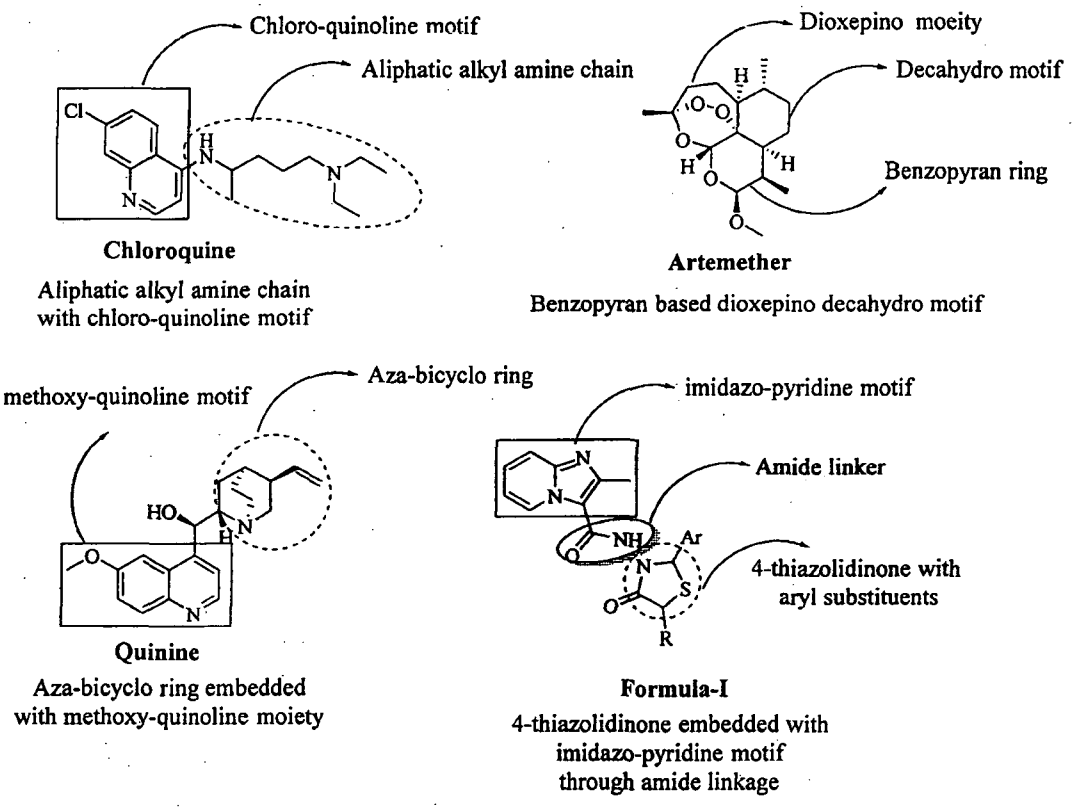
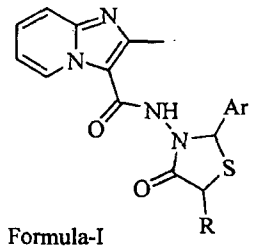


Fig. 2 Novel approach to strategically design the structural hybrid of Thiazolidin-3-yl-Imidazo-pyridine-3-carboxamide

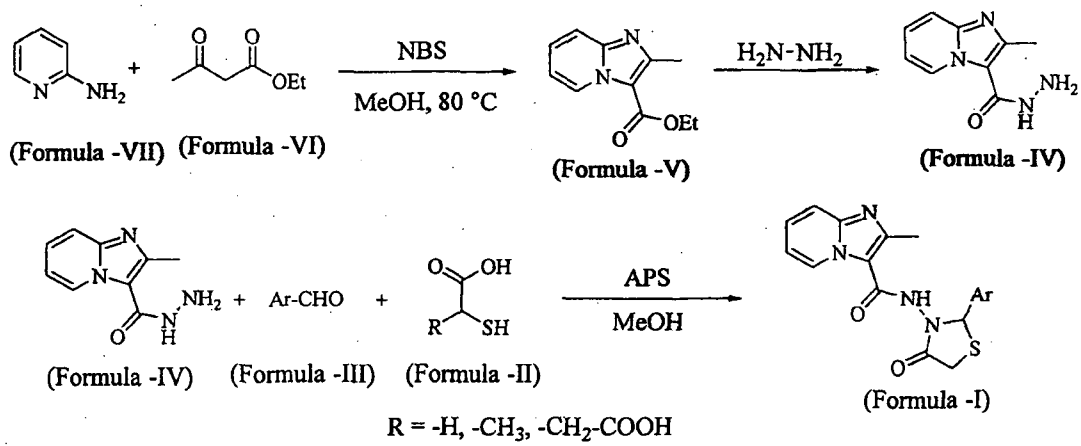
The present invention relates to a series of novel Thiazolidin-3-yl-Imidazo-pyridine-3-carboxamide scaffolds having antimalarial activity. The said compounds are screened to study the antimalarial potency against *P. falciparum*.



Formula-I

The -Ar in compound of formula 1 is aryl / heteroaryl ring which is specifically consisting of a substituted mono or di-substituents aryl / heteroaryl. Further the said aryl/heteroaryl is consisting of nitroaryl, halogen, *N,N*-dimethyl, cinnamyl, methyl and methoxy.

Further the present invention also relates to novel process for preparing the Thiazolidin-3-yl-Imidazo-pyridine-3-carboxamides thereof as per the process depicted in Scheme 1.



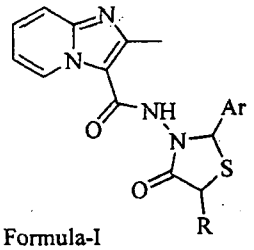
General Formula-I was obtained by the reaction of Formula-IV, substituted aromatic aldehydes (Formula-III), thioglycolic acid (Formula-II) and catalytic amount of ammonium persulphate (APS) in methanol, wherein Ar is defined as per Formula (I):

Furthermore, the present invention relates to the chemical composition of heterocyclic hybrids of Formula-I, demonstrating molecular docking to evaluate higher inhibitory potency against *P. falciparum* thereof.

All the synthesized compounds were screened for antimalarial activity in the Microcare laboratory & Tuberculosis Research Centre, Surat, Gujarat.

The *in-vitro* antimalarial assay was carried out according to the micro assay protocol of Rieckmann and co-workers with minor modifications (Rieckmann et al., *Lancet* 1978, *1*, 221-223). The cultures of *P. falciparum* 3D7 strain were maintained in medium RPMI 1640 supplemented with 25 mM HEPES, 1% D-glucose, 0.23% sodium bicarbonate and 10% heat inactivated human serum. The asynchronous parasites of *P. falciparum* were synchronized after 5% D-sorbitol treatment to obtain only the ring stage parasitized cells. The procedure is given as per the reference and the slides were microscopically observed to record maturation of ring stage parasites into trophozoites and schizonts in presence of different concentrations of the test agents. The test concentration which inhibited the complete maturation into schizonts was recorded as the minimum inhibitory concentrations (MIC). Quinine was used as the reference drug (positive control).

The invention relates to the development of antimalarial drugs based on completely new structures, such as the insertion of thaizolidinone to the 3rd position of imidazo-pyridine, as well as the integration of an amide linker. The amide linker, together with pharmacophores like thaizolidineone and imidazo-pyridine, will play a significant role in the establishment of antimalarial drugs in our innovation. For the first time we have develop the reported formula (I). Until now, no such structures are reported for the development of antimalarial drugs. In accordance with the objective of present invention to provide novel 2-methyl-*N*-(4-oxo-2-arylthiazolidin-3-yl)imidazo[1,2-*a*]pyridine-3-carboxamide (Formula-I).

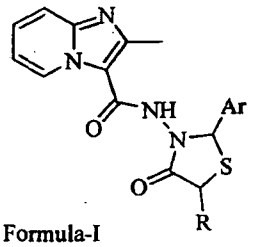


Formula-I

wherein,

Ar is defined as various derivatives of aromatic compounds as mentioned in the Table-1, metabolites thereof. Formula-I. or pharmaceutically acceptable salts, derivatives, metabolites thereof.

The specific compound of Formula-1 synthesized in accordance with the present invention is further elaborated here.



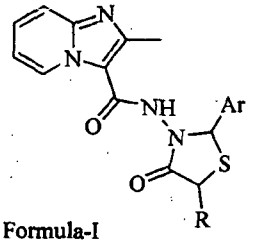
Formula-I

Table-1

Compounds	-Ar	-R
1 a	-3-Cl-C_6H_4	-H
1 b	-3-F-C_6H_4	-H
1 c	-2-OH-C_6H_4	-H
1 d	-4-OH-C_6H_4	-H
1 e	-3-OCH_3-4-OH-C_6H_3	-H
1 f	-2-CH_3-C_6H_4	-H
1 g	-2,3,4-$(OCH_3)_3$-C_6H_2	-H
1 h	-2-OCH_3-C_6H_4	-H
1 i	-3-OCH_3-C_6H_4	-H

1 j	-3-NO_2-C_6H_4	-H
1 k	-4-NO_2-C_6H_4	-H
1 l	-CH=CH-C_6H_5 / -C_8H_7	-H

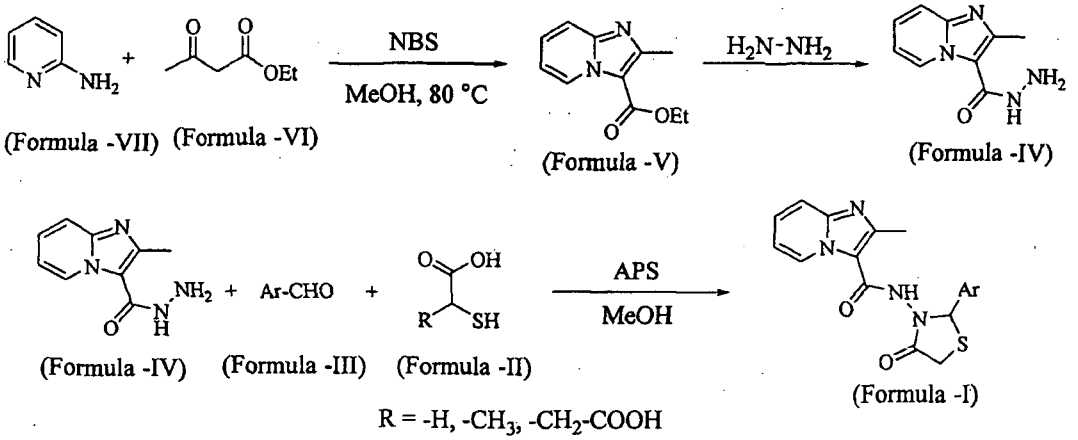
In one exemplary embodiment, wherein Formula-I is further defined as



Formula-I

wherein -R is either -H or $-CH_3$ or $-CH_2$-COOH. Aryl/heteroaryl ring is substituted by mono or di-substituents with nitro, halogen, *N,N*-dimethyl, cinnamyl, methyl and methoxy groups or pharmaceutically acceptable salts, derivatives, metabolites thereof.

The desirable product of formula (I) is obtained by the following one pot synthesis

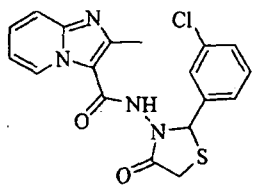


(Formula -VII) (Formula -VI) (Formula -V) (Formula -IV)

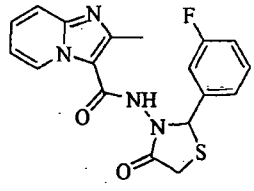
(Formula -IV) (Formula -III) (Formula -II) (Formula -I)

R = -H, -CH_3, -CH_2-COOH

General Formula-I was obtained by the reaction of Formula-IV, substituted aromatic aldehydes (Formula-III), thioglycolic acid (Formula-II) and catalytic amount of ammonium persulphate (APS) in methanol, wherein Ar is defined as per Formula (I):



1a



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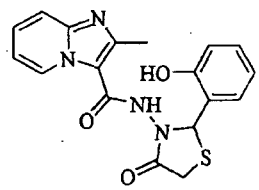
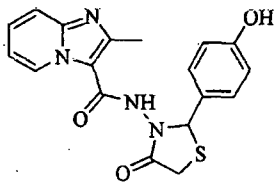
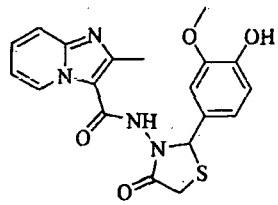


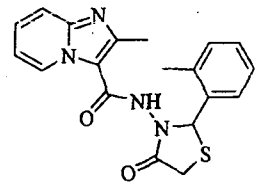
1c



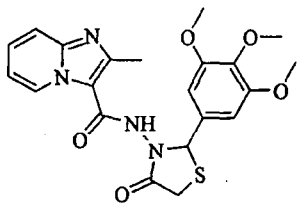
1d



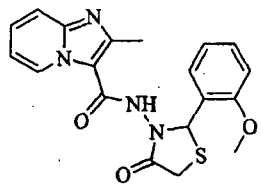
1e



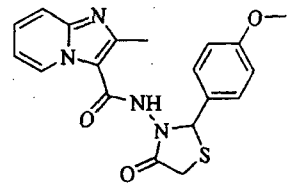
1f



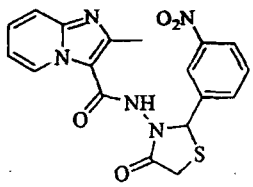
1g



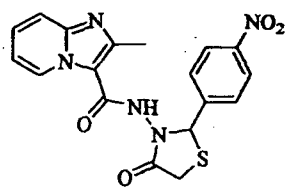
1h



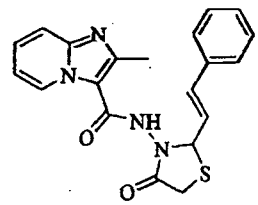
1i



1j



1k



1l

The compounds of Formula-I obtained as above are further purified by using alcohol (95%). The alcohol used for the purification of general compound of formula-I is selected but not limited to C1-C4 Alcohol namely methanol, ethanol, propanol, butanol or mixture of thereof.

