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Coumarin-Pyrazoline Derivatives: Their One-Pot Microwave Assisted Synthesis and Antimalarial Activity

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Abstract

The current work described the microwave assisted synthesis of various pyrazoline derivatives **(3a-3i)**. Equimolar quantities of different chalcones and substituted hydrazine (Acetyl hydrazine or Phenyl hydrazine) were irradiated under microwave conditions to give the title compounds.

These compounds were evaluated for *in vitro* antimalarial potential using schizont maturation inhibition assay using chloroquine sensitive 3D7 strain of *P. falciparum*. Amongst, all the compounds, the one with trimethoxy substitution (3g) was found to be most active with IC_{50} value of 11.63 µg/mL. From the results, one could infer that bulky groups favoured antimalarial activity. The derivatives were also studied for antimicrobial effect against bacteria (*Staphylococcus aureus* and *Escherichia coli*) and fungi (*Rhizopus oryza* and *Penicillum citrum*).

Keywords: Pyrazoline; Microwave; Antimalarial; Antimicrobial.

Introduction

Microwave assisted synthesis is a branch of green chemistry that has attained considerable attention in recent years. Chemical transformations involved *via* this technique are pollution free, eco-friendly and offer high yields together with simplicity in processing and handling [1]. This also offers enhanced speed, reproducibility and scalability with higher yields [2].

Heating reactions with conventional equipments such as oil baths, sand baths and heating mantles is not only slow but it creates hot surface on the reaction vessels leading to the decomposition of products, substrates and reagents over time. On the other hand, microwave energy is remotely introduced into the chemical reactor through walls. A properly designed vessel permits uniform heating of the sample, thereby generating fewer by-products and/or product decomposition [3].

Keeping in mind the urgent need for development of antimalarial agents and conserving the environment, scientists across the globe are engaged in microwave assisted synthesis. A few such developed compounds are shown in **Figure 1**. Christian *et al.* synthesized pyrimidine based compounds (**I**) and screened their antimalarial potential [4]. Ugwu *et al.* determined antimalarial activity of chalcone derivatives (**II**) [5]. Similarly, Vaidya *et al.* developed a series of aryl and heteroaryl chalcones (**III**) and assessed their antimalarial activity [6].

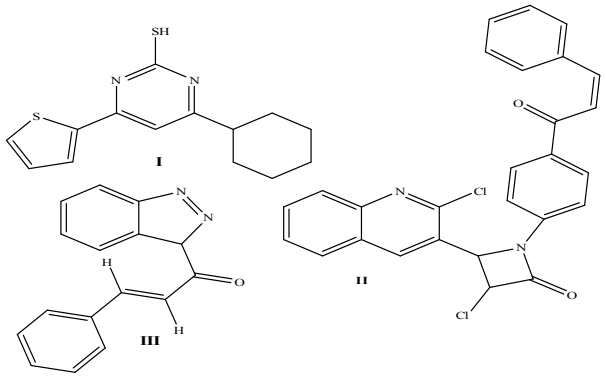

Fig. 1: Antimalarial compounds synthesized using microwave

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In the recent years, the mounting threat of resistance to existing antimicrobial agents, has heightened the urgency of development of effective agents with enhanced activity [7]. Coumarin is an important privileged structure for the development of antimicrobial agents and Novobiocin and chlorobiocin are shining example of it [8]. In addition pyrazoline is also reported to have antimicrobial activity [9-10].

Accordingly, we also designed a series of pyrazoline derivatives using microwave method and determined their antimalarial and antimicrobial potential. Earlier some scientist (Wanare *et al.*; Prasad *et al.*; Levai *et al.*) also reported some of the derivatives by using the conventional method [11-13]. Here, we reported the synthesis by microwave method which not only reduces the reaction time but also have lesser effect on the environment.

