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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

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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.

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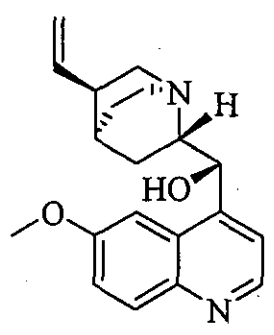
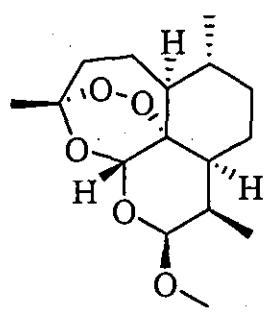
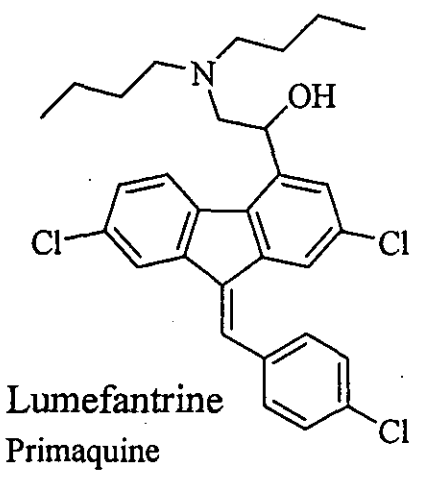
Mosquito control strategies (pesticide-treated nets and indoor residual spraying) had been extremely effective, but are currently ineffectual due to increased insecticide resistance. Similarly, first-line drug-based medications, especially Artemisinin combination therapy (ACTs), are at risk (Fernandez-Alvaro, 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).



Quinine

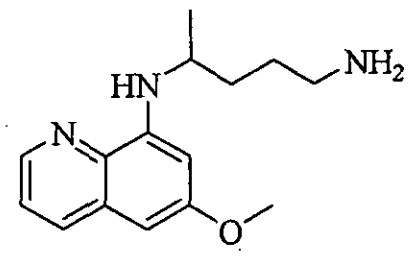


Artemether

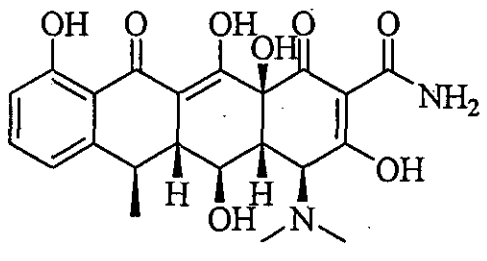


Lumefantrine

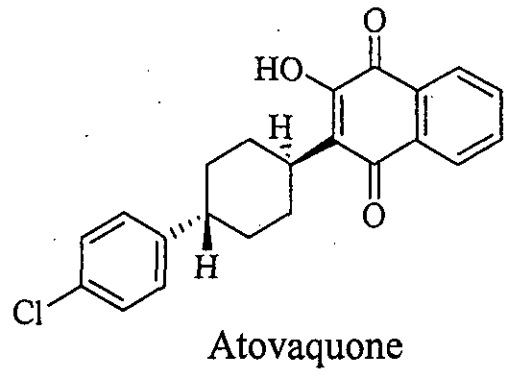
Primaquine



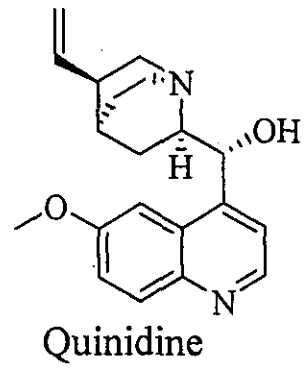
Primaquine



Doxycycline



Atovaquone



Quinidine

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

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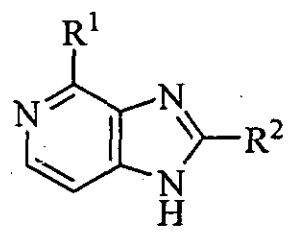
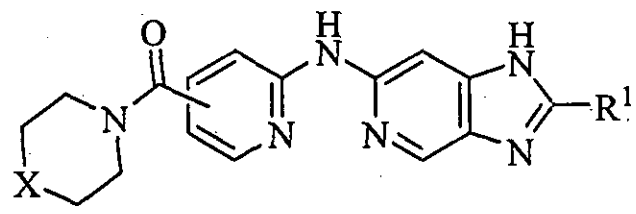
a]pyrazin-2(1*H*)-yl)-1*H*-imidazo[4,5-*c*]pyridines (Formula-2) (André Horatscheck et al., *J. Med. Chem.* 2020, *63* (21), 13013–13030). These reactions were carried out through multi-step reactions and characterized through various spectroscopic techniques. All synthesized compounds were screened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