The term "alkyl", is referred to C1-C3 alkyl such as $-CH_3$ or $-CH_2$-COOH

The term aryl is selected alone or in combination with aryl/heteroaryl ring is substituted by mono or di-substituents with nitro, halogen, *N,N*-dimethyl, cinnamyl, methyl and methoxy,

includes such aromatic radicals as phenyl, biphenyl, and benzyl, as well as fused aryl radicals such as naphthyl, anthryl, phenanthrenyl, fluorenyl, and indenyl and so forth.

The term "aryl" refers to an aromatic group for example, which is a 6 to 10 membered monocyclic or bicyclic ring system, which may be unsubstituted or substituted. Representative aryl groups may be phenyl, naphthyl etc. When said ring is substituted, the substituents are selected from halogen (e.g., F, Cl, Br, I), hydroxy, alkoxy, nitro.

The term "alkylaryl" or "arylalkyl" refers to alkyl-substituted aryl groups such as butylphenyl, propylphenyl, ethylphenyl, methylphenyl, 3,5-dimethylphenyl, *tert*-butylphenyl and so forth. The term "Haloaryl" refers to aryl radicals in which one or more substitutable positions has been substituted with a halo radical, examples include 4-fluorophenyl, 4- chlorophenyl, 4-bromophenyl and so forth.

The term "halogen" or "Halide" refers to fluorine, chlorine, bromine and iodine. Also included in the family of compounds of Formula-I and the pharmaceutically acceptable salts thereof. The phrase "pharmaceutically acceptable salts" connotes salts commonly used to form alkali metal salts and to form addition salts of free acids or free bases. The nature of the salt is not critical, provided that it is pharmaceutically acceptable. Suitable pharmaceutically acceptable acid addition salts of compounds of Formula-I may be prepared from an "acid" wherein the acid is selected from inorganic acid or from an organic acid. Examples of such "inorganic acids" are hydrochloric, hydrobromic, hydroiodic, nitric, carbonic, sulfuric, and phosphoric acid.

The following specific examples will be used to best describe our invention. These examples are provided to show the many specific and preferred embodiments and approaches in further detail. However, it should be noted that numerous alterations and modifications can be accomplished while remaining within the scope of the present invention. The Formula-I is

characterized by IR, ^{1}H NMR, ^{13}C NMR, and Mass spectroscopy. IR spectra revealed that the presence of the amine group was confirmed at 3190 cm^{-1}. The frequency at 1658 cm^{-1} and 1593 cm^{-1} confirmed carbonyl and cyano groups. Moreover, the presence of nitro group and C-S was validated at 1550 cm^{-1} and 686 cm^{-1}. The protons present in the claimed structure were confirmed by proton NMR. Peaks appeared at 2.58 ppm and 2.95-2.98 ppm showing the presence of methyl group and methylene group of 4-thiazolidinone moiety. Furthermore, methine proton of 4-thiazolidinone and amine was confirmed at 7.05 ppm and 11.52 ppm. The carbon skeleton of the claimed compound is also characterized by ^{13}C NMR spectroscopy. Peaks appeared at 16.3 ppm and 168.6 ppm revealed the presence of methyl group and carbonyl group while peaks appeared at 72.6 ppm and 174.1 ppm confirmed the methine carbon and the carbonyl group of 4-thiazolidinone. The mass spectra of the compound are in accordance with the claimed structure.

Example-1: Ethyl 2-methylimidazo[1,2-*a*]pyridine-3-carboxylate was prepared according to the literature method (Bhagat et al., *Tetrahedron Lett.* 2017, 37:3662-3666), (Formula VI).

Ethyl acetoacetate (1.05 mmol) reacted with N-bromo succinamide (1.2 mmol) in methanol (5 mL) at 80 °C for 30 min, followed by the addition of 2-aminopyridine (1.0 mmol) and heated at same temperature for another 30 min. Reaction mixture was cooled to room temperature and product generated was recrystallized from ethanol (95%). Yield: 56%; Solid; M.P. 66-68°C.

Example-2: Procedure for the synthesis of 2-methylimidazo[1,2-*a*]pyridine-3-carbohydrazide, (Formula V).

To a solution of formula V (1.0 mmol) in methanol (5 mL), hydrazine hydrate (20.0 mmol) was added and refluxed it for 2 h. Reaction mixture was then stirred for 15 minutes at room

temperature to furnish crystals of desired product. The completion of reaction was checked by TLC [n-hexane/ethyl acetate (V/V=3:2)]. Yield: 74%; Solid; M.P. 166-168°C.

Example-3: 2-Methyl-*N*-(4-oxo-2-arylthiazolidin-3-yl)imidazo[1,2-*a*]pyridine-3-carboxamide was prepared according to the literature method (Ebrahimi, *J. . Sulphur Chem.* 2016, 37:587-592), (Formula IV).

A mixture of formula IV (1 mmol), aromatic aldehydes (1 mmol), thioglycolic acid and APS (10 mol%) in methanol (10 mL) was refluxed for 60 min. The product formed was filtered off and washed with water and recrystallized from ethanol. The completion of the reaction was checked by TLC [n-hexane/ethyl acetate (V/V=1:4)].

Example-4: *N*-(2-(3-chlorophenyl)-4-oxothiazolidin-3-yl)-2-methylimidazo[1,2-*a*]pyridine-3-carboxamide **[1 a]**

A mixture of formula IV (1 mmol), 3-chlorobenzaldehyde (1 mmol), thioglycolic acid and APS (10 mol%) in methanol (10 mL) was refluxed for 60 min. Then do the further process as disclosed in example-3. Yield 66%; solid; M.P. 200-203 °C.

Example-5: *N*-(2-(3-fluorophenyl)-4-oxothiazolidin-3-yl)-2-methylimidazo[1,2-*a*]pyridine-3-carboxamide **[1 b]**

A mixture of formula IV (1 mmol), 3-fluorobenzaldehyde (1 mmol), thioglycolic acid and APS (10 mol%) in methanol (10 mL) was refluxed for 60 min. Then do the further process as disclosed in example-3. Yield 46%; solid; M.P. 172-176 °C.

Example-6: *N*-(2-(2-hydroxyphenyl)-4-oxothiazolidin-3-yl)-2-methylimidazo[1,2-*a*]pyridine-3-carboxamide **[1 c]**

A mixture of formula IV (1 mmol), 2-hydroxybenzaldehyde (1 mmol), thioglycolic acid and APS (10 mol%) in methanol (10 mL) was refluxed for 60 min. Then do the further process as disclosed in example-3. Yield 52%; solid; M.P. 182-186 °C.

Example-7: *N*-(2-(4-hydroxyphenyl)-4-oxothiazolidin-3-yl)-2-methylimidazo[1,2-*a*]pyridine-3-carboxamide **[1 d]**

A mixture of formula IV (1 mmol), 4-hydroxybenzaldehyde (1 mmol), thioglycolic acid and APS (10 mol%) in methanol (10 mL) was refluxed for 60 min. Then do the further process as disclosed in example-3. Yield 54%; solid; M.P. 203-206 °C.

Example-8: *N*-(2-(4-hydroxy-3-methoxyphenyl)-4-oxothiazolidin-3-yl)-2-methylimidazo[1,2-*a*]pyridine-3-carboxamide **[1 e]**

A mixture of formula IV (1 mmol), 4-hydroxy-3-methoxybenzaldehyde (1 mmol), thioglycolic acid and APS (10 mol%) in methanol (10 mL) was refluxed for 60 min. Then do the further process as disclosed in example-3. Yield 78%; solid; M.P. 167-170 °C.

Example-9: 2-methyl-*N*-(4-oxo-2-(*o*-tolyl)thiazolidin-3-yl)imidazo[1,2-*a*]pyridine-3-carboxamide **[1 f]**

A mixture of formula IV (1 mmol), 2-methylbenzaldehyde (1 mmol), thioglycolic acid and APS (10 mol%) in methanol (10 mL) was refluxed for 60 min. Then do the further process as disclosed in example-3. Yield 70%; solid; M.P. 184-187 °C.

Example-10: 2-methyl-*N*-(4-oxo-2-(3,4,5-trimethoxyphenyl)thiazolidin-3-yl)imidazo[1,2-*a*]pyridine-3-carboxamide **[1 g]**

A mixture of formula IV (1 mmol), 3,4,5-trimethoxybenzaldehyde (1 mmol), thioglycolic acid and APS (10 mol%) in methanol (10 mL) was refluxed for 60 min. Then do the further process as disclosed in example-3. Yield 80%; solid; M.P. 215-219 °C.

Example-11: *N*-(2-(2-methoxyphenyl)-4-oxothiazolidin-3-yl)-2-methylimidazo[1,2-*a*]pyridine-3-carboxamide **[1 h]**

A mixture of formula IV (1 mmol), 2-methoxybenzaldehyde (1 mmol), thioglycolic acid and APS (10 mol%) in methanol (10 mL) was refluxed for 60 min. Then do the further process as disclosed in example-3. Yield 37%; solid; M.P. 207-210 °C.

Example-12: *N*-(2-(3-methoxyphenyl)-4-oxothiazolidin-3-yl)-2-methylimidazo[1,2-*a*]pyridine-3-carboxamide **[1 i]**

A mixture of formula IV (1 mmol), 3-methoxybenzaldehyde (1 mmol), thioglycolic acid and APS (10 mol%) in methanol (10 mL) was refluxed for 60 min. Then do the further process as disclosed in example-3. Yield 61%; solid; M.P. 194-197 °C.

Example-13: 2-methyl-*N*-(2-(3-nitrophenyl)-4-oxothiazolidin-3-yl)imidazo[1,2-*a*]pyridine-3-carboxamide **[1 j]**

A mixture of formula IV (1 mmol), 3-nitrobenzaldehyde (1 mmol), thioglycolic acid and APS (10 mol%) in methanol (10 mL) was refluxed for 60 min. Then do the further process as disclosed in example-3. Yield 89%; solid; M.P. 223-226 °C.

Example-14: 2-methyl-*N*-(2-(4-nitrophenyl)-4-oxothiazolidin-3-yl)imidazo[1,2-*a*]pyridine-3-carboxamide **[1 k]**

A mixture of formula IV (1 mmol), 4-nitrobenzaldehyde (1 mmol), thioglycolic acid and APS (10 mol%) in methanol (10 mL) was refluxed for 60 min. Then do the further process as disclosed in example-3. Yield 84%; solid; M.P. 220-224 °C.

Example-15: 2-methyl-*N*-(4-oxo-2-styrylthiazolidin-3-yl)imidazo[1,2-*a*]pyridine-3-carboxamide **[1 l]**

A mixture of formula IV (1 mmol), cinnamaldehyde (1 mmol), thioglycolic acid and APS (10 mol%) in methanol (10 mL) was refluxed for 60 min. Then do the further process as disclose in example-3. Yield 80%; solid; M.P. 210-213 °C.

Antimalarial activity

All the synthesized compounds were screened for antimalarial activity in the Microcare laboratory & Tuberculosis Research Centre. The *in-vitro* antimalarial assay was carried out in 96 well microtiter plates according to the micro assay protocol of Rieckmann and co-workers with minor modifications (Rieckmann et al., Lancet 1978, *1*, 221-223). The cultures of *P. falciparum* 3D7 strain was maintained in medium RPMI 1640 supplemented with 25 mM HEPES, 1% D-glucose, 0.23% sodium bicarbonate and 10% heat inactivated human serum. The asynchronous parasites of *P. falciparum* were synchronized after 5% D-sorbitol treatment to obtain only the ring stage parasitized cells. For carrying out the assay, an initial ring stage parasitaemia of 0.8 to 1.5% at 3% haematocrit in a total volume of 200 µl of medium RPMI-1640 was determined by Jaswant Singh Bhattacharya (JSB) staining to assess the percent parasitaemia (rings) and uniformly maintained with 50% RBCs (O+). A stock solution of 5 mg/ml of each of the test samples was prepared in DMSO and subsequent dilutions were prepared with culture medium. The diluted samples in 20 µl volume were added to the test wells so as to obtain final concentrations (at five-fold dilutions) ranging between 0.4 µg/ml to 100 µg/ml in duplicate well containing parasitized cell preparation. The culture plates were incubated at 37 °C in a candle jar. After 36 to 40 h incubation, thin blood smears from each well were prepared and stained with JSB stain. The slides were microscopically observed to record maturation of ring stage parasites into trophozoites and schizonts in presence of different concentrations of the test agents. The test concentration which inhibited the complete maturation into schizonts was recorded as the minimum inhibitory concentrations (MIC). Quinine was used as the reference drug (positive control).

Structure activity relationship (SAR) and Biological screening of compound of Formulae I as per present invention:

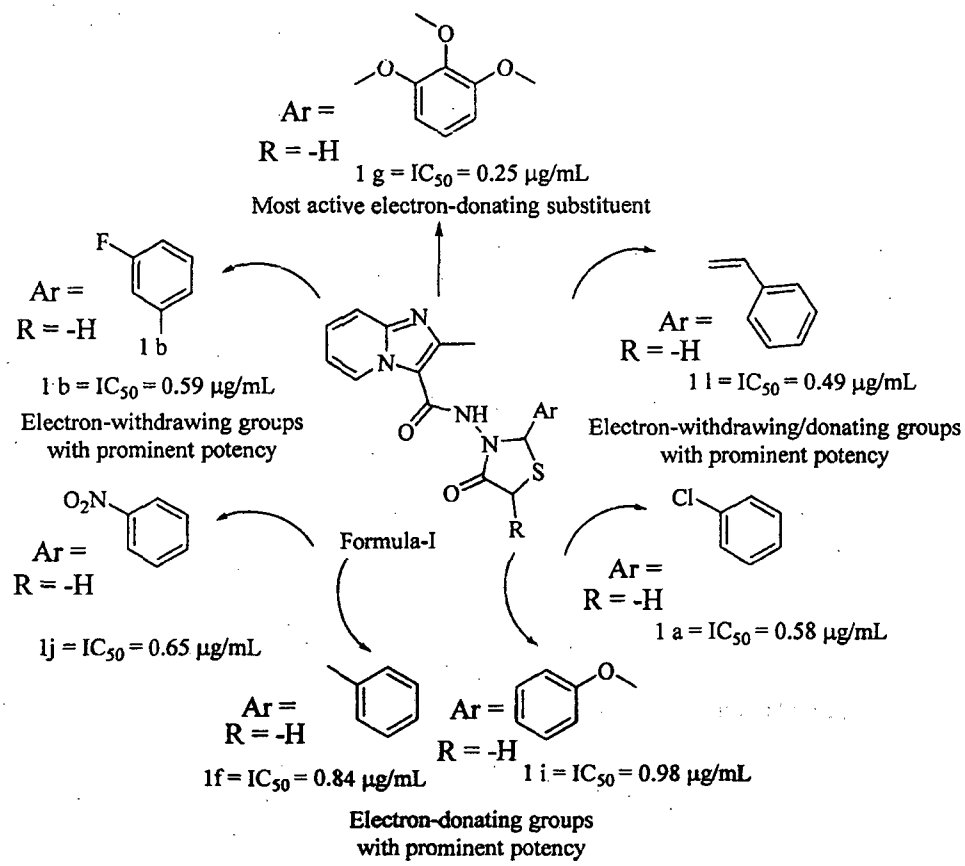


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Fig. 3: Schematic representation of innovation in the of structure-activity relationship based on Formula (I)

In this invention, we synthesized imidazo-pyridine hybrids bearing 4-thiazolidinone motif. To evaluate the structure activity relationship (SAR) of the synthesized hybrids, several electron-withdrawing and electron-donating functional groups were incorporated into the hybrid entity. The difference in antimalarial activity was mediated by the substitution pattern and electronic nature of the synthesized hybrids. Results of the antimalarial evaluation suggested that compound **1 g** was found to be the most active (MEAN IC_{50} = 0.25 µg/mL) against

P. falciparum. Compound **1 l** exhibited better potency than the standard drug quinine (MEAN IC_{50} = 0.26 µg/mL). Furthermore, compound 1 a demonstrated promising antimalarial activity. Compounds **1 b** showed a high level of inhibition against a malarial strain. The electron-withdrawing -NO_2 group is present at 3rd position, displayed better activity. Regardless, hybrids **1 f** and **1 I** was shown to be significant against the malarial pathogen. Furthermore, substituents **1 c, 1 d, 1 e,** and **1 k** showed high to moderate inhibitory potential. The remaining substituents were moderately effective against *P. falciparum*. It can be stated from the results that electron-donating methoxy group had enhanced the antimalarial potency of the synthesized entity.