Materials and Methods

Chemistry

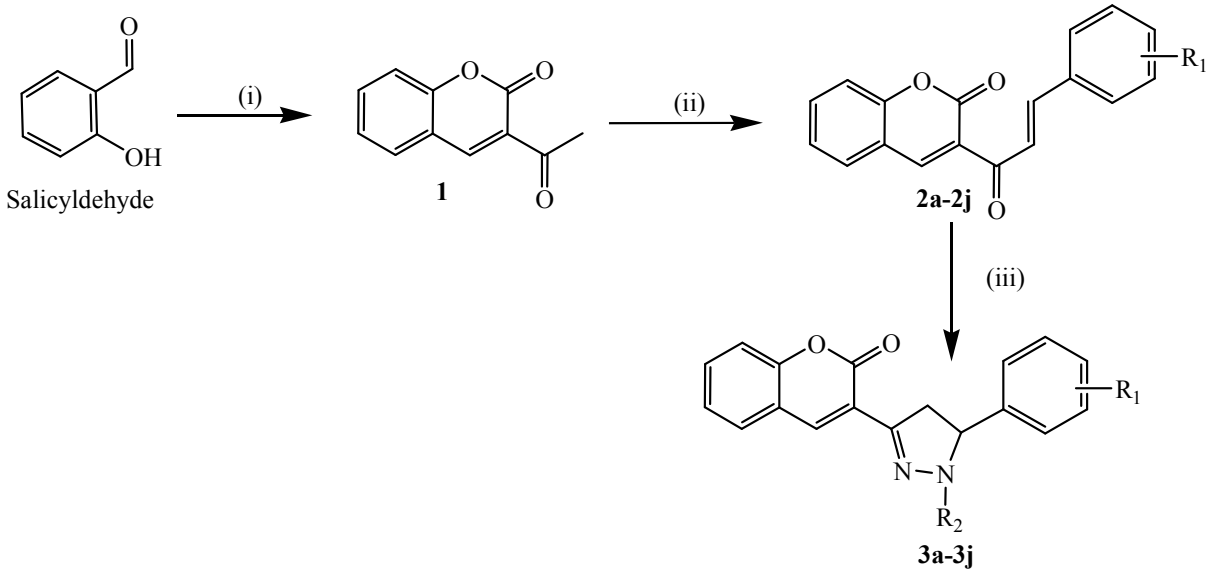
All the chemicals were used were of synthetic grade and were used without further purification. Melting points of all compounds were measured by open-end capillary method and are uncorrected. TLC was performed with silica gel 60 F254 (Merck) using Methanol: Chloroform (1:9 *v/v*) as solvent system and the spots were located either under ultra violet light or through the 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) and were found to be within the range of ±0.4% of theoretical values.

General Procedure for the Preparation of Various Pyrazolines **(3a-i)**

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

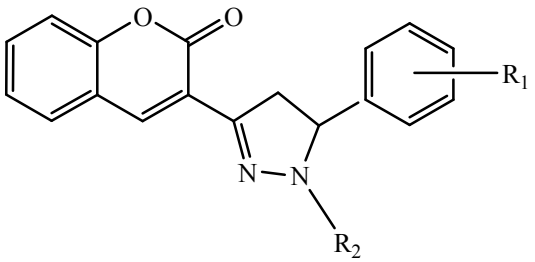
Procedure: Equimolar quantities of different chalcones (**2a-e**) and substituted hydrazine (Acetyl hydrazine or Phenyl hydrazine) were transferred to the microwave tube. These were then microwaved for the desired time (**Scheme 1**). After the completion of the reaction, the reaction mixture was poured 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**).



Reagents and conditions: (i) Ethylacetoacetate, ethanol, piperidine, reflux; (ii) Aldehyde, n-BuOH, reflux; (iii) Hydrazines, ethanol:acetic acid (1:1), Microwave (2-4 minutes)

SCHEME-I

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	3,4-$(OCH_3)_2$	$COCH_3$	4 minutes	60	170-172	168-170	0.60
3b	3,4,5-$(OCH_3)_3$	$COCH_3$	3 minutes	58	197-198	198-200	0.58
3c	4-CH_3	$COCH_3$	3 minutes	50	181-182	178-180	0.68
3d	4-Cl	$COCH_3$	4 minutes	56	203-204	204-206	0.64
3e	3-OCH_3	C_6H_5	3 minutes	60	174-176	175-177	0.63
3f	3,4-$(OCH_3)_2$	C_6H_5	2 minutes	72	148-150	147-149	0.68
3g	3,4,5-$(OCH_3)_3$	C_6H_5	3 minutes	60	169-170	172-174	0.70
3h	4-CH_3	C_6H_5	3 minutes	68	193-194	191-192	0.81
3i	4-Cl	C_6H_5	2 minutes	65	150-52	152-54	0.48

Solvent system used: Benzene: P.E:MeOH::9:1:0.1

In-vitro Antimalarial Activity of the Synthesized Compounds

Schizont Maturation Inhibition Assay was used to evaluate the *in-vitro* antimalarial activity of the synthesized compounds.

Procedure: *In vitro* drug sensitivity of the synthesized compounds was assessed using Trager and Jensen method as described by Dua *et al* [14]. Chloroquine sensitive 3D7 *P. falciparum* strains were used for the study. 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 drug. The inhibitory concentration value which kills 50% of the parasites (IC_{50}) was considered for antiplasmodial activity. IC_{50} values were determined using HN-NonLin V1.1 (Table 2).