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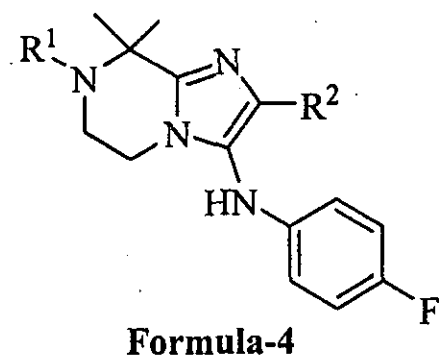
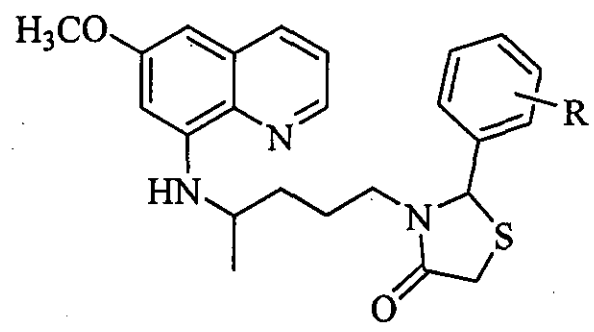


Formula-4

R^1, R^2 = Various substituents

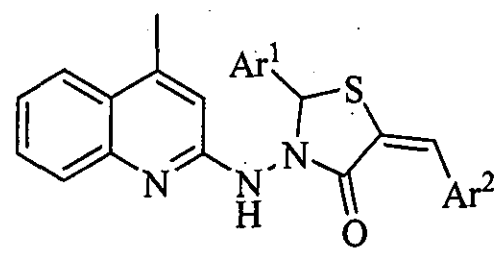
Anna Caroline C. Aguiar et al., have synthesized 3-(4-((6-methoxyquinolin-8-yl)amino)pentyl)-2-phenylthiazolidin-4-ones (Formula-5) (Anna Caroline C. Aguiar et al. *Malar. J.* 2017, *16* (110), 1–11) via one-pot synthesis. The synthesized compounds were evaluated for their *in-vitro* and *in-vivo* antimalarial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

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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)

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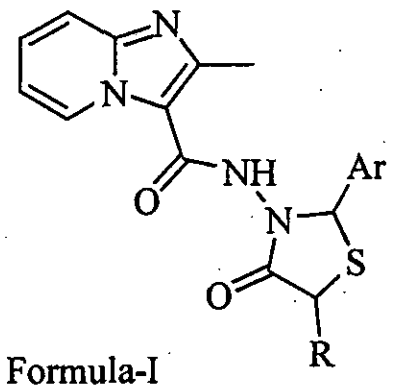
SUMMARY OF THE INVENTION

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:

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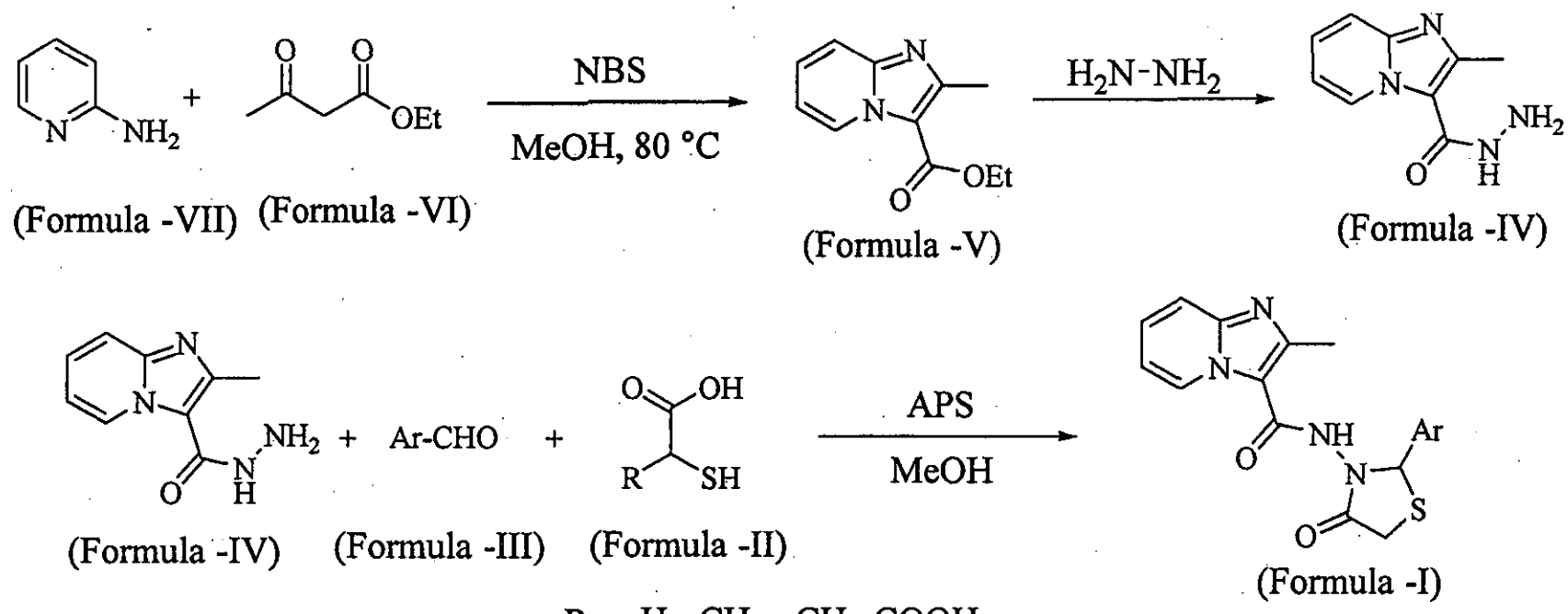

Scheme-1

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

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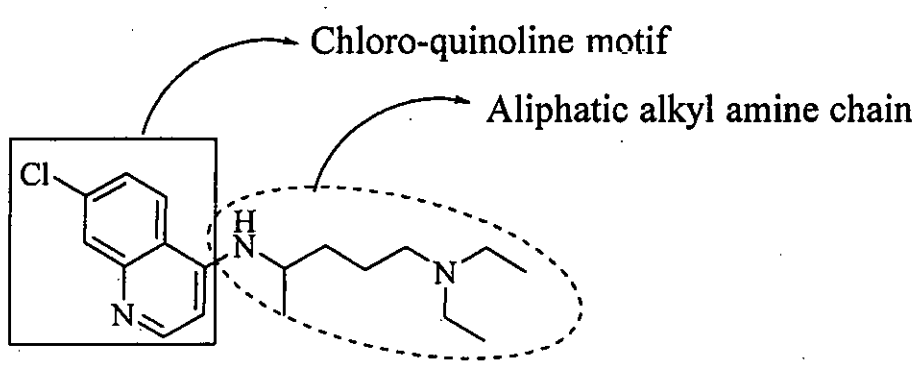
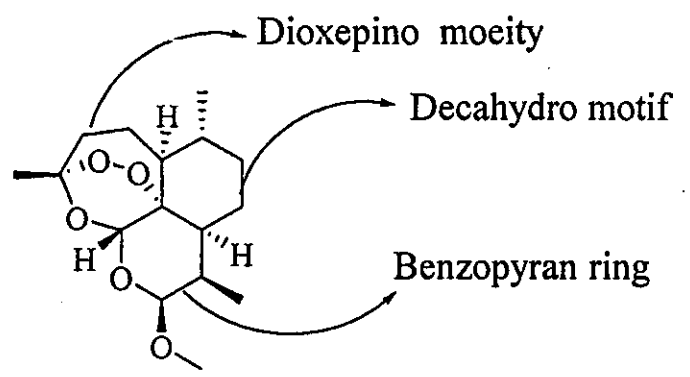
present invention relates to one-pot synthesis optimizing the in-process the isolation of intermediate steps to yield compound of Formula (I).

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.