Table-2: Results of the antimalarial activity (*Plasmodium falciparum 3D7*)

Sr No.	Compound or Formula (I), ID	-R	-Ar	Mean IC50, (µg/mL)
1	1a	-H	-3-Cl-C_6H_4	0.58 µg/mL
2	1b	-H	-3-F-C_6H_4	0.59 µg/mL
3	1c	-H	-2-OH-C_6H_4	1.30 µg/mL
4	1d	-H	-4-OH-C_6H_4	1.08 µg/mL
5	1e	-H	-3-OCH_3-4-OH-C_6H_3	1.43 µg/mL
6	1f	-H	-2-CH_3-C_6H_4	0.84 µg/mL
7	1g	-H	-2,3,4-$(OCH_3)_3$-C_6H_2	0.25 µg/mL
8	1h	-H	-2-OCH_3-C_6H_4	2.56 µg/mL
9	1i	-H	-3-OCH_3-C_6H_4	0.98 µg/mL
10	1j	-H	-3-NO_2-C_6H_4	0.65 µg/mL
11	1k	-H	-4-NO_2-C_6H_4	1.23 µg/mL
12	1l	-H	-CH=CH-C_6H_5 / -C_8H_7	0.49 µg/mL
Standard Drug: Quinine				0.268 µg/mL

In accordance with the present invention which offers novel approach in design and synthesize of a nitrogen and sulfur-containing heterocyclic hybrids consisting of Thiazolidin-

3-yl-Imidazo-pyridine-3-carboxamide of formula I that are synthesized using novel approach of one-pot synthesis optimizing the in-process isolation of intermediate compounds. The synthesized compounds are having commercial potential to offer potency to treat malaria specifically *P. falciparum*.

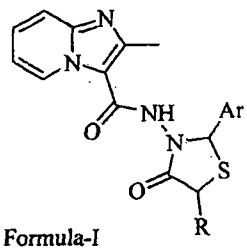
For, M K Bhavnagar University, Bhavnagar,

Date: 9th June, 2022

Prof. Nisheeth C. Desai

We claim,

1. The Thiazolidin-3-yl-Imidazo-pyridine-3-carboxamide of formula I, or its pharmaceutically acceptable salt, metabolites thereof,



Formula-I

wherein R is H or $-CH_3$, $-CH_2-COOH$.

Aryl/heteroaryl ring is substituted by mono or di-substituents with nitro, halogen, *N,N*-dimethyl, cinnamyl, methyl and methoxy groups or pharmaceutically acceptable salts, derivatives, metabolites thereof.

2. The Thiazolidin-3-yl-Imidazo-pyridine-3-carboxamide of formula I as claimed in claim-1 is,

   a. *N*-(2-(3-chlorophenyl)-4-oxothiazolidin-3-yl)-2-methylimidazo[1,2-*a*]pyridine-3-carboxamide

   b. *N*-(2-(3-fluorophenyl)-4-oxothiazolidin-3-yl)-2-methylimidazo[1,2-*a*]pyridine-3-carboxamide

   c. *N*-(2-(2-hydroxyphenyl)-4-oxothiazolidin-3-yl)-2-methylimidazo[1,2-*a*]pyridine-3-carboxamide

   d. *N*-(2-(4-hydroxyphenyl)-4-oxothiazolidin-3-yl)-2-methylimidazo[1,2-*a*]pyridine-3-carboxamide

   e. *N*-(2-(4-hydroxy-3-methoxyphenyl)-4-oxothiazolidin-3-yl)-2-methylimidazo[1,2-*a*]pyridine-3-carboxamide

f. 2-Methyl-*N*-(4-oxo-2-(*o*-tolyl)thiazolidin-3-yl)imidazo[1,2-*a*]pyridine-3-carboxamide

g. 2-Methyl-*N*-(4-oxo-2-(3,4,5-trimethoxyphenyl)thiazolidin-3-yl)imidazo[1,2-*a*]pyridine-3-carboxamide

h. *N*-(2-(2-methoxyphenyl)-4-oxothiazolidin-3-yl)-2-methylimidazo[1,2-*a*]pyridine-3-carboxamide

i. *N*-(2-(4-methoxyphenyl)-4-oxothiazolidin-3-yl)-2-methylimidazo[1,2-*a*]pyridine-3-carboxamide

j. 2-Methyl-*N*-(2-(3-nitrophenyl)-4-oxothiazolidin-3-yl)imidazo[1,2-*a*]pyridine-3-carboxamide

k. 2-Methyl-*N*-(2-(4-nitrophenyl)-4-oxothiazolidin-3-yl)imidazo[1,2-*a*]pyridine-3-carboxamide

l. 2-Methyl-*N*-(4-oxo-2-styrylthiazolidin-3-yl)imidazo[1,2-*a*]pyridine-3-carboxamide

3. The one-pot synthesis of Thiazolidin-3-yl-Imidazo-pyridine-3-carboxamide of formula I as claimed in claim-1 optimizing the in-process isolation of intermediate compounds of formulae IV, III and II in presence of a catalyst,

4. The one-pot synthesis of compound of formula 1 as claimed in claim 3 wherein the catalyst is ammonium persulphate (APS).

5. The Thiazolidin-3-yl-Imidazo-pyridine-3-carboxamide of formula I, or its pharmaceutically acceptable salt as claimed in claim 1 for the treatment of Malaria.

6. The Thiazolidin-3-yl-Imidazo-pyridine-3-carboxamide of formula I, or its pharmaceutically acceptable salt as claimed in claim 6 for the treatment of *Plasmodium falciparum*.

For, MK Bhavnagar University, Bhavnagar,

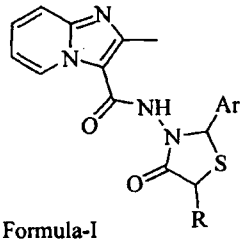
Date: 9th June, 2022

Prof. Nisheeth C. Desai

Thiazolidin-3-yl-Imidazo-pyridine-3-carboxamide as antimalarial agents

Abstract

In the present invention we have developed a series of hybrid molecules of 2-methyl-*N*-(4-oxo-2-arylthiazolidin-3-yl)imidazo[1,2-*a*]pyridine-3-carboxamides containing imidazo-pyridine embedded with 4-thiazolidinones and amide linker present on the 3rd position of imidazo-pyridine heterocyclic motifs (Formula-I). The synthetic procedure was performed via one pot reaction and process for preparation and formulas (IV), (III), and (II) are condensed together, optimizing the in-process isolation of intermediate compounds thereof. The synthesized hybrids were evaluated for antimalarial activity against *P. falciparum* by utilizing quinine as a standard drug. A tri-substituted derivative (2,3,4-$(OCH_3)_3$) was found to be higher potency than the standard drug.



Formula-I

For, MK Bhavnagar University, Bhavnagar,

Date: 9th June, 2022

Prof. Nisheeth C. Desai

सत्यमेव जयते

GOVERNMENT OF GUJARAT

LEGISLATIVE AND PARLIAMENTARY AFFAIRS DEPARTMENT

The Maharaja KrishnakumarSinhji Bhavnagar University Act, 1978.

(Gujarat Act No. 26 of 1978)

(As modified up to the 31st December, 2017)

H-2032- Book-07-2017.

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2017.

Protection of acts and orders.

68. All acts and orders in good faith done and passed by the University or any of its authorities, bodies or officers shall be final and no suit shall be instituted against or damage claimed from the University or its authorities, bodies or Officers for anything purporting to be done in pursuance of this Act and the Statutes, Ordinances, Regulations and Rules framed thereunder.

Construction of reference to the Bhavnagar University Act, 1978 and the Bhavnagar University in existing laws, instruments, etc.

Guj. 1 of 2012.

[1][68.A (1) As from the commencement of the Bhavnagar University (Amendment) Act, 2012 (hereinafter referred to as "the said Act"), any reference in any existing law or instrument or document-

(i) to the expression "the Bhavnagar University Act, 1978" shall be construed as if it were a reference to "the Maharaja Krishnakumarsinhji Bhavnagar University Act, 1978", and

(ii) to the expression "The Bhavnagar University" shall be construed as if it were a reference to "Maharaja Krishnakumarsinhji Bhavnagar".

(2) Any act done by, or any suit or other proceeding filed by or against the Bhavnagar University before the commencement of the said Act shall be deemed to have been done or, as the case may be, filed by or against Maharaja Krishnakumarsinhji Bhavnagar University.

Explanation:— For the purpose of this section "existing law" means any enactment of a Legislature or any other competent authority in relation to matters specified in List II and List III in the Seventh Schedule to the Constitution of India as in force in any part of the State of Gujarat immediately before commencement of the said Act and includes any statute, ordinance, rule, bye-law, regulation, order, notification, scheme form or other instrument having the force of law made, prescribed or issued under any such enactment.]

CHAPTER XII.

TRANSITORY PROVISIONS.

Transfer of certain Colleges, Departments and the Centres of the Saurashtra University to the University.

Guj. 39 of 1965.

69. (*1*) Notwithstanding anything contained in the Saurashtra University Act, 1965 or in the Statutes, Ordinances, Regulations, Rules and Orders made thereunder, the Colleges, Departments and the Centres specified in Schedule-II, shall, from the date of the commencement of this Act, cease to be the College, Departments or, as the case may be, Centres of the Saurashtra University, and shall be transferred to any vest in the University.

(*2*) The control and management of the Colleges, Departments and Centres referred to in sub-section (1) shall, with effect on and from the date of the commencement of this Act, stand transferred to the University and all properties and assets (whether movable or immovable) and liabilities of the Saurashtra University in relation thereto shall stand transferred to and vest in or devolve upon the University.

(*3*) Where before the date of the commencement of this Act, the Saurashtra Universtiy has made any contract in relation to the said Colleges Departments or Centres, such contracts shall be deemed to have been made by the University and any reference therein to the Saurashtra University shall be construed as reference to the University.

(*4*) Where immediately before the commencement of this Act, the Saurashtra University is a party to any legal proceedings with respect to any property and assets transferred to the University under this section or with respect to any of the rights, liabilities, or obligations of the Saurashtra University which have become the rights, labilities and obligations of the University, the University shall be deemed to be substituted for the Saurashtra University as a party to those proceedings and the proceedings shall continue accordingly.

1. Section 68A was inserted by Guj. 1 of 2012, s. 6.

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Fused Heterocycles: Synthesis of Some New Imidazo[1,2-*a*]-pyridine Derivatives

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Abstract: Some new thiazolidines and spirothiazolidines derived from hydrazones of 2-methylimidazo[1,2-*a*]pyridine-3-carboxylic acid hydrazide, a bioisosteric derivative of isoniazid, were synthesized and characterized by analytical, IR, ^{1}H- and ^{13}C-NMR and mass spectral data. Some of the newly synthesized compounds were screened for their antimycobacterial activities. None of the tested compounds showed significant *in vitro* antituberculous activity at 6.25 μg/mL (MIC rifampin 0.031 μg/mL).

Keywords: Imidazo[1,2-*a*]pyridine, hydrazones, thiazolidines, spirothiazolidines, antituberculous activity.

