

## Synthesis, antimalarial activity, and docking studies of monocarbonyl analogues of curcumin

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**Abstract.** The synthesis of five monocarbonyl analogues of curcumin is described. *In vitro* anti-malarial assay of the compounds was carried out and the effect of the substituents on the aryl ring has been described. The results show that all the five compounds exhibited some reasonable activity against the chloroquine-resistant plasmodium parasite. Molecular docking studies further confirmed the observed biological activity of the compounds.

**Keywords:** curcumin, monocarbonyl analogues, antimalarial activity, docking studies.

### 1. Introduction

Malaria is one of the world's most endemic diseases, affecting over 100 tropical and sub-tropical countries. *Plasmodium falciparum* remains the most dangerous of the five plasmodium species to man. In 2013, there were approximately 198 million cases of malaria, and estimated number of 584,000 deaths mainly in the African region [1]. Curcumin is a bis-benzylidene compound isolated from *Curcuma longa L* (Figure 1) which demonstrates an incredible potential as a lead compound [2-6]. However, the presence of the methylene group in its structure confers certain instability to the compound [7]. Consequently, a number of disadvantages in pharmacokinetics such as poor bioavailability and need for continuous use limits its application [8, 9]. Thus, more efforts are now directed toward the synthesis and biological assays of the monocarbonyl analogues, and preliminary results reveals that the compounds possess anticancer [10], antibacterial [11], anti-Cushing's disease [12], antifungal [13], anti-inflammatory [14], antitrypanosomal and Leishmania [15], and antimalarial activities [16]. In this work, a series of known monocarbonyl curcumin analogues are synthesized and evaluated for antimalarial activity among which the antimalarial activity of two have already been previously reported [16]. But as a proof of concept, it was decided to reassess them along with the other ones. Also, the computational studies were explored to further understand the interaction of the compounds with the target protein, a contribution that has not been reported for all the compounds.

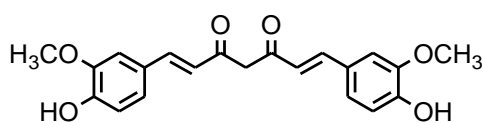


Figure 1. Chemical structure of curcumin

### 2. Experimental

#### 2.1. Materials and methods

All chemicals and solvents were purchased from Aldrich and used as received. <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on a Bruker AVANCE 400 spectrometer. IR spectra were recorded on a Perkin-Elmer FT-IR Spectrum BX spectrophotometer. Melting points were determined on an Electrothermal melting point apparatus and are uncorrected. Analytical TLC was carried out using precoated silica gel 60 F254 plates (0.2 mm thickness, Merck).

#### 2.2. Synthesis

##### General Procedure for the preparation of analogues 3a, b (Method A)

To a stirred solution of aromatic aldehyde (20 mmol, 2 equiv.) and acetone (10 mmol, 1 equiv.) in 15 ml of ethanol at -5 °C was added CF<sub>3</sub>CO<sub>2</sub>H (10 ml) drop wise over 30 minutes. The solution was then allowed to warm up to room temperature and left to stir for 10 h. On addition of dilute HCl, a precipitate is formed, which is then filtered and recrystallized to give the desired product.

**Compound 3a:** Yellow powder, 82% yield, mp 86-88°C. <sup>1</sup>H, <sup>13</sup>C NMR and IR spectra agreed with the literature [14b].

**Compound 3b:** Yellow powder, 100% yield, mp 241-244°C. <sup>1</sup>H, <sup>13</sup>C NMR and IR spectra agreed with the literature [7].

##### General Procedure for the preparation of analogues 3c, d, e (Method B)

The same procedure as in method A except that 40% NaOH solution in water was substituted for the CF<sub>3</sub>CO<sub>2</sub>H.

**Compound 3c:** Yellow powder, 81% yield, mp 260-265°C. <sup>1</sup>H, <sup>13</sup>C NMR and IR spectra agreed with the literature [17].

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**Compound 3d:** Yellow powder, 83% yield, mp 131-134°C.  $^1\text{H}$ ,  $^{13}\text{C}$  NMR and IR spectra agreed with the literature [10c].

**Compound 3e:** Yellow powder, 85% yield, mp 212-215°C.  $^1\text{H}$ ,  $^{13}\text{C}$  NMR and IR spectra agreed with the literature [10c].