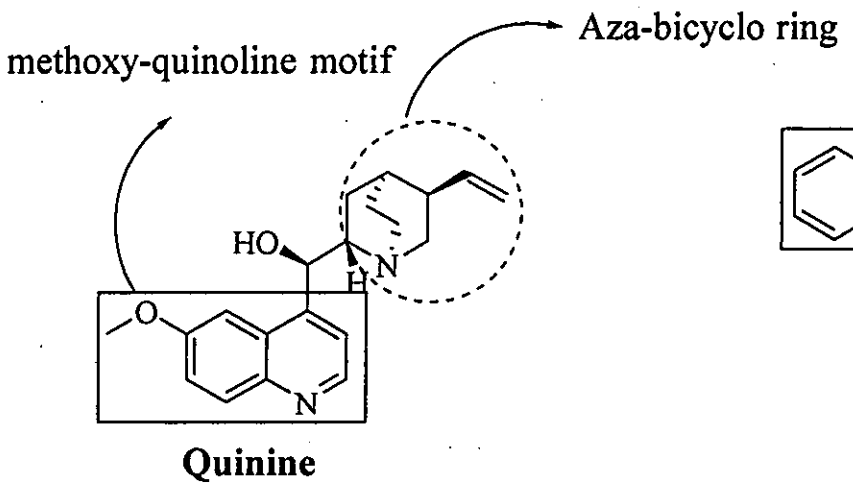
Chloroquine

Aliphatic alkyl amine chain
with chloro-quinoline motif



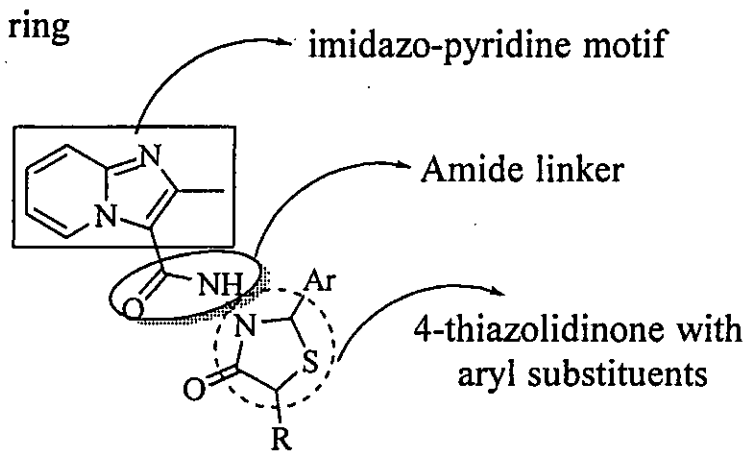
Artemether

Benzopyran based dioxepino decahydro motif



Quinine

Aza-bicyclo ring embedded
with methoxy-quinoline moiety



Formula-I

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

Our novel invention has a novel structural hybrid of imidazo-pyridine embedded with 4-thiazolidinone

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*.

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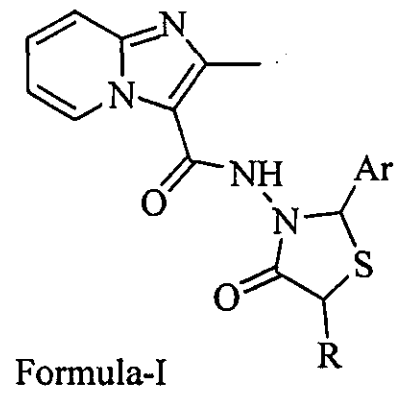
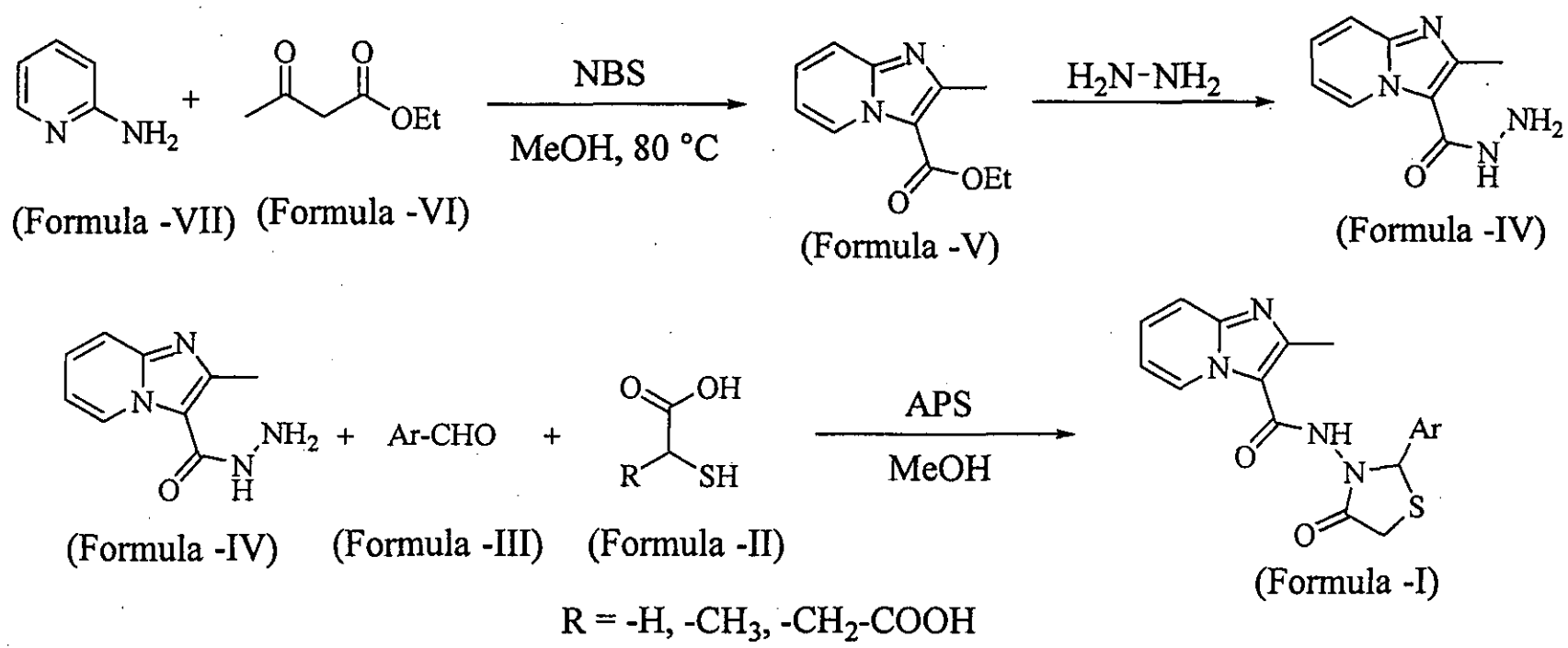


