*Original Article*

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# Microwave Assisted Synthesis and Antimalarial Activity of Coumarin-Pyrazoline Hybrids

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**Abstract**

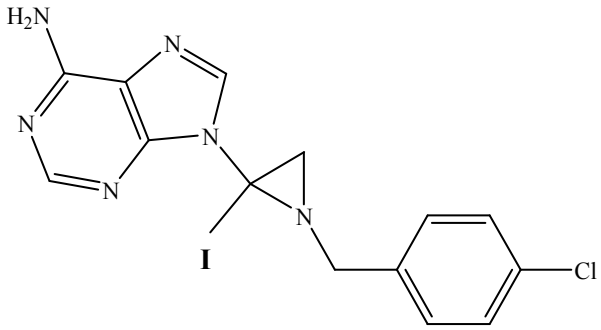
Pyrazline derivatives (3a-3i) were synthesized by using microwave irradiation *i.e.* by involving green chemistry. These compounds were taken for *in vitro* antimalarial activity using schizont maturation inhibition assay, using chloroquine as the standard against chloroquine sensitive, 3D7 strain of *P. falciparum*. Activity of the compounds was reported in terms of $IC_{50}$ values. Compound 3h with dimethylamino substitution was found to be most active with $IC_{50}$ value of 13.765 ìg/mL. From the results, it can be easily inferred that the bulky groups favoured antimalarial activity.

**Keywords:** Pyrazoline; Antimalarial; Microwave.

## Introduction

Malaria is one of the most common diseases of the world caused by parasites. *Plasmodium falciparum* is the major cause of severe malaria and death [1]. This disease affects nearly 40% of the global population [2]. Antimalarial agents comprise a major part of antiprotozoal drugs and have been in practice since ages. However, the recent increase in emergence of chloroquine resistant strains of *Plasmodium falciparum* has fuelled the drug development program against this old and widespread disease [3].

Microwave assisted organic synthesis has led to the revolutionization of organic synthesis. A number of compounds can be prepared in a fraction of time. Thereby, this microwave technique has rapidly gained acceptance as a valuable tool for accelerating the process of drug discovery and development. Microwave radiation is non-ionizing form of energy that does not alter the molecular structure of compounds and provides only thermal activation. Heating effect leads to organic transformations *via* dielectric polarization [4]. Following this approach, D'hooghe *et al.* synthesized aziridine based compounds (I) and determined their antimalarial potential (Figure 1) [5].




**I**

**Fig. 1:** Antimalarial compound

Following the same approach of microwave assisted synthesis, a series of pyrazolines was synthesized and screened for antimalarial potential. The molecules were earlier synthesized by Xin-Hua Liu *et al.* and Suresh Khode *et al.* [6,7]. Xin-Hua *et al.*

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synthesized the acetyl derivatives of pyrazoline by refluxing α,β-unsaturated ketone with hydrazine monohydrate using acetic acid for 2 h. Similarly, the other set was developed by Suresh Khode by refluxing coumarin chalcone and phenylhydrazine in pyridine for 6 h. The microwave method not only reduces the reaction time but also has lesser effects on environment.

## Subjects and Methods

*Experimental Section*

All the chemicals procured were of synthetic grade. These were used without any further purification. Melting points of all the compounds were measured by open-end capillary method and are uncorrected. TLC was performed with silica gel 60 F254 (Merck) using Methanol: Chloroform (1:9 *v/v*) as the solvent system. The spots were observed either under ultra violet light or by exposure to iodine vapours.

The IR spectra were recorded using Bruker alpha-T spectrophotometer. $^1$H-NMR spectra were recorded on a Bruker Avance-400MHz in $CDCl_3$ or DMSO-*d6* with tetramethylsilane (TMS) as internal standard. The mass spectra (MS) were recorded on Waters SYNAPT UPLC-MS/MS working on Mass Linux V4.1 software. Spectral data are consistent with assigned structures. Elemental analyses were performed using CHNS Elementor (Vario EL-III). These values were within the range of ±0.4% of theoretical values.

General Method for Synthesis of various pyrazoline derivatives (3a-i).

Different pyrazolines have been synthesized by using microwave (Model: CEM synthesis system, model no. 925245 Discover Bench Mate, 240v/50Hz).

*Procedure*: Equimolar quantities of different chalcones (2a-i) and substituted hydrazine (Acetyl hydrazine or Phenyl hydrazine) were transferred to the microwave tube. These were then microwaved for the desired time. After the completion of the reaction, the reaction mixture was poured in ice-cold water. The solid separated was filtered and purified by column (MeOH:Chloroform::1:9 *v/v*) to get pure pyrazoline derivatives (Table 1).

