SYNTHESIS AND ANTIMALARIAL ACTIVITY OF SOME NEW 3-PHENYL-2-THIOXOTHIAZOLIDIN-4-ONE DERIVATIVES

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Received: 28 Dec 2016, Revised and Accepted: 20 Mar 2017

ABSTRACT

Objective: Current therapies to treat *P. falciparum* malaria are heavily reliant on artemisinin-based combinations. However, resistance to artemisinin has recently been identified, and resistance to key artemisinin partner drugs is already widespread. Therefore, there is an urgent need for new antimalarial drugs with improved attributes over older therapies. The objective of this research work is to synthesize new antimalarial agents more effective against clinically relevant malarial strains.

Methods: In present work, a series of ten 3-phenyl-2-thioxothiazolidin-4-one (MF1-MF10) derivatives, were synthesized by Knoevenagel condensation of N-phenyl rhodanine (I) with substituted aromatic or hetero aromatic aldehydes using microwave irradiation. N-phenyl rhodamine (Ⅰ) was synthesized by a conventional reaction involving methyl-2-mercaptoacetate (1) and phenyl isothiocyanate in presence of triethylamine. All the synthesized compounds were characterized by various spectroscopic techniques and evaluated for *in-vitro* antimalarial activity by micro dilution technique against resistant strains of *Plasmodium falciparum*.

Results: The antimalarial activity data showed that six compounds (MF5, MF6, MF7, MF8 and MF9) exhibited IC50 values ranging from 1.0-1.30 µg/ml, three compounds (MF6, MF7 and MF8) displayed IC50 values in the range of 0.9-1.0 µg/ml. Compound MF9 showed most significant result with maximum activity (IC50 = 0.85µg/ml).

Conclusion: The antimalarial activity results revealed that compound MF5 possess potent activity and could be identified as a promising lead for further investigation.

Keywords: *P. falciparum*, 3-phenyl-2-thioxothiazolidin-4-one, Antimalarial activity

INTRODUCTION

Malaria remains one of the most important infectious disease problems in the world, accounting for an estimated 212 million cases and up to 429,000 deaths in 2015. Malaria is caused by five species of parasites belonging to the genus *Plasmodium*. Four of these, *P. falciparum, P. vivax*, *P. malariae* and *P. ovale* are human malaria species that are spread from one person to another via the bite of female mosquitoes of the genus *Anopheles*. [1] *Plasmodium falciparum* is the most lethal protozoan parasite of the genus which is responsible for malaria complications such as cerebral malaria or severe anaemia. [2, 3] At present, no effective vaccines are available due to the high mutability of the genome of *P. falciparum*,[4] meanwhile, resistance of malaria parasites has also quickly developed to a variety of quinoline analogs (e.g., chloroquine), antifolates (e.g., sulfadoxine-pyrimethamine) and inhibitors of electron transport (e.g., atovaquone). What's worse, resistance to artemisinin has now emerged.[5, 6] Accordingly, the discovery of new effective drugs to counter the spread of malaria parasites that are resistant to existing agents, especially acting on multi-targets, is an urgent need. The development of drug resistance has become a major health concern and has stimulated the search for alternative antimalarial agents. In this perspective rhodamine nucleus offers an alternative due to presence of wide spectrum of activities such as antibacterial [7], anti-inflammatory [8], antiviral [9, 10], antidiabetic [11], anticancer [12], tyrosinase inhibitors [8] and antimalarial [13] and are frequently associated with low toxicity and they can be considered as a privileged scaffold and an ideal framework for the design of compounds that can interact with different targets as their inherent affinity for several biological targets [14]. In present work, a series of ten new 3-phenyl-2-thioxothiazolidin-4-one (MF1-MF10) derivatives were synthesized and evaluated for their *in-vitro* antimalarial activities against resistant strain of *Plasmodium falciparum*. Herein synthesis and antimalarial activity of some new 3-phenyl-2-thioxothiazolidin-4-one derivatives is reported.

MATERIALS AND METHODS

Melting points were determined by the open capillary method and are uncorrected. The progress of the reactions was monitored by thin layer chromatography (TLC) with ethyl acetate: hexane (1:1 v/v) as eluent. TLC was carried out on precoated plates (silica gel 60, F254) and visualized with UV light. Column chromatography was performed on silica gel (100-200). Antón Paar, Monowave 300, Microwave Synthesis Reactor was used for microwave-assisted synthesis. Infrared spectra were determined as KBr pellets on a Shimadzu IR affinity-1 model 1400 spectrophotometer and are expressed in cm⁻¹. ¹H NMR spectra were recorded on a Bruker's Avance-III FT NMR spectrometers using CDCl₃ as a solvent; chemical shifts are expressed in δ ppm. HRMS spectral data were obtained with a Bruker micro, TOF QII high-resolution mass spectrometer and both the above analysis were performed in Indian Institute of science and research technology (IISER, Bhopal); IR analyses were performed in Department of Pharmacy, S. G. S. I. T. S., Indore M. P.