Formula-I

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.

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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).

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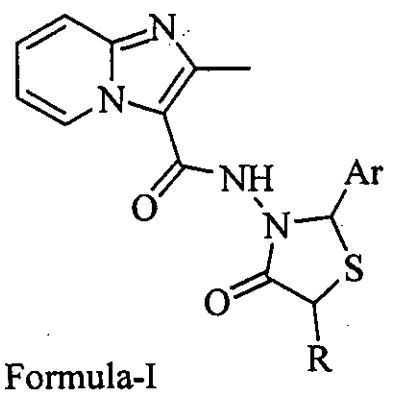
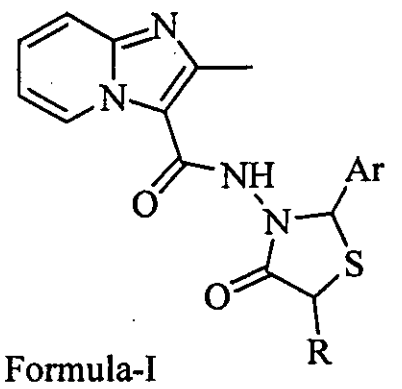


Formula-I

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

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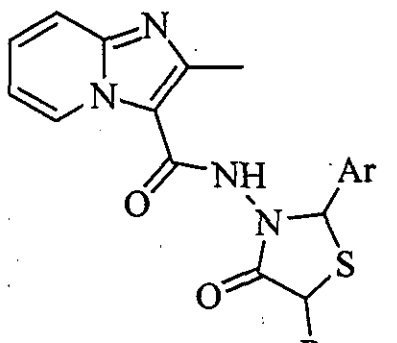
1 j	-3-NO_2-C_6H_4	-H
1 k	-4-NO_2-C_6H_4	-H
1 l	-CH=CH-C_6H_5 / -C_8H_7	-H