Introduction

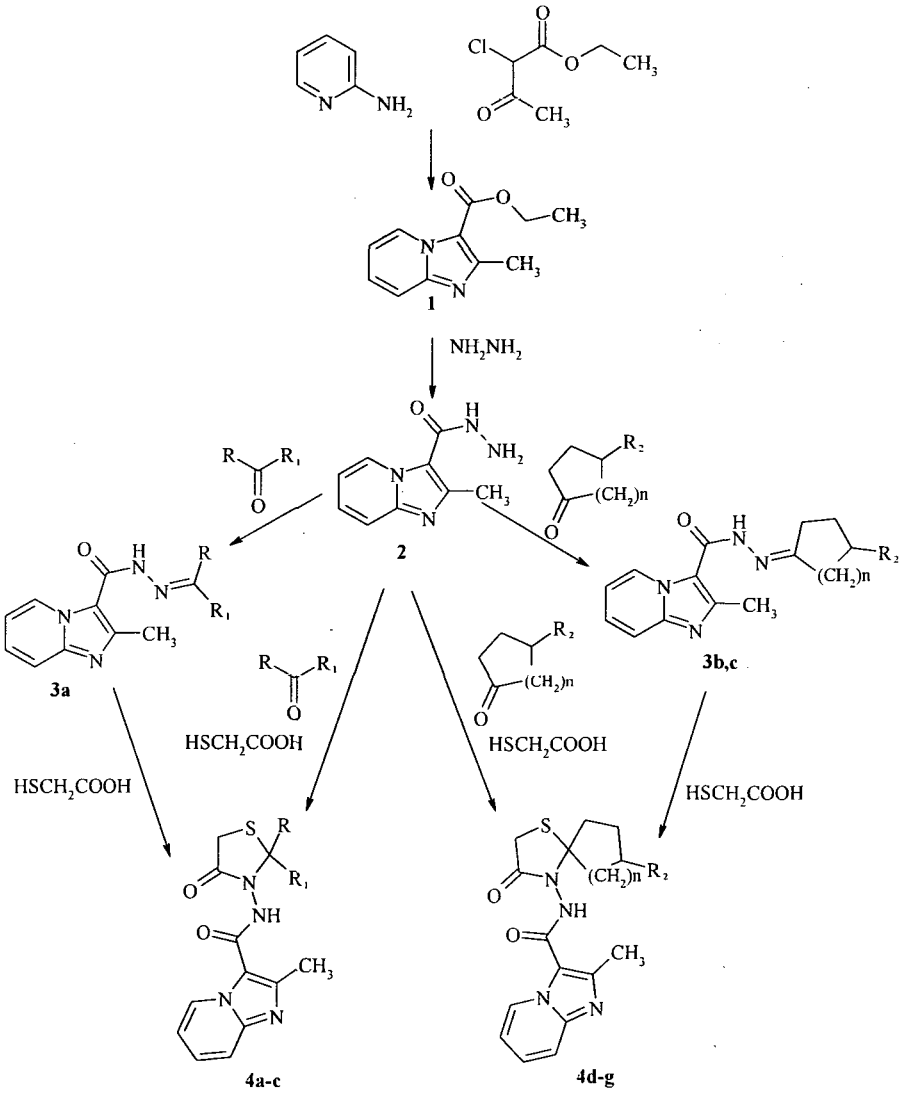
Mycobacterium tuberculosis infects over one-third of the world's population and causes almost three million deaths every year [1]. Isonicotinic acid hydrazide (isonazid) is one of the primary drugs used in combination with ethambutol, rifampin, streptomycin and pyrazinamide to treat tuberculosis, but the treatment of this disease is still a major health problem due to multi-drug resistant bacterial strains and new antimycobacterial agents, different from available first-line drugs, are urgently needed. As part of our studies on imidazo[1,2-*a*]pyridine we have recently reported the synthesis of some imidazo[1,2-*a*]pyridine-3-carboxylic acid hydrazides and related compounds and their antimycobacterial activities [2]. Continuing our search for new antimycobacterial agents we have now

synthesized some new ketone-hydrazones **3a-c**, thiazolidines **4a-c** and spiro compounds **4d-g** incorporating an imidazo[1,2-*a*]pyridine moiety. These compounds were characterized by their elemental and spectral analyses (IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra).

Results and Discussion

The synthetic pathway followed in the preparation of the compounds is outlined in Scheme 1. The starting materials, ethyl 2-methylimidazo[1,2-*a*]pyridine-3-carboxylate (**1**) and 2-methylimidazo[1,2-*a*]pyridine-3-carboxylic acid hydrazide (**2**), were obtained by previously described methods [3,4].

Scheme 1



Condensation of **2** with the appropriate ketones in ethanol yielded the corresponding ketone-hydrazones **3**. The hydrazones were reacted with mercaptoacetic acid in dry benzene (Method A) to give cyclocondensation products **4b,d** and **e** in 69.8-72.3 % yields. On the other hand, refluxing a mixture of **2** and the appropriate ketone together with mercaptoacetic acid in dry benzene (Method B) also produced the target compounds **4** but in higher yields (69.7-99.1 %), except in the case of **4b** (55.5 %). All the compounds were characterized by their physical data and elemental analyses (Table 1), IR, ^{1}H- and ^{13}C-NMR and EI mass spectra.

Table 1. Some physical and analytical data of compounds **3** and **4**

Comp.	R	R_1	R_2	n	M.p. (°C)	Yield %	Formula (molecular weigh)	Analysis (calcd./found)(%)		
								C	H	N
3a	CH_3	C_2H_5	-	-	120-5	75.8	$C_{13}H_{16}N_4O$	63.91	6.60	22.94
							(244.30)	63.81	6.96	22.55
3b	-	-	-	1	162-6	62.1	$C_{14}H_{16}N_4O.1.5H_2O$	59.35	6.76	19.78
							(283.61)	**60.84**	**6.96**	**19.70**
3c	-	-	-	2	76-8	63.8	$C_{15}H_{18}N_4O.2H_2O$	58.81	7.24	18.29
							(306.33)	58.94	7.56	18.21
4a	CH_3	CH_3	-	-	222-5	87.3	$C_{14}H_{16}N_4O_2S.H_2O$	52.16	5.63	17.38
						(Method B)	(322.38)	52.70	6.04	17.30
4b	CH_3	C_2H_5	-	-	138-43	69.8	$C_{15}H_{18}N_4O_2S.H_2O$	53.56	5.99	16.65
						(Method A)	(336.39)	53.45	6.10	16.83
						55.5				
						(Method B)				
4d	-	-	-	1	137-43	75.5	$C_{16}H_{18}N_4O_2S.H_2O$	55.15	5.79	16.08
						(Method A)	(348.42)	55.10	5.82	15.92
						80.0				
						(Method B)				
4e	-	-	-	2	258-65	77.3	$C_{17}H_{20}N_4O_2S$	59.28	5.85	16.27
						(Method A)	(344.43)	58.97	5.77	16.10
						99.1				
						(Method B)				
4f	-	-	CH_3	2	154-6	72.3	$C_{18}H_{22}N_4O_2S.0.5H_2O$	58.85	6.31	15.26
						(Method B)	(367.46)	58.64	7.26	15.42
4g	-	-	C_2H_5	2	142-6	81.7	$C_{19}H_{24}N_4O_2S.2H_2O$	55.86	6.91	13.71
						(Method B)	(408.52)	55.44	6.56	12.09

The IR spectra of the starting materials **3** showed C=O bands in the 1654-1679 cm^{-1} region. A new strong band at 1690-1710 cm^{-1} in the spectra of **4** provided firm support for ring closure. The most significant evidence for the reaction was the presence of two doublets (dd, 2H, J=16 Hz) at about 3.61 and 3.68 in the ^{1}H-NMR spectrum of **4b** [6]. In the spectra of **4a,c-g**, the same protons were observed as singlets (2H) at about 3.40-3.72 ppm due to the lack of chirality. ^{13}C-NMR and DEPT (135) spectra of the prototypes (**4b,d** and, **e**) were also studied and are detailed. Signals at about 71.44-76.59 ppm, which are not seen in DEPT spectra, were assigned to the quarternary (spiro) carbon atoms. According to the data obtained from DEPT and HETCOR experiments the signals at about 28.80-29.72 ppm were assigned to the CH_2 group located in the thiazolidine moiety [7]. The mass spectra of all the compounds were relatively simple and showed (except for **4g**) the peaks due to molecular ions.

Antituberculous Activity

Primary screening was conducted at 6.25 μg/mL against *M. tuberculosis* H_{37}Rv. The *M. tuberculosis* H_{37}Rv was grown in a medium containing a radiolabeled substrate. Labeled CO_2 produced was detected and quantitated with a BACTEC 460 automatic radiometric system. Compounds giving inhibitions < 90 % (MIC > 6.25 μg/mL, MIC rifampin 0.031 μg/mL) were not evaluated further [5]. None of the compounds showed antituberculous activity at the tested concentration.

Acknowledgements

We thank Dr. Joseph A. Maddry from the Tuberculosis Antimicrobial Acquisition and Coordination Facility (TAACF), National Institute of Allergy and Infectious Diseases Southern Research Institute, Birmingham, AL (USA) for the *in vitro* evaluation of antituberculous activity. This work was supported by Istanbul University Research Fund Project No. T-452/071197.

Experimental

General

Melting points determined with a Buchi 530 melting point apparatus in open capillaries and are uncorrected. IR (KBr disks) and ^{1}H- and ^{13}C-NMR spectra (DMSO-d_6) were recorded on Perkin Elmer Model 1600 and Bruker AC 200 and DPX 400 instruments, respectively. Microanalyses were carried out on a Carlo Erba 1106 elemental analyzer. All starting materials were purchased E. Merck (Darmstadt, Germany).

Ethyl 2-methyimidazo[1,2-a]pyridine-3-carboxylate (**1**) [3].

2-Aminopyridine (0.01 mol) was heated under reflux with ethyl 2-chloroacetoacetate (0.1 mol) in 96 % C_2H_5OH (25 mL) for 6h and then cooled. Excess C_2H_5OH was evaporated *in vacuo*. The residual red oil was partitioned between ether-water. After drying, the ether extracts were evaporated and the residual oil was allowed to crystallize. M.p. 69 °C, yield 45.05%.

2-Methylimidazo[1,2-a]pyridine-3-carboxylic acid hydrazide (**2**) [4].

Ethyl 2-methylimidazo[1,2-*a*]pyridine-3-carboxylate (0.01 mol) was heated under reflux with H_2NNH_2 (0.1 mol) in 96% C_2H_5OH (15 mL) for 5h and then cooled. The crystals formed were washed with H_2O, dried and recrystallized from C_2H_5OH (96 %). M.p.180 °C, yield 27.16 %.

General procedure for preparation of 2-methylimidazo[1,2-a]pyridine-3-carboxylic acid (alkylidene / cycloalkylidene) hydrazides **3a-c**.

2-Methylimidazo[1,2-*a*]pyridine-3-carboxylic acid hydrazide (**2**, 0.01 mol), the appropriate ketone (0.011 mol), a drop of conc. H_2SO_4 and 96 % C_2H_5OH (20 mL) were heated under reflux for 6h. The crude products which precipitated on cooling were filtered and recrystallized from an C_2H_5OH-H_2O mixture.

2-Methylimidazo[1,2-a]pyridine-3-carboxylic acid sec-butylidenehydrazide (**3a**): IR: 1654 (C=O) cm^{-1}; ^{1}H-NMR: δ (ppm) = 1.04 (3H, t, CH_2CH_3), 1.98 (3H, s, CH_3), 2.28 (2H, q, CH_2CH_3), 2.53 (3H, s, 2-CH_3), 7.01 (1H, t, 6-H), 7.38 (1H, t, 7-H), 7.58 (1H, d, 8-H), 8.88 (1H, d, 5-H), 10.03 (1H, s, CONH); EIMS (%) = 244 (M^+, 38), 159 (100).

2-Methylimidazo[1,2-a]pyridine-3-carboxylic acid cyclopentylidenhydrazide (**3b**): IR: 1670 (C=O) cm^{-1}; ^{1}H-NMR: δ (ppm) = 1.68-1.83 (4H, m, cyclopentylidene-3H,4H), 2.34-2.49 (4H, m, cyclopentylidene-2H,5H), 2.54 (3H, s, 2-CH_3), 7.00 (1H, t, 6-H), 7.40 (1H, t, 7-H), 7.58 (1H, d, 8-H), 8.89 (1H, d, 5-H), 9.91 (1H, s, CONH); EIMS (%) = 256 (M^+, 100).

2-Methylimidazo[1,2-a]pyridine-3-carboxylic acid cyclohexylidenhydrazide (**3c**): IR: 1679 (C=O) cm^{-1}; ^{1}H-NMR: δ (ppm) = 1.4-1.78 (6H, m, cyclohexylidene 3H,4H,5H), 2.21-2.31 (2H, m, cyclohexylidene-2H,6H, axial), 2.33-2.60 (2H, m, cyclohexylidene-2H,6H, equatorial), 2.52 (3H, s, 2-CH_3), 7.01 (1H, t, 6-H), 7.37 (1H, t, 7-H), 7.56 (1H, d, 8-H), 8.86 (1H, d, 5-H), 10.28 (1H, s, CONH); EIMS (%) = 270 (M^+, 72), 78 (100).

General procedures for preparation of 2-methylimidazo[1,2-a]pyridine-3-carboxylic acid amides **4 a-g**.

Method A

A mixture of **3a-c** (0.01 mol) and $HSCH_2COOH$ (0.15 mol) was heated under reflux for 6h in dry benzene (30 mL) using a Dean-Stark trap for removal of water of condensation. Excess benzene was evaporated *in vacuo*. The residue was triturated with saturated $NaHCO_3$ until CO_2 evaluation ceased and then allowed to stand overnight. The solid thus obtained was filtered, washed with H_2O and recrystallized from an C_2H_5OH-H_2O mixture.

Method B

The appropriate ketone (0.011 mol) was added to a solution of **2** (0.01 mol) in dry benzene (30 mL) and the mixture was heated under reflux for 1.5h using a Dean-Stark trap. After cooling $HSCH_2COOH$ (0.15 mol) was added dropwise to the solution and the resulting mixture was refluxed for 6h. The compounds were purified using the procedure described under Method A.

2-Methylimidazo[1,2-a]pyridine-3-carboxylic acid (2,2-dimethyl-4-oxo-1,3-thiazolidin-3-yl)amide (**4a**): IR: 1662 (CONH), 1690 (thiazolidine C=O) cm^{-1}; ^{1}H-NMR: δ (ppm) = 1.36 (6H, s, $-C(CH_3)_2$), 2.44 (3H, s, 2-CH_3), 3.52 (2H, s, CH_2S), 6.88 (1H, t, 6-H), 7.25 (1H, t, 7-H), 7.42 (1H, d, 8-H), 8.65 (1H, d, 5-H), 9.81 (1H, s, CONH); EIMS (%) = 304 (M^+, 3), 156 (100).

2-Methylimidazo[1,2-a]pyridine-3-carboxylic acid (2-ethyl-2-methyl-4-oxo-1,3-thiazolidin-3-yl)amide (**4b**): IR: 1662 (CONH), 1690 (thiazolidine C=O) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.04 (3H, t, CH_2CH_3), 1.66 (3H, s, C-CH_3), 1.76-1.84,1.92-1.99 (1H, 1H, 2m, CH_2CH_3), 2.60 (3H, s, 2-CH_3), 3.61, 3.68 (1H, 1H, dd, J=16 Hz, CH_2S), 6.93 (1H, t, 6-H), 7.34 (1H, t, 7-H), 7.46 (1H, d, 8-H), 9.22 (1H, d, 5-H), 7.93 (1H, s, CONH); ^{13}C-NMR δ(ppm) = 168.67/161.73 (thiazolidine CO and CONH), 148.19/146.57 (imidazopyridine C_2 and C_{8a}), 128.19 (imidazopyridine C_5), 127.80 (imidazopyridine C_7), 117.14 (imidazopyridine C_8), 114.33 (imidazopyridine C_3), 71.44 (thiazolidine C_2), 34.72 ($\underline{C}H_2CH_3$), 29.72 (thiazolidine C_3), 28.32 (CH_3), 16,73 (2-CH_3), 9.53 ($CH_2\underline{C}H_3$); EIMS (%) = 318 (M^+,100).