Antimicrobial Activity

The newly prepared compounds were screened for their antibacterial activity against *E. coli* (ATCC-8739) and *S. aureus* (ATCC-29737) strains at a concentration of 100 µg/mL by turbidity method [15] using norfloxacin as standard. Antifungal activity of the compounds was determined against *R. oryza* (NRRL-21498) and *P. citrum* (NCIM-768) using fluconazole as standard. The results of antimicrobial effect of compounds were reported as minimal inhibitory concentrations (MICs, µg/mL), and are shown in **Table 2**.

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

Comp no.	Antimalarial (IC_{50}; µg/mL)	Antibacterial (MIC; µg/mL)		Antifungal (MIC; µg/mL)	
		S. aureus	*E. coli*	*R. oryza*	*P. citrum*
3a	23.080	25	50	50	50
3b	22.314	25	25	25	50
3c	24.589	25	50	50	100
3d	31.344	50	50	>100	100
3e	15.230	**12.5**	50	25	50
3f	12.417	**12.5**	25	**12.5**	25
3g	**11.633**	**10**	**12.5**	**12.5**	25
3h	14.388	25	50	100	100
3i	16.003	50	50	50	100
Chloroquine [16]	0.002	--	--	--	--
Norfloxacin	--	6.25	6.25	--	--
Fluconazole	--	--	--	6.25	6.25

Result and Discussion

Chemistry

A series of compounds (**3a-3i**) were synthesized in accordance with the method outlined in **Scheme I**. In the first step, acetyl coumarin was prepared utilizing the principle of Knoevenagel condensation. 3-Acetylcoumarin was taken to the next step and was reacted with different aromatic aldehydes resulting in the formation of chalcones (**2a-2e**). The mechanism in the formation of chalcones involved Claisen Schmidt reaction. Further these, chalcones were used for the synthesis of pyrazolines (**3a-i**). Synthesis of pyrazolines was afforded using microwave irradiation. The solid product obtained after irradiation was purified using column to get pure derivatives. Reaction time for all the products was in the range of 2-4 minutes. The synthesized compounds under microwave irradiation give the same compound as that of conventional method but with lesser time, higher yield, free from impurities and eco-friendly economic conditions

Following purification using column, melting point of the compounds was recorded. IR, NMR and mass spectra were recorded for these compounds.

Lactone ring was found in the region of 1732-1721cm^{-1} and C=N stretching was observed in the region of 1600-1593 cm^{-1}. In ^{1}H NMR spectra, the three protons of pyrazoline ring appeared as doublets of doublets. Other aromatic protons appeared at their expected chemical shift and integral values in ^{1}H NMR. M^{+}+1 values in the mass spectra also supported the results.

In-vitro Antimalarial Activity

These compounds were evaluated for *in vitro* antimalarial potential using schizont maturation inhibition assay using chloroquine sensitive 3D7 strain of *P. falciparum*. Results for antimalarial activity were reported in terms of IC_{50} values. Amongst, all the compounds, the one with trimethoxy substitution (**3g**) was found to be most active with IC_{50} value of 11.63 mg/mL.

Decrease in methoxy group leads to decrease in activity as exemplified by results of compound **3e** and **3f** with IC_{50} values of 15.23 and 12.41. Replacement of acetyl group by phenyl group further increases the activity. From the results, one could infer that bulky groups favoured antimalarial activity.

Antimicrobial Activity

The compounds were also evaluated for their antimicrobial activity using turbidity method against bacteria *Staphylococcus aureus* (ATCC-29737) and *Escherichia coli* (ATCC-8739) and the fungal strains of *Rhizopus oryza* (NRRL-21498) and *Penicillum citrum* (NCIM-768) using Norfloxacin and Fluconazole as standard. The results revealed that most of the newly synthesized compounds exhibited promising antibacterial activities. In addition to malaria, compound **3g** was also found to be active against both bacterial and fungal strains with MIC of 10 and 12.5 mg/mL against *S. aureus* and *E. coli*; 12.5 against *R. oryza* respectively. Compound **3f** was active against only *S. aureaus* and *R. oryza* with MIC of 12.5 mg/mL.

Conclusion

Compound **3g** with trimethoxy substitution was found to have significant antimalarial and antimicrobial activity. An increase in bulkiness resulted in profound increase in activity. Further studies regarding target identification is in progress at our end.

Conflict of Interest

The authors have declared no conflicts of interest.

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