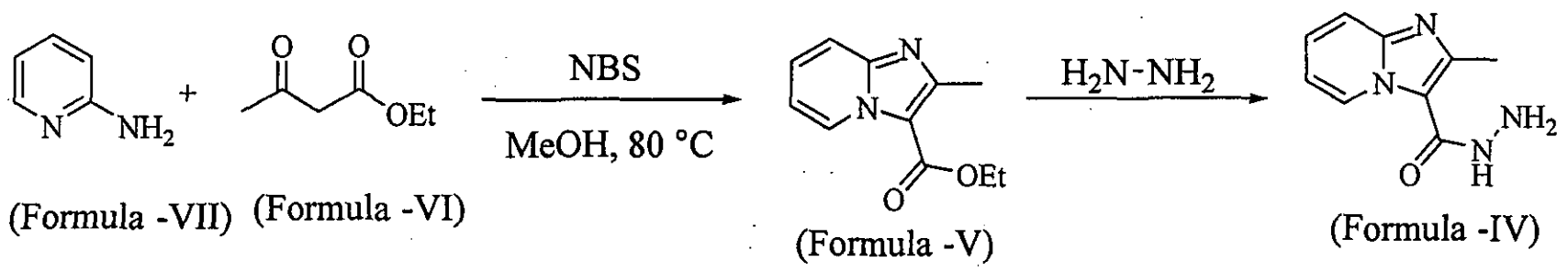
In one exemplary embodiment, wherein Formula-I is further defined as



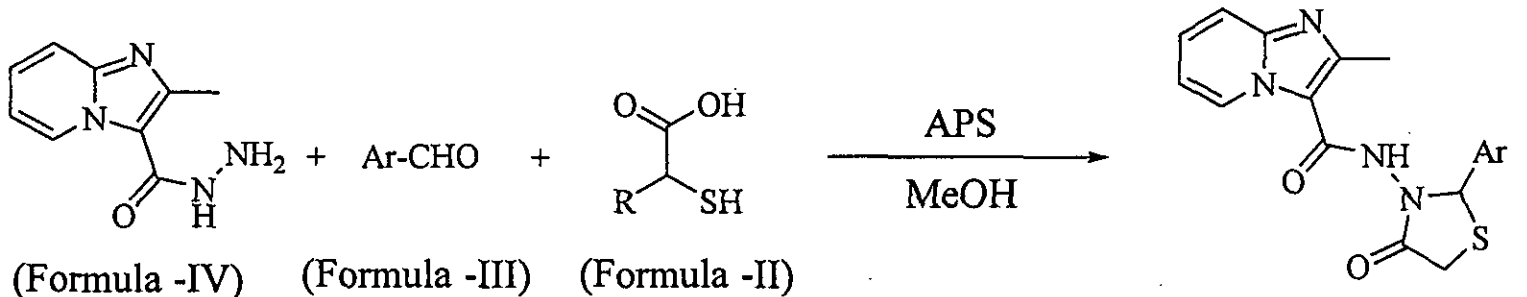
Formula-I

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):

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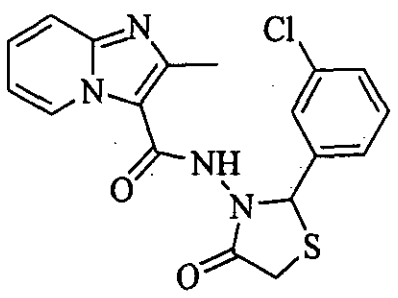

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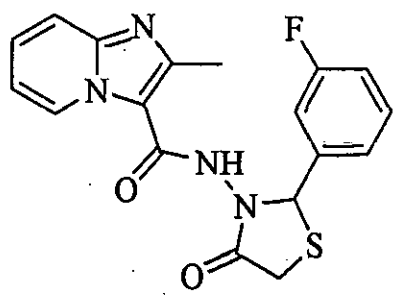

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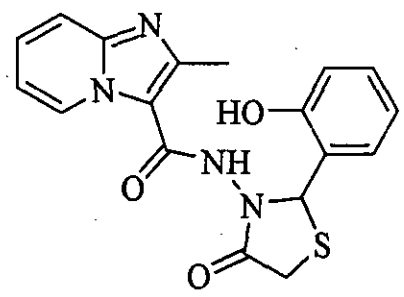

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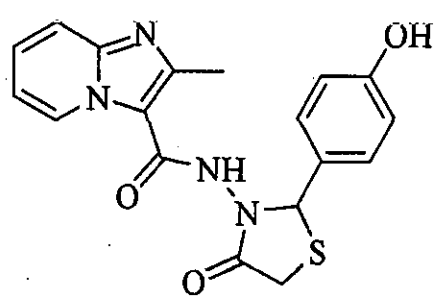

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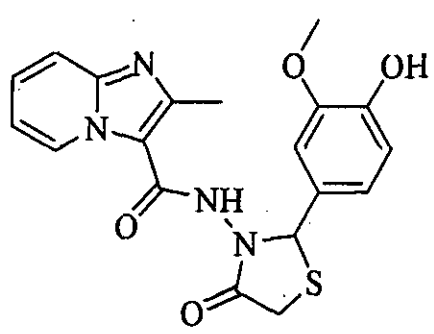