2-Methylimidazo[1,2-a]pyridine-3-carboxylic acid (2,2-diethyl-4-oxo-1,3-thiazolidin-3-yl)amide (**4c**): IR: 1662 (CONH), 1690 (thiazolidine C=O) cm^{-1}; ^{1}H-NMR: δ (ppm) = 0.8 (6H, t, CH_2CH_3), 1.50-1.65 (4H, m, CH_2CH_3), 2.40 (3H, s, 2-CH_3), 3.40 (2H, s, CH_2S), 6.64 (1H, t, 6-H), 7.22 (1H, t, 7-H), 7.40 (1H, d, 8-H), 8.66 (1H, d, 5-H), 9.72 (1H, s, CONH); EIMS (%) = 332 (M^+, 4.5), 46 (100).

2-Methylimidazo[1,2-a]pyridine-3-carboxylic acid (3-oxo-1-thia-4-azaspiro[4.4]non-4-yl)amide (**4d**): IR: 1662 (CONH), 1691 (spiro[4.4]nonane C=O) cm^{-1}; ^{1}H-NMR: δ (ppm) = 1.67-1.97 (4H, m, spiro-7H,8H), 2.15-2.21 (2H, m, spiro-6H,9H axial), 2.23-2.40 (2H, m, spiro-6H,9H equatorial), 2.64 (3H, s, 2-CH_3), 3.72 (2H, s, CH_2S), 7.05 (1H, t, 6-H), 7.46 (1H, t, 7-H), 7.62 (1H, d, 8-H), 8.90 (1H, d, 5-H), 9.98 (1H, s, CONH); ^{13}C-NMR δ (ppm) = 168.67/161.73 (spiro[4.4]nonane C_3 and CONH), 148.05/146.62 (imidazopyridine C_2 and C_{8a}) , 128.25 (imidazopyridine C_5), 127.85 (imidazopyridine C_7), 117.12 (imidazopyridine C_8), 114.74 (imidazopyridine C_3), 114.34 (imidazopyridine C_6), 76.79 (C_5), 39.22 (spiro[4.4]nonane C_6 and C_9), 29.72 (spiro[4.4]nonane C_2), 23.62 (spiro[4.4]nonane C_7 and C_8), 16.75 (2-CH_3); EIMS (%)= 330 (M^+, 66.45), 90 (100).

2-Methylimidazo[1,2-a]pyridine-3-carboxylic acid (3-oxo-1-thia-4-azaspiro[4.5]dec-4-yl)amide (**4e**): IR: 1673 (CONH), 1709 (spiro[4.5]decane C=O) cm^{-1}; ^{1}H-NMR: δ (ppm) = 1.05-2.54 (10H, m, spiro-6H,7H,8H,9H,10H), 2.67 (3H, s, 2-CH_3), 3.64 (2H, s, CH_2S), 7.07 (1H, t, 6-H), 7.44 (1H, t, 7-H), 7.62 (1H, d, 8-H), 8.90 (1H, d, 5-H), 9.93 (1H, s, CONH); ^{13}C-NMR δ (ppm) = 168.67/161.73 (spiro[4.5]decane C_3 and CONH), 148.00/146.00 (imidazopyridine C_2 and C_{8a}), 128.29 (imidazopyridine C_5), 127.84 (imidazopyridine C_7), 117.11 (imidazopyridine C_8), 114.80 (imidazopyridine C_3), 114.37 (imidazopyridine C_6), 73.04 (spiro[4.5]decane C_5), 28.80 (spiro[4.5]decane C_2), 24.90 (spiro[4.5]decane C_8), 23.76 (spiro[4.5]decane C_6 and C_9), 23.62 (spiro[4.5]decane C_6 and C_{10}), 16.78 (2-CH_3); EIMS (%) = 344 (M^+, 92.4), 160 (100).

2-Methylimidazo[1,2-a]pyridine-3-carboxylic acid (8-methyl-3-oxo-1-thia-4-azaspiro[4.5]dec-4-yl) amide (**4f**): IR: 1662 (CONH), 1693 (spiro[4.5]decane C=O) cm^{-1}; ^{1}H-NMR: δ (ppm) = 0.67 (3H, s, CH_3), 1.28-1.63 (9H, m, spiro-6H,7H,8H,9H,10H), 2.43 (3H, s, 2-CH_3), 3.43 (2H, s, CH_2S), 6.85 (1H, t, 6-H), 7.22 (1H, t, 7-H), 7.40 (1H, d, 8-H), 8.67 (1H, d, 5-H), 9.79 (1H, s, CONH); EIMS (%) = 358 (M^+, 4), 46 (100).

2-Methylimidazo[1,2-a]pyridine-3-carboxylic acid (8-ethyl-3-oxo-1-thia-4-azaspiro[4.5]dec-4-yl) amide (**4g**): IR: 1672 (CONH), 1710 (spiro[4.5]decane C=O) cm^{-1}; ^{1}H-NMR: δ (ppm) = 0.84 (3H, s, CH_2CH_3), 1.05-1.98 (11H, m, spiro-6H,7H,8H,9H,10H, CH_2CH_3), 2.64 (3H, s, 2-CH_3), 3.64 (2H, s, CH_2S), 6.99 (1H, t, 6-H), 7.37 (1H, t, 7-H), 7.67 (1H, d, 8-H), 8.86 (1H, d, 5-H), 9.99 (1H, s, CONH); EIMS (%) = 46 (100).

In vitro evaluation of antituberculous activity [5]

A primary screen was conducted at 6.25 μg/mL against *M. tuberculosis* $H37R_V$ in BACTEC 12B medium using a BACTEC 460 radiometric system. Compounds **3a-c, 4b,d-e**, chosen as prototypes, did not show *in vitro* antituberculous activity at 6.25 μg/mL (MIC rifampin 0.031 μg/mL).

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Exhibit 4

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Novel arylidene derivatives of quinoline based thiazolidinones: Synthesis, *in vitro*, *in vivo* and *in silico* study as antimalarials

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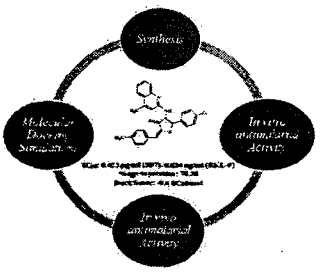
HIGHLIGHTS

- Synthesis and characterization of arylidene derivatives of quinoline based thiazolidinones.
- Evaluation for *in vitro* antimalarial potential against CQ-sensitive and CQ-resistant strains of *P. falciparum*.
- Top five potent compounds were further evaluated *in vivo* against *P. berghei*.
- Docking studies have been performed in the active site of *P. falciparum* lactate dehydrogenase.
- **5g** was found to be most promising candidate with 73.38% of suppression.

GRAPHICAL ABSTRACT



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1. Introduction

Malaria is a common and life-threatening disease transmitted via the bites of infected female *Anopheles* mosquitoes and caused by protozoan parasites of the genus *Plasmodium*. The prevalent species responsible for it are *Plasmodium falciparum* and *Plasmodium vivax* and the most lethal parasite is former one. The parasitic multiplication begins in the liver, infecting erythrocytes where a cyclic asexual replication begins with the cycles of fever and chills as symptoms of malaria. This infected person with severe illness can result to death within hours to days; if untreated (Warhurst et al., 2003; Mishra et al., 2017). According to the World Health Organisation (WHO) report, 212 million new cases of malaria worldwide in 2015 were reported (range 148–304 million). The WHO African Region accounted for most global cases of malaria (90%), followed by the South-East Asia Region (7%) and the Eastern Mediterranean Region (2%) (WHO, World Malaria Report, 2016). The surge in resistance of malaria parasites particularly in *P. falciparum* is an important factor in the persistence of this disease as a major worldwide public health threat (Sinha et al., 2014). The existing chemotherapy lacks satisfaction and effectiveness due to the side effects associated to long-term treatments. The existing drugs come across shortcomings like drug resistance and strain sensitivity for the clinically accessible chemotherapy (Sahu et al., 2016; Manohar et al., 2014; Teixeira et al., 2014).

Quinoline derived drugs like chloroquine, amodiaquine, quinine, quinidine, mefloquine, primaquine, lumefantrine, and halofantrine have long been used against malaria and all these shows

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potent activity against the erythrocytic stage of infection (Olson et al., 1999). Primaquine also kills intrahepatic forms and gametocytes. The drugs act by accumulating in the parasite food vacuole and forming a complex with heme that prevents crystallization in the *Plasmodium* food vacuole. Heme polymerase activity is restraint, resulting in accumulation of cytotoxic-free heme (Foley and Tilley, 1998; Bekhit et al., 2012). The appearance of drug-resistant strains of the malaria parasite was the distressing outcome of efforts for the development of insecticide-resistant mosquitos (Kumar et al., 2015).

Several biological activities are associated with a five-membered ring thiazolidine which is an important pharmacophore. 4-Thiazolidinones having wide range of biological activities are evolved from thiazolidine with a carbonyl group at the position 4. Substitution of group to the carbon atom present at position 2 shows marked difference in structure and physicochemical properties of 4-thiazolidones (Singh et al., 2010; Dorn et al., 1995). Rigid molecules present in the nitrogen containing heterocyclic skeleton (thiazolidine-4-one) show biologically active scaffold for the design of new antimalarial drugs active against *Plasmodium* malaria parasite (Rojas Ruiz et al., 2011; Kumar et al., 2014; Rosenthal et al., 2002). In the current study, novel thiazolidinone-quinoline hybrids and their corresponding arylidene derivatives were prepared in good yield. Structure-Activity relationship has also been established to get a deep insight into the effect of different substitutions on antimalarial potential of the series. This work also includes the docking simulation of the active agents among the series to get an idea about the ligand receptor interaction. We, herein present study, reported the synthesis, *in vitro*, *in vivo* and *in silico* screening of synthesized series for antimalarial potential.

2. Experimental

2.1. Synthetic strategy adopted

The synthetic protocol followed for the synthesis of compounds under study has been outlined in Scheme 1.

2.1.1. General experimental procedure for synthesis of hydrazone (3a-e)

The whole synthesis is outlined in Scheme 1. To a mixture of 2-hydrazino-4-methylquinoline, **compound 2** in EtOH (20 ml) was added in an equimolar amount of various aromatic aldehyde and refluxed in the presence of 1–2 drop of glacial acetic acid for 6 h. The resulting solution was poured on crushed ice to yield hydrazones in high yield (Unsal-Tan et al., 2012; Kumar et al., 2007).

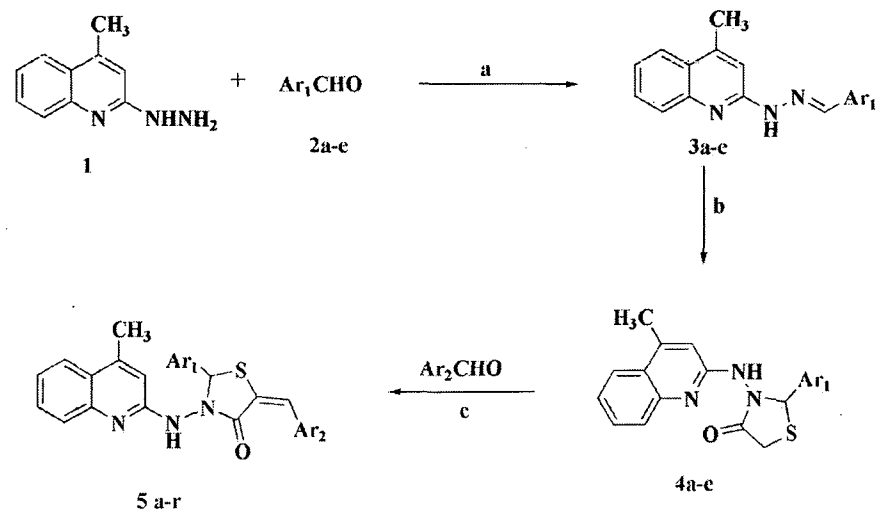
2.1.1.1. 1-Benzylidene-2-(4-methylquinolin-2-yl)hydrazine ***(3a).*** Yield 73%, mp 153–154 °C IR (υ, cm^{-1}): 3354.20 (N-H), 2919.50 (C-H), 1612.10 (C=N); ^{1}H NMR (400 MHz, $CDCl_3$, δ): 8.59 (s, br, 1H, NH), 8.23 (s, 1H, quinoline ring), 7.43–7.89 (m, 9H, aromatic), 7.28 (s, 1H, -N=CH), 2.74 (s, 3H, CH_3 of Qu-ring).

2.1.1.2. 1-(4-chlorobenzylidene)-2-(4-methylquinolin-2-yl)hydrazine ***(3b).*** Yield 71%, mp 166–168 °C IR (υ, cm^{-1}): 3361.12 (N-H), 2921.17 (C-H), 1613.24 (C=N), 653.27 (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$, δ): 8.61 (s, br, 1H, NH), 8.26 (s, 1H, quinoline ring), 7.83 (d, 2H), 7.65–7.89 (m, 4H), 7.29 (s, 1H, -N=CH), 6.98 (d, 2H), 2.78 (s, 3H, CH_3).

2.1.1.3. 1-(4-methylbenzylidene)-2-(4-methylquinolin-2-yl)hydrazine ***(3c).*** Yield 79%, mp 172–174 °C IR (υ, cm^{-1}): 3349.11 (N-H), 2929.41 (C-H), 1609.81 (C=N); ^{1}H NMR (400 MHz, $CDCl_3$, δ): 8.61(s, br, 1H, NH), 8.23 (s, 1H, quinoline ring), 7.86 (d, 2H), 7.55–7.78 (m, 4H), 7.26 (s, 1H, -N=CH), 6.99 (d, 2H), 2.73 (s, 3H, CH_3 of Qu-ring), 2.31 (s, 3H, CH_3).