**Table 1:** List of pyrazolines (3a-i) along with their physical parameters

| Comp no. | Substitution | | Reaction time | Yield | Reported m.p | Observed m.p | $R_f$ |
|---|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | | (%) | (°C) | (°C) | |
| **3a** | 2-Cl | $COCH_3$ | 3 minutes | 58 | 184-86 | 181-82 | 0.70 |
| **3b** | 2,4-$Cl_2$ | $COCH_3$ | 4 minutes | 62 | 197-99 | 199-200 | 0.68 |
| **3c** | 4-F | $COCH_3$ | 4 minutes | 60 | 209-210 | 207-08 | 0.62 |
| **3d** | 4-OH | $COCH_3$ | 2 minutes | 62 | 167-68 | 165-66 | 0.56 |
| **3e** | 2,4-$Cl_2$ | $C_6H_5$ | 3 minutes | 64 | 178-80 | 177-78 | 0.90 |
| **3f** | 4-F | $C_6H_5$ | 3 minutes | 56 | 108-10 | 105-06 | 0.76 |
| **3g** | 2-$NO_2$ | $C_6H_5$ | 3 minutes | 66 | 160-62 | 159-60 | 0.62 |
| **3h** | 4-N$(CH_3)_2$ | $C_6H_5$ | 2 minutes | 60 | 134-36 | 133-34 | 0.56 |
| **3i** | 4-OH | $C_6H_5$ | 3 minutes | 62 | 150-52 | 151-52 | 0.72 |

*In-Vitro Antimalarial Activity of the Synthesized Compounds*

Synthesized compounds were assessed for *in vitro* antimalarial activity using Schizont.

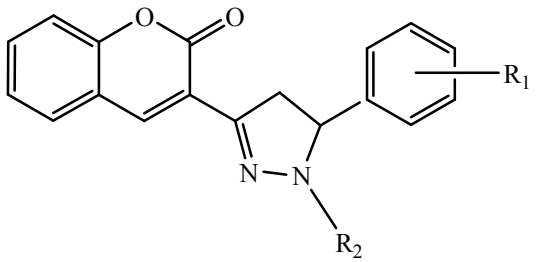
*Maturation Inhibition Assay*

*Procedure*: *In vitro* drug sensitivity of the synthesized compounds was assessed using Trager and Jensen method as described by Dua *et al.* [8] (Table 2). Antimalarial activity was determined against Chloroquine sensitive 3D7 strain of *Plasmodium falciparum*. Culture was maintained in $A^+$ erythrocytes using RPMI 1640 medium supplemented with $AB^+$ human serum (10%), sodium bicarbonate (0.2%), HEPES buffer (25 mM) and gentamycin (50 µg/mL). The culture was treated with different drug concentrations. After 72 h of incubation, blood smears were prepared and stained with JSB I and JSB II. Percentage maturation of schizonts against control was determined. Chloroquine was used as the standard reference. The inhibitory concentration value which kills 50% of the parasites ($IC_{50}$) was determined using HN-NonLin V1.1.

## Results

In the present study, we report the synthesis of pyrazoline-coumarin hybrids using microwave irradiation. The compounds were evaluated for their *in-vitro* antimalarial activity by Schizont maturation inhibition assay (Table 2). The results indicate that compounds with bulky substitutions are better inhibitors. The best active compound (3h) exhibited an $IC_{50}$ of 13.765 µg/mL.

**Table 2:** *In-vitro* antimalarial activity of the synthesized pyrazolines (3a-i)




| Comp no. | Substitution | | $IC_{50}$ (µg/mL) | Comp no. | Substitution | | $IC_{50}$ (µg/mL) |
|---|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | | | $R_1$ | $R_2$ | |
| 3a | 2-Cl | $COCH_3$ | 30.650 | 3b | 2,4-$Cl_2$ | $COCH_3$ | 38.711 |
| 3c | 4-F | $COCH_3$ | 29.291 | 3d | 4-OH | $COCH_3$ | 29.155 |
| 3e | 2,4-$Cl_2$ | $C_6H_5$ | 16.577 | 3f | 4-F | $C_6H_5$ | 15.368 |
| 3g | 2-$NO_2$ | $C_6H_5$ | 13.996 | **3h** | **4-N($CH_3$)$_2$** | **$C_6H_5$** | **13.765** |
| 3i | 4-OH | $C_6H_5$ | 18.793 | Chloroquine | | | 0.002 [9] |

## Discussion

Synthesis of pyrazline derivatives (3a-3i) involved different steps. The first step towards the synthesis of 3-Acetyl coumarin (1) involved the reaction between salicyladehyde and ethylacetoacetate utilizing the principle of Knoevenagel reaction. Reaction of the formed coumarin with different aldehydes lead to the formation of chalcones (2a-2i). This reaction involved the mechanism of Claisen Schmidt reaction. The final reaction for preparation of pyrazoline derivatives (3a-3i) was carried using microwave irradiation, the method involving green chemistry. Chalcones were reacted with substituted hydrazines in microwave tubes. Purification of the compounds was performed using column. Total reaction time for the final step varied between 2-4 minutes.

% yield, melting point and $R_f$ values were recorded for all the synthesized compounds. Further, identity was ascertained using spectral techniques *viz.* IR, NMR and mass. Lactone ring was observed in the region of 1728-1693 $cm^{-1}$. Stretching for C=N was seen in the spectral region of 1600-1592 $cm^{-1}$. NMR and mass spectral data also supported the confirmation of identity.

These compounds were taken for *in vitro* antimalarial activity using schizont maturation inhibition assay, using chloroquine as the standard against chloroquine sensitive, 3D7 strain of *P. falciparum*. Activity of the compounds was reported in terms of $IC_{50}$ values. Compound 3h with dimethylamino substitution was found to be most active with $IC_{50}$ value of 13.765 µg/mL. From the results, it can be easily inferred that the bulky groups favoured antimalarial activity.

## Conclusion

It is quite evident from the results that presence of presence of bulky groups in the compounds shored up the antimalarial potential of the compounds. Further studies involving target identification are under progress.

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