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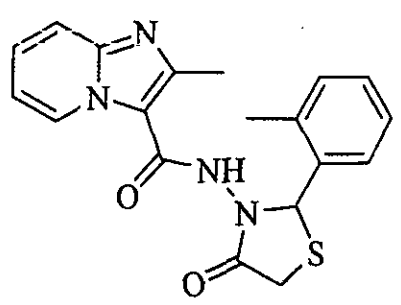

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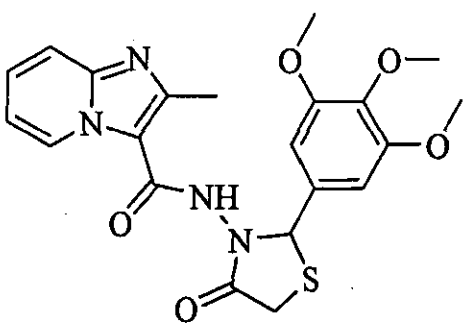

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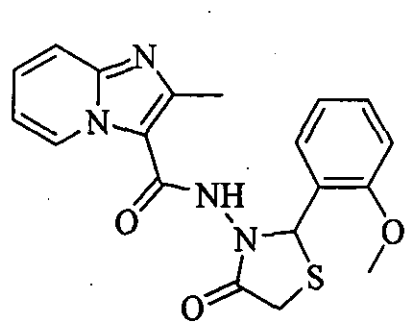

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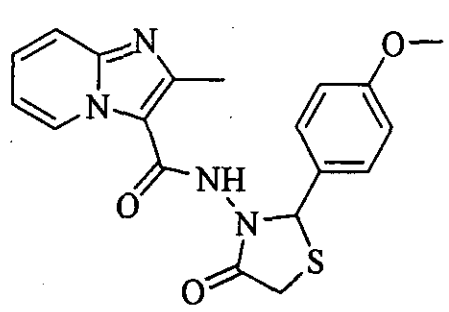

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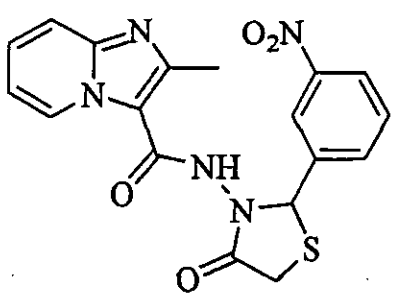

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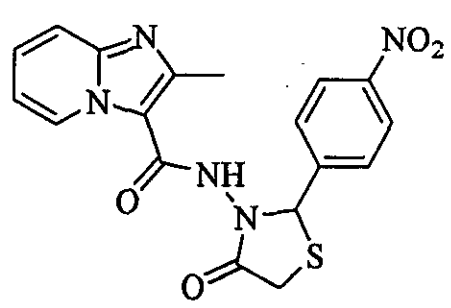

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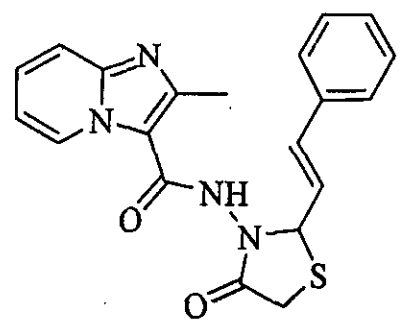

1l

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,

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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

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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

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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]**

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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.

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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]**

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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).