2.1.1.4. 1-(4-methoxybenzylidene)-2-(4-methylquinolin-2-yl)hydrazine ***(3d).*** Yield 81%, mp 151–153 °C IR (υ, cm^{-1}): 3327.33 (N-H), 2932.45 (C-H), 1615.11 (C=N); ^{1}H NMR (400 MHz, $CDCl_3$, δ): 8.64 (s, br, 1H, NH), 8.23 (s, 1H, quinoline ring), 7.81 (d, 2H), 7.68–7.90 (m, 4H), 7.28 (s, 1H, -N=CH), 6.98 (d, 2H), 3.89 (s, 3H, OCH_3), 2.79 (s, 3H, CH_3).

2.1.1.5. 1-(4-methylquinolin-2-yl)-2-(thiophen-2-ylmethylene)hydrazine ***(3e).*** Yield 69%, mp 140–142 °C IR (υ, cm^{-1}): 3341.29 (N-H), 2922.17 (C-H), 1613.62 (C=N); ^{1}H NMR (400 MHz, $CDCl_3$, δ): 8.59 (s, br, 1H, NH), 8.17 (s, 1H, quinoline ring), 7.21 (s, 1H, -N=CH), 6.83–7.56 (m, 7H), 2.70 (s, 3H, CH_3).



Scheme 1. Synthesis of arylidene derivatives (**5 a-r**): **a)** 1–2 drops Glacial acetic acid, Ethanol, reflux, 6 h. **b)** Thioglycolic acid, 1,4-Dioxane, $ZnCl_2$, reflux, 8–10 h. **c)** Glacial acetic acid, Sodium acetate, reflux, 12 h.

*2.1.2. General experimental procedure for synthesis of thiazolidinone **(4a-e)***

To a solution of compound (3) (0.01 mol) in 1,4 dioxane (50 ml) was added mercapto acetic acid (0.015 mol) with stirring and a little amount of anhydrous $ZnCl_2$ was added. The mixture was refluxed for 10–12 h, after the completion of reaction, it was cooled and the excess solvent distilled and poured into sodium bicarbonate solution to neutralize it. The solid product was filtered and washed with cold water. The resulting solid was recrystallized in ethanol (99%) (Desai and Dodiya, 2014; Nagalakshmi et al., 2013).

*2.1.2.1. 3-(4-methylquinolin-2-ylamino)-2-phenylthiazolidin-4-one **(4a)**.* Yield 51%, mp 202–204 °C IR (υ, cm^{-1}): 3406.34 (N-H), 1656.17 (C=O); ^1H NMR (400 MHz, DMSO-d_6, δ): 8.46 (s, 1H, NH), 8.16 (s, 1H, H_3 of quinoline), 7.33–7.90 (m, 9H), 6.77 (s, 1H, thiazolidinone, 2nd position), 5.29 (s, 2H, Thiazolidinone, 4th position), 2.63 (s, 3H, CH_3 of quinoline).

*2.1.2.2. 2-(4-chlorophenyl)-3-(4-methylquinolin-2-ylamino)thiazolidin-4-one **(4b)**.* Yield 54%, mp 230–232 °C IR (υ, cm^{-1}): 3409.46 (N-H), 1659.32 (C=O); ^1H NMR (400 MHz, DMSO-d_6, δ): 8.52 (s, 1H, NH), 8.14 (s, 1H, H_3 of quinoline), 7.20–7.85 (m, 8H), 6.65 (s, 1H, thiazolidinone, 2nd position), 5.33 (s, 2H, Thiazolidinone, 4th position), 2.68 (s, 3H, CH_3 of quinoline).

*2.1.2.3. 3-(4-methylquinolin-2-ylamino)-2-p-tolylthiazolidin-4-one **(4c)**.* Yield 49%, mp 236–238 °C IR (υ, cm^{-1}): 3411.43 (N-H), 1659.22 (C=O); ^1H NMR (400 MHz, DMSO-d_6, δ):8.41 (s, 1H, NH), 8.19 (s, 1H, H_3 of quinoline), 7.15–7.95 (m, 8H), 6.73 (s, 1H, thiazolidinone, 2nd position), 5.06 (s, 2H, Thiazolidinone, 4th position), 2.65 (s, 3H, CH_3 of quinoline), 2.35 (s, 3H, CH_3 of phenyl).

*2.1.2.4. 2-(4-methoxyphenyl)-3-(4-methylquinolin-2-ylamino)thiazolidin-4-one **(4d)**.* Yield 57%, mp 220–222 °C IR (υ, cm^{-1}): 3410.58 (N-H), 1654.63 (C=O); ^1H NMR (400 MHz, DMSO-d_6, δ): 8.42 (s, 1H, NH), 8.26 (s, 1H, H_3 of quinoline), 7.06–7.80 (m, 8H), 6.54 (s, 1H, thiazolidinone, 2nd position), 5.15 (s, 2H, Thiazolidinone, 4th position), 4.35 (s, 3H, OCH_3 of phenyl), 2.67 (s, 3H, CH_3 of quinoline).

*2.1.2.5. 3-(4-methylquinolin-2-ylamino)-2-(thiophen-2-yl)thiazolidin-4-one **(4e)**.* Yield 42%, mp 200–202 °C IR (υ, cm^{-1}): 3399.18 (N-H), 1659.47 (C=O); ^1H NMR (400 MHz, DMSO-d_6, δ): 8.48 (s, 1H, NH), 8.21 (s, 1H, H_3 of quinoline), 7.16–7.85 (m, 7H), 5.23 (s, 2H, Thiazolidinone, 4th position), 6.67 (s, 1H, thiazolidinone, 2nd position), 2.56 (s, 3H, CH_3 of quinoline).

2.1.3. General experimental procedure for synthesis of arylidine derivatives

A well-stirred solution of 3-(4-methylquinolin-2-ylamino)-2-arylthiazolidin-4-ones **(4a-e)** in 20 ml glacial acetic acid was buffered with sodium acetate, 0.66 g (8 mmol) followed by addition of substituted arylaldehyde (6 mmol). The solution was refluxed for 12 h and then poured into ice-cold water to yield titled compounds, **5a-r**. The resulting product was purified by recrystallization from dioxane (Omar et al., 2010; Deep et al., 2014).

*2.1.3.1. 5-Benzylidene-3-(4-methylquinolin-2-ylamino)-2-phenylthiazolidin-4-one **(5a)**.* Yield 62%, mp 212–214 °C IR (ν, Cm^{-1}): 1527 (=C-H), 2974 (=C-H), 1565 (C=N); ^1H NMR (400 MHz, DMSO-d_6, δ): 8.25 (s, 1H, NH), 8.11 (s, 1H, H_3 of quinoline), 6.82 (s, 1H, thiazolidinone, 2nd position), 6.9–7.85 (m, 14H), 5.88 (s, 1H, =CH-Ar), 2.70 (s, 3H, CH_3 of quinoline); MS: m/z = 424.35 (M+1); Anal. Calcd for $C_{26}H_{21}N_3OS$: C, 73.73; H, 5.00; N, 9.92. Found: C, 73.81; H, 5.02; N, 9.89.

*2.1.3.2. 5-(4-chlorobenzylidene)-3-(4-methylquinolin-2-ylamino)-2-phenylthiazolidin-4-one **(5b)**.* Yield 56%, mp 219–221 °C IR (ν, cm^{-1}): 1525 (=C-H), 2975 (=C-H), 1569 (C=N); ^1H NMR (400 MHz, DMSO-d_6, δ): 8.24 (s, 1H, NH), 8.13 (s, 1H, H_3 of quinoline), 6.88 (s, 1H, thiazolidinone, 2nd position), 6.78–7.67 (m, 13H), 5.82 (s, 1H, =CH-Ar), 2.73 (s, 3H, CH_3 of quinoline); MS: m/z = 458.73 (M+1); Anal. Calcd for $C_{26}H_{20}ClN_3OS$: C, 68.19; H, 4.40; N, 9.18. Found: C, 68.21; H, 4.46; N, 9.25.

*2.1.3.3. 5-(4-methylbenzylidene)-3-(4-methylquinolin-2-ylamino)-2-phenylthiazolidin-4-one **(5c)**.* Yield 60%, mp 199–201 °C IR (ν, cm^{-1}): 1526 (=C-H), 2972 (=C-H), 1571 (C=N); ^1H NMR (400 MHz, DMSO-d_6, δ):8.23 (s, 1H, NH), 8.11 (s, 1H, H_3 of quinoline), 6.92–7.83 (m, 13H), 6.86 (s, 1H, thiazolidinone, 2nd position), 5.80 (s, 1H, =CH-Ar), 2.69 (s, 3H, CH_3 of quinoline), 2.45 (s, 3H, CH_3 of phenyl). MS: m/z = 438.47 (M+1); Anal. Calcd for $C_{27}H_{23}N_3OS$: C, 74.11; H, 5.30; N, 9.60. Found: C, 74.19; H, 5.37; N, 9.71.

*2.1.3.4. 5-(4-methoxybenzylidene)-3-(4-methylquinolin-2-ylamino)-2-phenylthiazolidin-4-one **(5d)**.* Yield 63%, mp 205–206 °C IR (ν, cm^{-1}): 1529 (=C-H), 2976 (=C-H), 1573 (C=N); ^1H NMR (400 MHz, DMSO-d_6, δ): 8.23 (s, 1H, NH), 8.17 (s, 1H, H_3 of quinoline), 6.83 (s, 1H, thiazolidinone, 2nd position), 6.77–7.69 (m, 13H), 5.83 (s, 1H, =CH-Ar), 4.03 (s, 3H, OCH_3 of phenyl), 2.67 (s, 3H, CH_3 of quinoline); MS: m/z = 454.35 (M+1); Anal. Calcd for $C_{27}H_{23}N_3O_2S$: C, 71.50; H, 5.11; N, 9.26. Found: C, 71.53; H, 5.16; N, 9.21.

*2.1.3.5. 5-Benzylidene-2-(4-chlorophenyl)-3-(4-methylquinolin-2-ylamino)thiazolidin-4-one **(5e)**.* **Yield 63%, mp 205–206 °C IR (ν, cm^{-1}): 1531 (=C-H), 2972 (=C-H), 1571 (C=N); ^1H NMR (400 MHz, DMSO-d_6, δ): 8.27 (s, 1H, NH), 8.19 (s, 1H, H_3 of quinoline), 7.1–7.92 (m, 13H), 6.79 (s, 1H, thiazolidinone, 2nd position), 5.84 (s, 1H, =CH-Ar), 2.65 (s, 3H, CH_3 of quinoline); MS: m/z = 458.35 (M+1); Anal. Calcd for $C_{26}H_{20}ClN_3OS$: C, 68.19; H, 4.40; N, 9.18. Found: C, 68.21; H, 4.46; N, 9.25.**

*2.1.3.6. 5-(4-chlorobenzylidene)-2-(4-chlorophenyl)-3-(4-methylquinolin-2-ylamino) thiazolidin -4-one **(5f)**.* Yield 61%, mp 217–218 °C IR (ν, cm^{-1}): 1527 (=C-H), 2971 (=C-H), 1573 (C=N); ^1H NMR (400 MHz, DMSO-d_6, δ): 8.29 (s, 1H, NH), 8.18 (s, 1H, H_3 of quinoline), 7.12–7.97 (m, 12H), 6.75 (s, 1H, thiazolidinone, 2nd position), 5.87 (s, 1H, =CH-Ar), 2.68 (s, 3H, CH_3 of quinoline). MS: m/z = 492.45 (M+1); Anal. Calcd for $C_{26}H_{19}Cl_2N_3OS$: C, 63.42; H, 3.89; N, 8.53. Found: C, 63.52; H, 3.93; N, 8.55.

*2.1.3.7. 5-(4-methylbenzylidene)-2-(4-chlorophenyl)-3-(4-methylquinolin-2-ylamino) thiazolidin-4-one **(5g)**.* Yield 59%, mp 221–223 °C IR (ν, cm^{-1}): 1528 (=C-H), 2968 (=C-H), 1571 (C=N); ^1H NMR (400 MHz, DMSO-d_6, δ): 8.33 (s, 1H, NH), 8.17 (s, 1H, H_3 of quinoline), 7.14–7.93 (m, 12H), 6.79 (s, 1H, thiazolidinone, 2nd position), 5.80 (s, 1H, =CH-Ar), 2.65 (s, 3H, CH_3 of quinoline), 2.47 (s, 3H, CH_3 of phenyl); MS: m/z = 492.45 (M+1); Anal. Calcd for $C_{27}H_{22}ClN_3OS$ C, 68.71; H, 4.70; N, 8.90. Found: C, 68.76; H, 4.73; N, 8.94.

*2.1.3.8. 5-(4-methoxybenzylidene)-2-(4-chlorophenyl)-3-(4-methylquinolin-2-ylamino) thiazolidin-4-one **(5h)**.* Yield 65%, mp 198–201 °C IR (ν, cm^{-1}): 1532 (=C-H), 2976 (=C-H), 1577 (C=N); ^1H NMR (400 MHz, DMSO-d_6, δ): 8.31 (s, 1H, NH), 8.20 (s, 1H, H_3 of quinoline), 6.88–7.87 (m, 12H), 6.72 (s, 1H, thiazolidinone, 2nd position), 5.84 (s, 1H, =CH-Ar), 4.12 (s, 3H, OCH_3 of phenyl), 2.71 (s, 3H, CH_3 of quinoline); MS: m/z = 488.34 (M+1); Anal. Calcd for $C_{27}H_{22}ClN_3O_2S$; C, 66.45; H, 4.54; N, 8.61. Found: C, 66.47; H, 4.53; N, 8.64.

2.1.3.9. 5-Benzylidene-3-(4-methylquinolin-2-ylamino)-2-p-tolylthiazolidin-4-one ***(5i)***. Yield 54%, mp 213–215 °C IR (*v*, cm^{-1}): 1530 (=C-H), 2974 (=C-H), 1573 (C=N); ^1H NMR (400 MHz, DMSO-d_6, δ): 8.26 (s, 1H, NH), 8.15 (s, 1H, H_3 of quinoline), 7.1–7.92 (m, 13H), 6.71 (s, 1H, thiazolidinone, 2nd position), 5.87 (s, 1H, =CH-Ar), 2.66 (s, 3H, CH_3 of quinoline), 2.34 (s, 3H, CH_3 of phenyl). MS: *m/z* = 438.47 (M+1); Anal. Calcd for $C_{27}H_{23}N_3OS$: C, 74.11; H, 5.30; N, 9.60. Found: C, 74.19; H, 5.37; N, 9.71.