General method for synthesis of N-phenyl Rhodanine [15]

A mixture of phenyl isothiocyanate (0.11 mmol), methyl-2-mercaptoacetate (0.1 mmol) and Et,N (0.03 mmol) in CH₂Cl₂ was stirred for 1 hour. Excess isothiocyanate was removed by amino- methylated polystyrene resin (0.015 mmol). The solution was filtered and concentrated to give N-phenyl rhodamine (I).

General method for Synthesis of MF1-MF10

A mixture of N-phenyl rhodanine (1) (0.2 mmol), substituted aromatic/heteroaromatic aldehydes (0.2 mmol), and three drops of piperidine in absolute ethanol (5 ml) were thoroughly mixed in a glass vial (G10/G30). The reaction mixture was then heated with microwave irradiation at 100 °C for 25 min (table 1). After cooling, the solid mass was placed in 50 ml of cold ethanol and crushed ice.
The slurry was filtered to give solid mass and dried under vacuum to give corresponding MF, to MF₃ derivatives.

### Table 1: Experiment setting and method for microwave assisted synthesis

<table>
<thead>
<tr>
<th>Step</th>
<th>Program</th>
<th>Temperature °C</th>
<th>Time mm:ss</th>
<th>Cooling</th>
<th>Stirrer Speed Rpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Heat as fast as possible</td>
<td>100</td>
<td>-</td>
<td>Off</td>
<td>600</td>
</tr>
<tr>
<td>2.</td>
<td>Hold</td>
<td>-</td>
<td>25:00</td>
<td>Off</td>
<td>600</td>
</tr>
<tr>
<td>3.</td>
<td>Cool down</td>
<td>55</td>
<td>0</td>
<td>On</td>
<td>600</td>
</tr>
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</table>

(Z)-5-benzylidene-3-phenyl-2-thioxothiazolidin-4-one (MF₁)

Yellow crystal; IR (KBr) cm⁻¹: 3044.77 (+=CH, stretch), 2932.54 (+=CH, stretch, aromatic), 1710.93 (C=O), 1607.74 (C=S), 1495.86 (C=C, aromatic), 1781.20 (C=C, bend, aromatic), 1675.21 (C=N); ¹H NMR (CDCl₃): 8.79 (d, 2H, Pyridine), 7.75 (d, 2H, N-Phenyl), 7.67 (s, 1H, =CH), 7.54 (t, 2H, Pyridine), 7.47 (t, 3H, N-Phenyl); HRMS (ESI⁺) (m/z): [M+1], 304.

(Z)-5-(pyridin-2-ylmethylene)-2-thioxothiazolidin-4-one (MF₂)

Orange crystal; IR (KBr) cm⁻¹: 3070.81 (+=CH, stretch), 3016.8 (C=C, aromatic), 1718.85 (C=O), 1592.31 (C=S), 1543.12 (C=C, aromatic), 807.24 (C=C, bend, aromatic), 1693.57 (C=N); ¹H NMR (CDCl₃): 8.76 (d, 2H, Pyridine), 7.66 (s, 1H, =CH), 7.36 (d, 2H, Pyridine), 7.51 (t, 3H, N-Phenyl), 7.27 (t, 2H, N-Phenyl); HRMS (ESI⁻) (m/z): [M-1], 299.

(Z)-5-(4-(dimethylamino)benzylidene)-3-phenyl-2-thioxothiazolidin-4-one (MF₃)

Orange crystal; IR (KBr) cm⁻¹: 3083.34 (+=CH, stretch), 2924.21 (+=CH, stretch, aromatic), 1735.94 (C=O), 1684.89 (C=S), 1583.63 (C=C, aromatic), 841 (C=C, bend, aromatic); ¹H NMR (CDCl₃): 7.89 (s, 1H, =CH), 7.47 (d, 2H, N-Phenyl), 7.43 (t, 3H, N-Phenyl), 7.32 (d, 2H, Phenyl), 7.24 (d, 2H, Phenyl), 3.06 (s, 6H CH₃); HRMS (ESI⁻) (m/z): [M-1], 341.

In vitro antimalarial evaluation

**Assay protocol**

All the synthesized compounds were screened for in vitro antimalarial activity at Microcare laboratory and TRC, Surat, Gujarat. The in vitro antimalarial assay was carried out in 96 well microtiter plates according to the microassay protocol of Rieckmann and co-workers with minor modifications. All the cultures of *P. falciparum* strains were maintained in medium RPMI 1640 supplemented with 25m MHEPES, 1% D-glucose, 0.23% sodium bicarbonate and 10% heat-inactivated human serum. The asynchronous parasites of *P. falciparum* which inhibited the complete maturation into schizonts was recorded as the IC₅₀ value of test compounds. The test concentration was determined by samples, prepared in DMSO and their subsequent dilutions were prepared with culture medium, then diluted samples were added to the test wells so as to obtain final concentrations ranging between 0.4µg/ml-100µg/ml in duplicate well-containing parasite cell preparation. The culture plates were incubated at 37 °C in a candle jar, after 36-40 h of incubation; thin blood smear slides were prepared from each well and stained with JSB stain. The slides were observed under a microscope 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 IC₅₀ value of test compounds.
RESULTS AND DISCUSSION

Chemistry

The 3-phenyl-2-thioxothiazolidin-4-one (MF<sub>1</sub>-MF<sub>10</sub>) derivatives describe in present research work are shown in table 2. N-Phenyl Rhodanine (1) was synthesized by reacting methyl thioglycolate with phenyl isothiocyanate at room temperature as outlined in scheme 1.