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

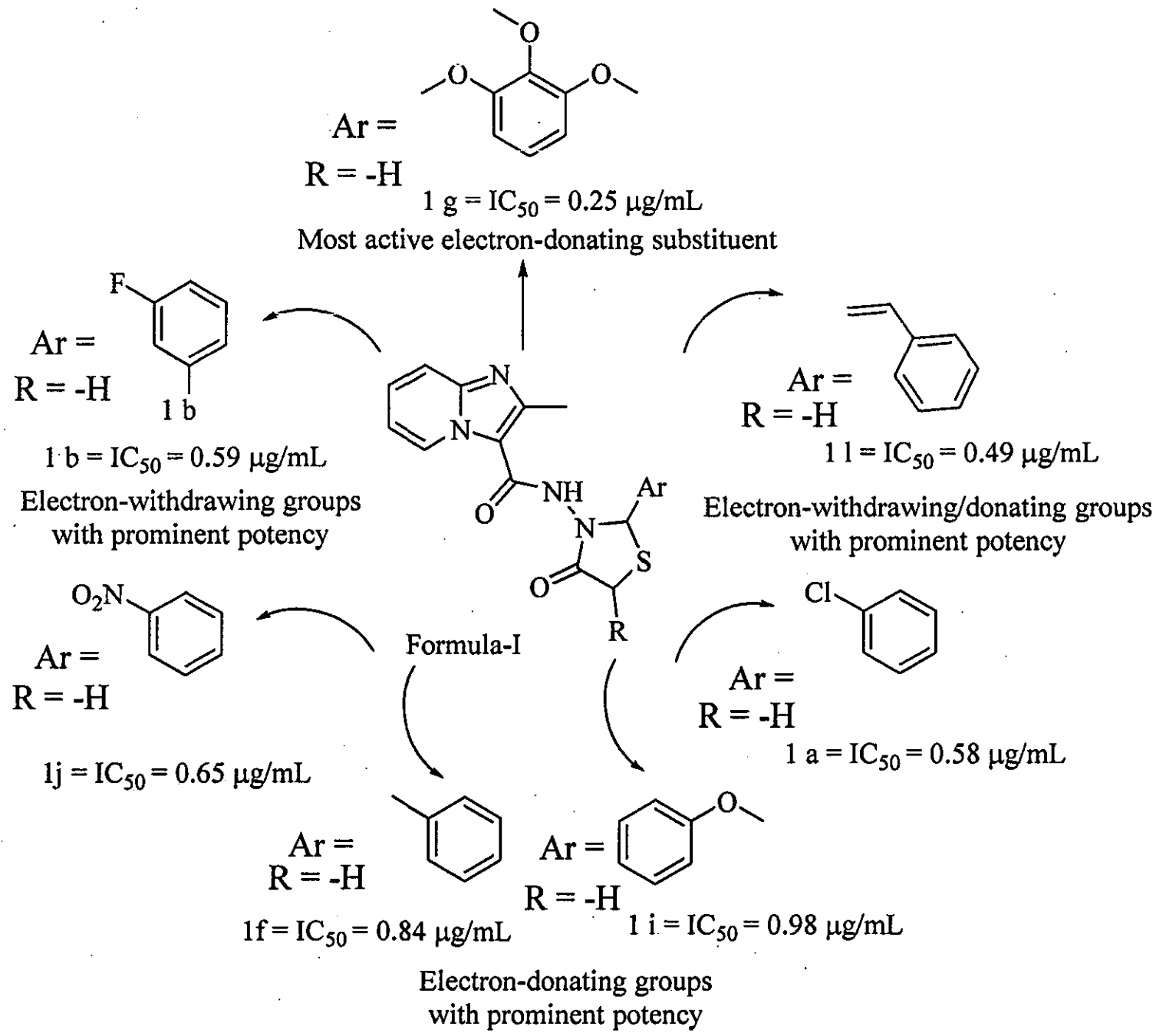

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Fig. 3: Schematic representation of innovation in the of structure-activity relationship based on Formula (I)

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

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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-

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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

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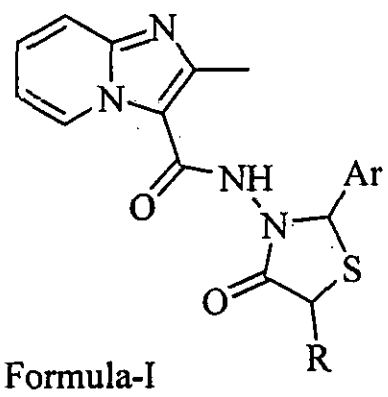
We claim,

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

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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*.

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For, MK Bhavnagar University, Bhavnagar,

Date: 9th June, 2022

Prof. Nisheeth C. Desai

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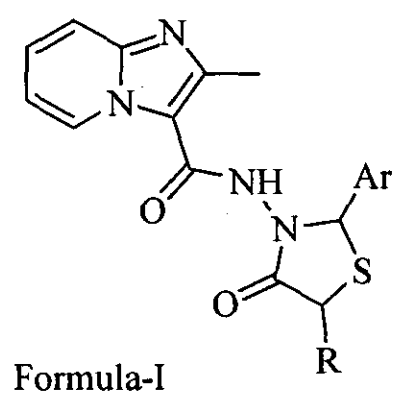
Thiazolidin-3-yl-Imidazo-pyridine-3-carboxamide as antimalarial agents

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

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