2.1.3.10. 5-(4-chlorobenzylidene)-3-(4-methylquinolin-2-ylamino)-2-p-tolylthiazolidin-4-one ***(5j)***. Yield 61%, mp 219–220 °C IR (*v*, cm^{-1}): 1529 (=C-H), 2967 (=C-H), 1569 (C=N); ^1H NMR (400 MHz, DMSO-d_6, δ): 8.28 (s, 1H, NH), 8.12 (s, 1H, H_3 of quinoline), 7.12–7.96 (m, 12H), 6.72 (s, 1H, thiazolidinone, 2nd position), 5.80 (s, 1H, =CH-Ar), 2.64 (s, 3H, CH_3 of quinoline), 2.32 (s, 3H, CH_3 of phenyl). MS: *m/z* = 492.45 (M+1); Anal. Calcd for $C_{27}H_{22}ClN_3OS$ C, 68.71; H, 4.70; N, 8.90. Found: C, 68.76; H, 4.73; N, 8.94.

2.1.3.11. 5-(4-methylbenzylidene)-3-(4-methylquinolin-2-ylamino)-2-p-tolylthiazolidin-4-one ***(5k)***. Yield 49%, mp 209–211 °C IR (*v*, cm^{-1}): 1525 (=C-H), 2965 (=C-H), 1567 (C=N); ^1H NMR (400 MHz, DMSO-d_6, δ): 8.36 (s, 1H, NH), 8.12 (s, 1H, H_3 of quinoline), 6.98–7.81 (m, 12H), 6.59 (s, 1H, thiazolidinone, 2nd position), 5.68 (s, 1H, =CH-Ar), 2.68 (s, 3H, CH_3 of quinoline), 2.41 (s, 3H, CH_3 of phenyl), 2.34 (s, 3H, CH_3 of phenyl). MS: *m/z* = 452.35 (M+1); Anal. Calcd for $C_{28}H_{25}N_3OS$; C, 74.47; H, 5.58; N, 9.31. Found: C, 74.56; H, 5.62; N, 9.36.

2.1.3.12. 5-(4-methoxybenzylidene)-3-(4-methylquinolin-2-ylamino)-2-p-tolylthiazolidin-4-one ***(5l)***. Yield 54%, mp 206–207 °C IR (*v*, cm^{-1}): 1526 (=C-H), 2969 (=C-H), 1572 (C=N); ^1H NMR (400 MHz, DMSO-d_6, δ): 8.29 (s, 1H, NH), 8.15 (s, 1H, H_3 of quinoline), 7.12–7.96 (m, 12H), 6.61 (s, 1H, thiazolidinone, 2nd position), 5.73 (s, 1H, =CH-Ar), 4.25 (s, 3H, OCH_3 of phenyl), 2.71 (s, 3H, CH_3 of quinoline), 2.38 (s, 3H, CH_3 of phenyl). MS: *m/z* = 468.53 (M+1); Anal. Calcd for $C_{28}H_{25}N_3O_2S$; C, 71.92; H, 5.39; N, 8.99. Found: C, 71.99; H, 5.45; N, 9.02.

2.1.3.13. 5-Benzylidene-2-(4-methoxyphenyl)-3-(4-methylquinolin-2-ylamino)thiazolidin-4-one ***(5m)***. Yield 61%, mp 202–203 °C IR (*v*, cm^{-1}): 1527 (=C-H), 2974 (=C-H), 1576 (C=N); ^1H NMR (400 MHz, DMSO-d_6, δ): 8.37 (s, 1H, NH), 8.20 (s, 1H, H_3 of quinoline), 7.1–7.92 (m, 13H), 6.71 (s, 1H, thiazolidinone, 2nd position), 5.74 (s, 1H, =CH-Ar), 4.27 (s, 3H, OCH_3 of phenyl), 2.68 (s, 3H, CH_3 of quinoline), MS: *m/z* = 454.35 (M+1); Anal. Calcd for $C_{27}H_{23}N_3O_2S$: C, 71.50; H, 5.11; N, 9.26. Found: C, 71.53; H, 5.16; N, 9.21.

2.1.3.14. 5-(4-chlorobenzylidene)-2-(4-methoxyphenyl)-3-(4-methylquinolin-2-ylamino) thiazolidin-4-one ***(5n)***. Yield 56%, mp 199–201 °C IR (*v*, cm^{-1}): 1526 (=C-H), 2972 (=C-H), 1575 (C=N); ^1H NMR (400 MHz, DMSO-d_6, δ): 8.33 (s, 1H, NH), 8.21 (s, 1H, H_3 of quinoline), 6.92–7.94 (m, 12H), 6.74 (s, 1H, thiazolidinone, 2nd position), 5.79 (s, 1H, =CH-Ar), 4.27 (s, 3H, OCH_3 of phenyl), 2.67 (s, 3H, CH_3 of quinoline). MS: *m/z* = 488.34 (M+1); Anal. Calcd for $C_{27}H_{22}ClN_3O_2S$; C, 66.45; H, 4.54; N, 8.61. Found: C, 66.47; H, 4.53; N, 8.64.

2.1.3.15. 5-(4-methylbenzylidene)-2-(4-methoxyphenyl)-3-(4-methylquinolin-2-ylamino)thiazolidin-4-one ***(5o)***. Yield 53%, mp 210–211 °C IR (*v*, cm^{-1}): 1522 (=C-H), 2976 (=C-H), 1577 (C=N); ^1H NMR (400 MHz, DMSO-d_6, δ): 8.38 (s, 1H, NH), 8.16 (s, 1H, H_3 of quinoline), 6.98–7.90 (m, 12H), 6.72 (s, 1H, thiazolidinone, 2nd position), 5.72 (s, 1H, =CH-Ar), 4.18 (s, 3H, OCH_3 of phenyl), 2.62 (s, 3H, CH_3 of quinoline), 2.38 (s, 3H, CH_3 of phenyl). MS: *m/z* = 468.53 (M+1); Anal. Calcd for $C_{28}H_{25}N_3O_2S$; C, 71.92; H, 5.39; N, 8.99. Found: C, 71.99; H, 5.45; N, 9.02.

2.1.3.16. 5-(4-methoxybenzylidene)-2-(4-methoxyphenyl)-3-(4-methylquinolin-2-ylamino)thiazolidin-4-one ***(5p)***. Yield 52%, mp 229–231 °C IR (*v*, cm^{-1}): 1527 (=C-H), 2976 (=C-H), 1573 (C=N); ^1H NMR (400 MHz, DMSO-d_6, δ):8.26 (s, 1H, NH), 8.12 (s, 1H, H_3 of quinoline), 6.98–7.88 (m, 12H), 6.69 (s, 1H, thiazolidinone, 2nd position), 5.68 (s, 1H, =CH-Ar), 4.26 (s, 3H, OCH_3 of phenyl), 4.17 (s, 3H, OCH_3 of phenyl), 2.72 (s, 3H, CH_3 of quinoline). MS: *m/z* = 484.25 (M+1); Anal. Calcd for $C_{28}H_{25}N_3O_3S$; C, 69.54; H, 5.21; N, 8.69. Found: C, 69.58; H, 5.28; N, 8.73.

2.1.3.17. 5-Benzylidene-3-(4-methylquinolin-2-ylamino)-2-(thiophen-2-yl)thiazolidin-4-one ***(5q)***. Yield 67%, mp 189–191 °C IR (*v*, cm^{-1}): 1526 (=C-H), 2979 (=C-H), 1569 (C=N); ^1H NMR (400 MHz, DMSO-d_6, δ): 8.34 (s, 1H, NH), 8.13 (s, 1H, H_3 of quinoline), 6.93–7.75 (m, 12H), 6.72 (s, 1H, thiazolidinone, 2nd position), 5.81 (s, 1H, =CH-Ar), 2.76 (s, 3H, CH_3 of quinoline). MS: *m/z* = 430.19 (M+1); Anal. Calcd for $C_{24}H_{19}N_3OS_2$; C, 67.11; H, 4.46; N, 9.78. Found: C, 67.18; H, 4.51; N, 9.83.

2.1.3.18. 5-(4-methylbenzylidene)-3-(4-methylquinolin-2-ylamino)-2-(thiophen-2-yl)thiazolidin-4-one ***(5r)***. Yield 54%, mp 182–183 °C IR (*v*, cm^{-1}): 1531 (=C-H), 2971 (=C-H), 1573 (C=N); ^1H NMR (400 MHz, DMSO-d_6, δ): 8.36 (s, 1H, NH), 8.17 (s, 1H, H_3 of quinoline), 6.98–7.96 (m, 12H), 6.92 (s, 1H, thiazolidinone, 2nd position), 5.84 (s, 1H, =CH-Ar), 2.78 (s, 3H, CH_3 of quinoline), 2.36 (s, 3H, CH_3 of phenyl). MS: *m/z* = 444.61 (M+1); Anal. Calcd for $C_{25}H_{21}N_3OS_2$; C, 67.69; H, 4.77; N, 9.47. Found: C, 67.68; H, 4.77; N, 9.51.

2.2. In vitro antimalarial activity

Schizont Maturation Inhibition (SMI) assay was performed against Chloroquine sensitive strain, 3D7 and Chloroquine resistant strain, RKL9 of *Plasmodium falciparum* in RPMI medium (Rieckmann et al., 1978). The cultures of both strains were synchronized using 5% aqueous sorbitol solution. All stages except trophozoites (ring form) of parasite were degenerated and removed by centrifugation at 1500 rpm for 5 min. The test solutions were prepared in 0.1 ml DMSO and further diluted to produce concentration of 1.98–500 μg/ml with RPMI 1640 medium. 96 well plates were used for both cultures and inoculated with synchronized parasites. The plates were kept for 24–30 h in 5% CO_2 incubator at 37 °C. Thick smear was prepared for each well, stained with Giemsa stain and examined microscopically. EC_{50} was calculated for evaluation of antimalarial potential (Saini et al., 2016).

2.3. In vivo antimalarial screening

Acute toxicity study was performed by following OECD guidelines 423. Overnight fasted 4–6 week old using Swiss Albino Mice were used. The mice was observed for any symptom of toxicity such as change in colour of eyes, fur, urination, defecation, posture alteration, aggressiveness or any lethal response for first 4 h and examined for 24 h. If no lethal response was found, then second animal was administered with higher dose and observed as earlier. The animal were given dose for 14 days and kept under observation for the calculation of LD_{50} (Ecobichon, 1977).

In vivo antimalarial potential was investigated by 4-day suppressive test using Swiss Albino Mice of 4–6 week (22±5 g, 5 animal/group) against *P. berghei*. Each mice was inoculated with 0.2 ml of 1×10^6 parasitized RBC (from donor mice) intraperitoneally on day 0. Two hour post-infection, all the test groups were

administered with test drug at 200 mg/kg and standard at 5 mg/kg for 4 consecutive days. On day 4, thin smears were prepared from tail vein and were stained using Giemsa stain for evaluation of parasitemia microscopically. The mortality was observed for 7 days to check the survival rate. Percentage of parasitemia inhibition was calculated by (A-B)/A × 100, where A is the parasitemia of negative control (without any treatment) and B is the parasitemia of each test group (Devi et al., 2001; Manohar et al., 2012).

2.4. Docking study

In an effort to get insight the factors determining the bioactivity of novel arylidene derivatives of thiazolidinone-quinoline hybrids, docking simulations were performed in the active sight of *Plasmodium falciparum* lactate dehydrogenase (Singh et al., 2016; Prathiban et al., 2015). The molecular docking study was done by AutodockVina and autodock tools using Lamarckian Genetic Algorithm (Trott and Olson, 2010). The crystal structure of protein (PDB ID: 1CET) was obtained from Protein Data Bank (www.rcsb.org) (Kaushik et al., 2015). The structures of five ligands were prepared using ChemDrawUltra 8.0.3. Then the structures were converted into required. pdbqt format using ADT 1.5.6. During protein preparation, all the water molecules were removed; polar hydrogen and partial charges were added and saved as. pdbqt. The active grid was generated for docking with size 40 × 40 × 40 along x, y & z centres, 25.8, 26.829 & 9.405 respectively with 0.375 Å grid spacing. Further, ADT and Python were used for visualisation and identification of residues involved in binding.

3. Result and discussion

3.1. Chemistry

The synthetic protocol was successfully followed for the synthesis of thiazolidinone and their respective arylidene derivatives. The first step, compounds **(3a-e)** were prepared in good yield by condensation of 2-hydrazino-4-methylquinoline with different aromatic aldehyde in acidic conditions. In next step, hydrazone intermediates were converted into thiazolidinone **(4a-e)** by reacting with thioglycolic acid in dioxane. Finally the target compounds, 5-Arylidene-3-(4-methylquinolin-2-ylamino)-2-arylthiazolidin-4-one, were obtained by reacting equimolar amount of **(4a-e)** and aryl aldehyde in Glacial acetic acid.

Characteristic data from FTIR and ^{1}H NMR was studied for the progression of reaction. A strong band in range of 3200–3400 cm^{-1} is attributed to secondary amine present in all the intermediates and final compounds. Similarly, ^{1}H NMR of all the derivatives were supported by the presence of one broad singlet corresponding to NH nearly at δ 8.3–8.6 and a sharp singlet for proton at 3rd position in quinoline ring around δ 8.2. Characteristic peak in FTIR of compounds **(4a-e)** was observed in range of 1650–1660 cm^{-1} due to the presence of carbonyl group. In ^{1}H NMR, two distinct singlets corresponding to C-CH_2-S and N-CH-S at δ 5.29 and 6.77 ppm respectively, confirmed the synthesis of thiazolidinone ring. Further, disappearance of singlet at δ 5.29 for two protons with emergence of new singlet in δ 5.88 for single proton authenticated the progression of reaction from thiazolidinone to target arylidene derivatives. Eventually, all the structures of synthesized compounds were found in accordance with mass and elemental analysis.