The intermediates 1 upon Knoevenagel condensation with suitably substituted aromatic/hetero aromatic aldehydes under microwave heating condition in presence of piperidine produced 3-phenyl-2-thioxothiazolidin-4-one (MF<sub>1</sub>-MF<sub>10</sub>) derivatives. This reaction generated a double bond that produced E and Z isomers. Similar analogs are reported to exist predominantly as Z-isomers. [8, 16] It is presumed that the derivatives synthesised here are mainly Z-isomers.

Methyl-2-mercaptoacetate (1) N-Phenyl Rhodanine (1); MF<sub>1</sub>-MF<sub>10</sub>

Scheme 1: Reagents and Conditions (i) Phenyl Isothiocynates, TEA, CH<sub>2</sub>Cl<sub>2</sub>, rt, 1h; (ii) Piperidine, ethanol, MW,100 °C, 25 Min

Table 2: Structure, molecular formula, molecular weight, % yield, melting point and antimalarial activity (IC<sub>50</sub>µg/ml) of MF<sub>1</sub>-MF<sub>10</sub> derivatives

<table>
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<tr>
<th>Comp. code</th>
<th>Substituent Ar</th>
<th>Molecular Formula</th>
<th>Molecular Weight</th>
<th>Melting Point °C</th>
<th>% yield</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; µg/ml</th>
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<tr>
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<td>-</td>
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<td>0.268</td>
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Antimalarial activity

All the compounds were screened for intra-erythrocytic in vitro antimalarial activity against resistance strains of Plasmodium falciparum by using chloroquine and quinine as reference drugs. The results of antimalarial activity are summarised in table 2. Among the ten evaluated compounds, six compounds exhibited IC<sub>50</sub> values ranging from 1.0-1.30 (MF<sub>1</sub>, MF<sub>2</sub>, MF<sub>3</sub>, MF<sub>4</sub>, MF<sub>5</sub>, MF<sub>6</sub>). Three compounds displayed IC<sub>50</sub> values in the range of 0.9-1.0 (MF<sub>7</sub>, MF<sub>8</sub>, MF<sub>9</sub>). The compound MF<sub>6</sub> showed the most significant result with maximum activity (IC<sub>50</sub> = 0.85µg/ml). Variations of the different substituent on the aromatic ring and replacement of aromatic ring with heterocyclic ring have been explored to ascertain the structure-activity relationship among the synthesised compounds. With reference to the compound MF<sub>1</sub> (IC<sub>50</sub> 1.16 µg/ml) substitution with chloro [compound MF<sub>2</sub>, IC<sub>50</sub> 0.9 µg/ml] or N,N dimethyl [compound MF<sub>9</sub>, IC<sub>50</sub> 0.94µg/ml] at para position of phenyl ring appeared to potentiate antimalarial activity while fluoro [compound MF<sub>5</sub>, IC<sub>50</sub> 1.28 µg/ml] appeared to marginal reduction in activity. Compounds with 3-nitro (compound MF<sub>6</sub>, IC<sub>50</sub> 0.90 µg/ml) substitution with chloro (compound MF<sub>7</sub>, IC<sub>50</sub> 1.16 µg/ml) substitution with chloro (compound MF<sub>8</sub>, IC<sub>50</sub> 1.16 µg/ml) substitution with chloro (compound MF<sub>9</sub>, IC<sub>50</sub> 1.22 µg/ml) substitution with chloro (compound MF<sub>10</sub>, IC<sub>50</sub> 0.94µg/ml) in the case of Thiophen (compound MF<sub>1</sub>, IC<sub>50</sub> 1.06 µg/ml) leads to significant increase in antimalarial activity.
CONCLUSION
There is an urgent need for discovery of new and effective antimalarial agents after widespread development of resistance to currently available antimalarial drugs. As part of our research, we have synthesized a series of ten 3-phenyl-2-thioxothiazolidin-4-one (MF\textsubscript{1}-MF\textsubscript{10}) derivatives, by Knoevenagel condensation of N-phenyl rhodanine (I\textsubscript{1}) with substituted aromatic or hetero aromatic aldehydes using microwave irradiation. After spectral confirmation, all the compounds were screened for in vitro antimalarial activity against resistant strain of plasmodium falciparum. One compound MF\textsubscript{9} showed most significant result with maximum activity (IC\textsubscript{50}= 0.85\,\mu g/ml), thus it could be useful as a structural lead for future development of novel antimalarial molecules.

ACKNOWLEDGMENT
The authors are thankful to Director, S. G. S. I. T. S., Indore for providing facilities for successful completion of above work.

CONFLICTS OF INTERESTS
Authors have none to declare

REFERENCES

How to cite this article