3.2. In vitro antimalarial evaluation and structure-activity relationship

The antimalarial potential of entire set of synthesized analogues was assessed by *in vitro* antimalarial assay against Chloroquine-sensitive, 3D7 and Chloroquine-resistant, RKL9 strains of *Plasmodium falciparum*. The activity results of the target molecules have been displayed in Table 1. All compounds exhibited good antimalarial potency with EC_{50} range 0.432–2.672 μg/ml against 3D7 while against RKL-9 EC_{50} varies from 0.824 to 11.451 μg/ml. EC_{50} value for all the synthesized derivatives against both Chloroquine sensitive and Chloroquine resistant strain has also been represented graphically in Fig. 1. Compound **5g** was found to be most potent among the series with EC_{50} of 0.423 μg/ml and 0.824 μg/ml against 3D7 and RKL-9 respectively. Compound **5r** displayed maximum EC_{50} against both the strains and hence considered as least active compound of the series. Compounds **5b, 5e, 5g, 5j** and **5n** were found to be five most potent analogues among the series with EC_{50} (3D7/RKL-9) values of 0.731/1.617, 0.734/2.011, 0.423/0.824, 0.562/0.992 and 0.632/1.211 μg/ml respectively. From *in vitro* study, we have concluded the results in structure-activity relationship.

3.2.1. Structure-activity relationship

It has been revealed that the presence of chloro group at *p*-position of either ring led to the development of best candidate for malaria as compounds **5b, 5e, 5g, 5h, 5j** and **5n** were found to have EC_{50} less than 1 μg/ml. Unfortunately, 5f, substitution of both rings with chloro group didn't meet the expectation of enhanced potency and was found to be less active than above given (substitution with chloro group at one ring only) derivatives. Incorporation of methyl group along with chloro group was proven to be best match and hence **5g** was the most potent derivative among the series. Furthermore, it was also indicated that the presence of methyl group was more preferred as compared to methoxy group at *p*-position of phenyl rings. The effect of ring size was also established by replacing phenyl ring with five membered sulphur containing thienyl ring, the activity was reduced by many folds, even compound **5r**, least active among the series, belongs to this category. The complete Structure-activity relationship study regarding various substitution around arylidene derivatives suggested that *p*-position of rings should be occupied by chloro group at one ring while methyl ring at another to get remarkable antimalarial activity. The SAR study of series is depicted in Fig. 2.

3.3. In vivo antimalarial activity

No mortality was observed during acute toxicity study. At the end of study, the dose calculated for all the synthetic derivatives was 200 mg/kg. Among the synthesized analogues **5 a-r**, five most potent compounds, **5b, 5e, 5g, 5j** and **5n**, were further selected for *in vivo* antimalarial evaluation. Compounds were screened against *P. berghei* in swiss albino mice using 4-day suppressive test. Compound **5g**, bearing *p*-chloro group in one ring and *p*-methyl group in another, showed best activity with 73.38% of parasitemia inhibition. Four mice out of five were found to be alive on 7th day. Antimalarial potential displayed by 5 selected compounds has been presented in Table 2. Microscopic examination of thin smears of control group, standard (Chloroquine), **5g** (most active) and **5e** (least active) from *in vivo* study has been displayed in Fig. 3. Further the plausible route for antimalarial activity of compounds was studied through *in-silico* approach.

Table 1
In vitro antimalarial activity of synthetic derivatives **5(a-r)** against CQ-sensitive (3d7) and CQ-resistant (RKL-9) strain of *P. falciparum*.

S. No.	Compounds	Ar_1	Ar_2	EC_{50} (3D7, μg/ml)	EC_{50} (RKL-9, μg/ml)
1.	5a	C_6H_5-	C_6H_5-	1.012	3.121
2.	5b	C_6H_5-	*p*-Cl C_6H_4-	0.731	1.617
3.	5c	C_6H_5-	*p*-$CH_3C_6H_4$-	1.212	4.723
4.	5d	C_6H_5-	*p*-$OCH_3C_6H_4$-	1.230	5.123
5.	5e	*p*-Cl C_6H_4-	C_6H_5-	0.734	2.011
6.	5f	*p*-Cl C_6H_4-	*p*-Cl C_6H_4-	0.783	2.026
7.	5g	*p*-Cl C_6H_4-	*p*-$CH_3C_6H_4$-	0.423	0.824
8.	5h	*p*-Cl C_6H_4-	*p*-$OCH_3C_6H_4$-	0.791	2.114
9.	5i	*p*-$CH_3C_6H_4$-	C_6H_5-	1.501	6.726
10.	5j	*p*-$CH_3C_6H_4$-	*p*-Cl C_6H_4-	0.562	0.992
11.	5k	*p*-$CH_3C_6H_4$-	*p*-$CH_3C_6H_4$-	1.732	9.001
12.	5l	*p*-$CH_3C_6H_4$-	*p*-$OCH_3C_6H_4$-	1.621	7.992
13.	5m	*p*-$OCH_3C_6H_4$-	C_6H_5-	1.414	6.023
14.	5n	*p*-$OCH_3C_6H_4$-	*p*-Cl C_6H_4-	0.632	1.211
15.	5°	*p*-$OCH_3C_6H_4$-	*p*-$CH_3C_6H_4$-	1.536	7.231
16.	5p	*p*-$OCH_3C_6H_4$-	*p*-$OCH_3C_6H_4$-	1.801	9.373
17.	5q	2-thienyl	C_6H_5-	1.931	9.921
18.	5r	2-thienyl	*p*-$CH_3C_6H_4$-	2.672	11.451
19.	CQ	–	–	0.375	0.80

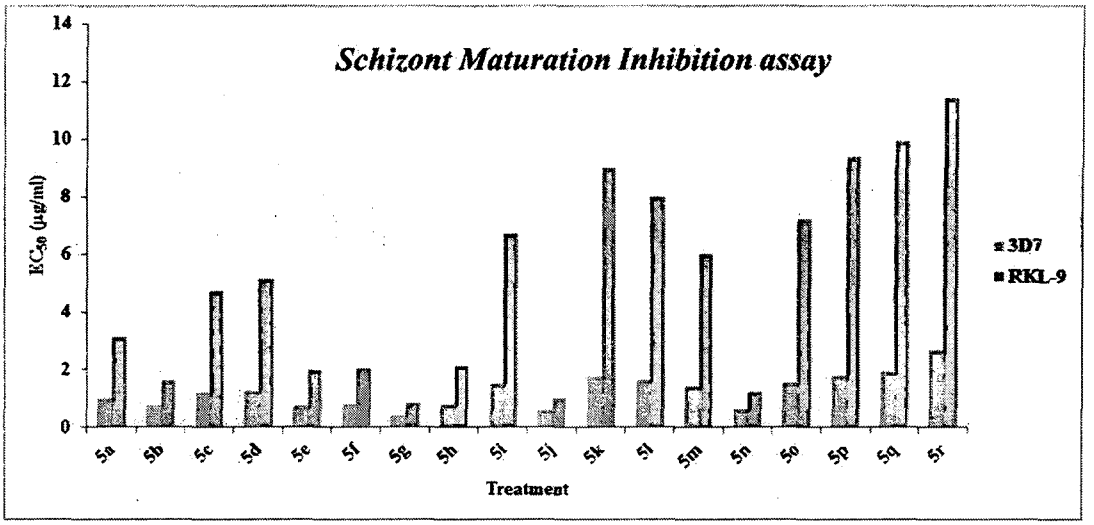


Fig. 1. EC_{50} of synthesized derivatives, **5a-r**, against 3D7 and RKL-9 of *Plasmodium falciparum*.

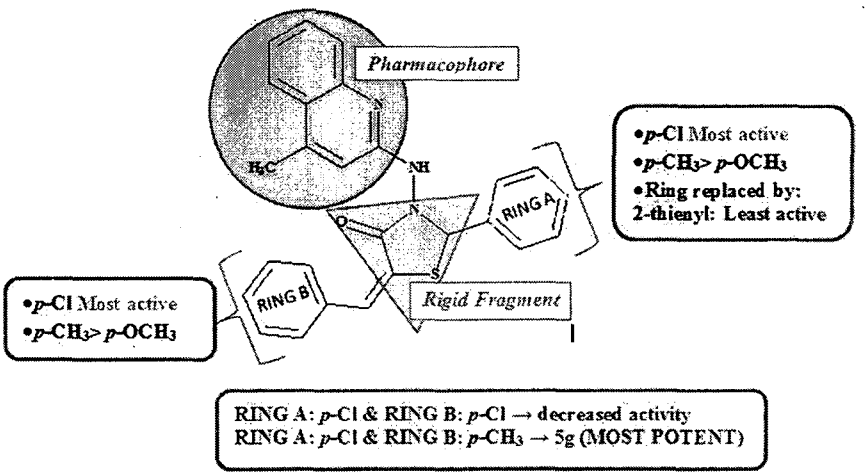


Fig. 2. Structure-Activity Relationship of Arylidene derivatives of Thiazolidinone-quinoline hybrids.

Table 2
Effect of five selected compounds on parasitemia of *P. berghei* infected mice.

S. No.	Drug treatment	Dose\kg	No. Of animals	%parasitemia	Percentage inhibition	Survival on 7th day
1.	Control	–	5	49.62 ± 0.304	–	0/5
2.	Standard	5 mg/kg	5	–	100	5/5
3.	5b	200 mg/kg[a]	5	29.43 ± 0.312	40.68**	2/5
4.	5e	200 mg/kg[a]	5	35.72 ± 0.403	28.01	3/5
5.	5g	200 mg/kg[a]	5	13.21 ± 0.126	73.38**	4/5
6.	5j	200 mg/kg[a]	5	17.34 ± 0.301	65.05**	4/5
7.	5n	200 mg/kg[a]	5	21.21 ± 0.116	57.26**	3/5

N = 5, Values are expressed as Mean ± SEM and analyze by ANOVA. **p < .01(significant). Values are compared with control group.
[a] Dose calculated by acute toxicity method.

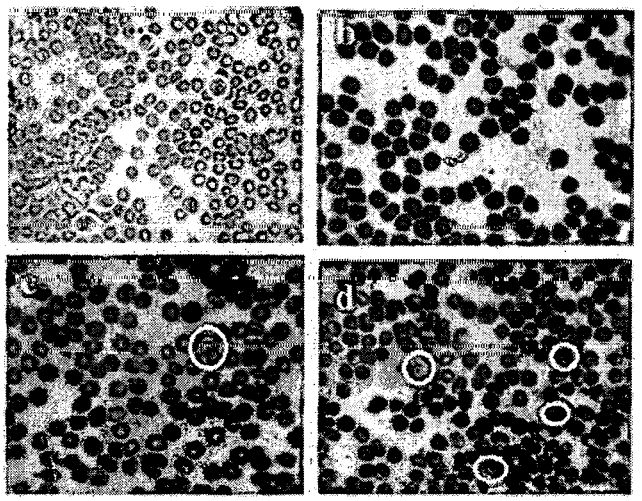


Fig. 3. Photomicrographs of blood smears of different groups showing parasitemia (encircled) (a) parasite infected RBCs in control group (b) parasitemia after treatment with standard drug, chloroquine c) parasitemia after treatment with **5g** (Most active) c) parasitemia after treatment with **5e** (Least active).

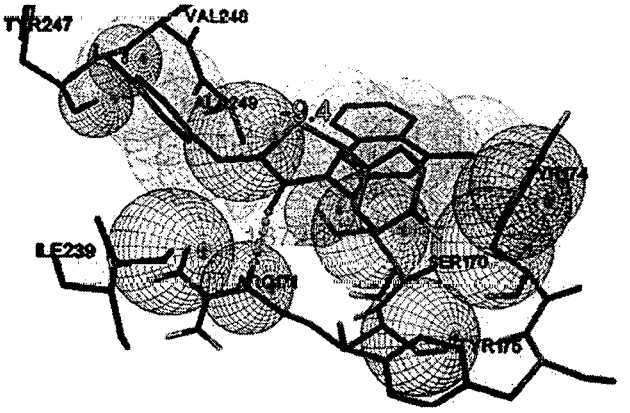


Fig. 4. Binding interaction of most potent synthesized ligand, **5g**, with active site of Lactate dehydrogenase (PDB ID: 1CET).

3.4. Docking study

Anaerobic life cycle of parasite *Plasmodium falciparum* is supported by *Plasmodium falciparum* Lactate Dehydrogenase (*Pf*LDH), being the terminal enzyme that leads the regeneration of NAD^+ from NADH for continual glycolysis. From the literature, it has been revealed that inhibition of *Pf*LDH may lead a pathway for designing of antimalarial agents and this has been illustrated in the present study by docking simulations using 1CET, *Pf* Lactate dehydrogenase enzyme complex as target protein. The docked confirmation and results of ligands in binding pocket is demonstrated in Table 3. The main amino acid residue that has played a vital role in interaction of ligands with target protein was ARG171. The biological evaluation was corroborated by *in silico* study as compound **5g** formed a stable interaction with *Pf*LDH enzyme having binding affinity −9.4 kCal/mol and hydrogen bond as well, depicted in Fig. 4. The results from docking study justified our preceding research of arylidene derivatives of quinoline-thiazolidinone hybrids as antimalarial agents.

4. Conclusion

In summary, we have synthesized novel series comprised of arylidene derivatives of quinoline-thiazolidinone hybrids, where compound **5g** exhibited promising *in vitro* antimalarial potency against both 3D7 and RKL-9 strains of *Plasmodium falciparum*. It also showed highest suppression of parasitemia against *P. berghei* during *in vivo* antimalarial screening. The present study also portrayed the utility of docking simulations to get a deep insight of interaction of synthesized scaffold with target proteins. From structure-activity relationship, **5g** may be utilised as a lead molecule for further investigation to improve their pharmacological potential. Thus, the current study serves as an encouragement for the development of new hybrids of thiazolidinone pharmacophore as antimalarial agent.

Conflicts of interest

The authors report no conflict of interest.

Table 3
Docking simulations of **5b**, **5e**, **5g**, **5j** and **5n** in active site of Lactate dehydrogenase receptor (PDB ID: 1CET).

S. No.	Compound	Dock Score (kCal/mol)	Number of hydrogen bonds	Amino Acid involved	Group of ligand involved
1.	5b	−9.0	1	ARG171	O of C=O (thiazolidinone)
2.	5e	−9.0	–	–	–
3.	5g	−9.4	1	ARG171	O of C=O (thiazolidinone)
4.	5j	−9.2	1	ARG171	O of C=O (thiazolidinone)
5.	5n	−9.2	–	–	–

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