### 2.3. Antimalarial assay

A test tube containing 5% parasitaemia was charged with a test solution (0.1 ml) and the culture medium (0.2 ml) and mixed thoroughly. The sensitivity of the parasites to each test compound with the serial concentrations of 10, 5, 2.5, and 1.25 mg/ml was determined under microscope at 37 °C after 24 h, 48 h and 72 h of incubation. In order to supply the 95% of nitrogen, 2% of oxygen and 3% of carbon dioxide necessary for the incubation, the test tubes were all transferred into a jar containing a burning candle and then replaced the cover until the flame stopped burning. The preparation of the control groups was performed by mixing the culture media with the positive erythrocytes to serve as the negative control. While the culture media plus positive erythrocytes and artemether (a known antimalarial) as the positive control [18].

At the end of 24, 48, and 72 h incubation periods, an aliquot of each culture medium was smeared on a slide,

stained using Geimsa staining technique and then observed under a microscope. The mean number of erythrocytes that appeared red-pink was estimated and average percentage elimination determined. Calculation of the percentage elimination of the parasites after incubation is taken as the activity of each test compound. This has been carried out using the following relation:

$$\% = \frac{N}{N_x}$$

where:

% = percentage activity of the samples,

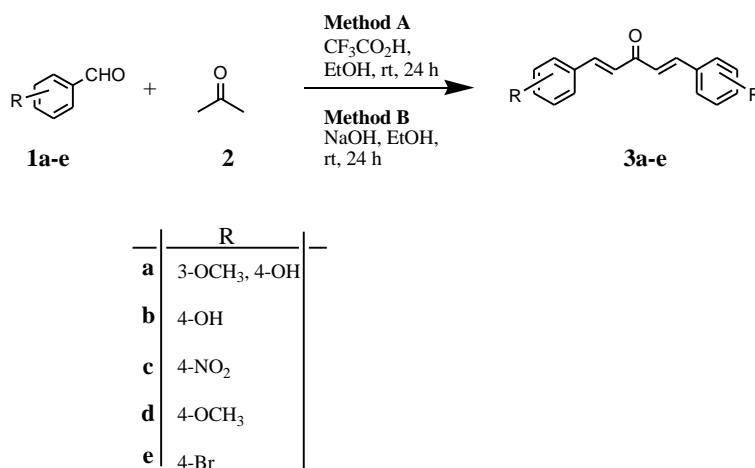
$N$  = total number of cleared red blood cells,

$N_x$  = total number of parasite in the red blood cells.

## 3. Results and discussion

### 3.1. Chemistry

Five monocarbonyl curcumin analogues, **3a-e** were synthesized (Scheme 1) by condensation of aromatic aldehyde with acetone following standard procedure [14] with modification in the case of compounds **3a,b** [10a]. The spectroscopic (IR,  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra) data of the compounds were consistent with the reported values [7, 10c, 14b, 17].



Scheme 1. Synthesis of monocarbonyl curcumin derivatives

Stereochemical configuration of the compounds was established to be *E* based on the coupling constant (16 Hz) of the two protons attached to the olefinic bond of the 1,5-diphenyl-1,4-pentadiene-3-ones.

### 3.2. Antimalarial activity

A modified method of the procedure by Hanne and co-workers was used for antimalarial activity studies [18]. The synthesized compounds were screened for *in vitro* antimalarial activity against the parasitaemia of *P. falciparum* using artemether drug as positive control [18], and the activity is expressed as percentage elimination of the parasites after incubation period in accordance with the reported procedure (Table 1) [19].

The preliminary structure-activity-relationship revealed that the presence of a methoxy group on the aryl ring confers strong potency on **3d** with 57% elimination of the parasites. However introducing a hydroxyl substituent onto **3a** slightly reduced the elimination potency of the parasites to 55%. A further decrease in activity is observed in the case of the nitro derivative (51%). The hydroxyl derivative, **3b** exhibits moderate elimination potency (48%) while the least of all the compounds appears to be the bromo substituted derivative, **3e** (42.6%). It might be inferred that all the steric and electronic factors responsible for enhancing the activity did not favor the bromo derivative.

Table 1. Antimalarial activity of the synthesized compounds **3a-e**

| <b>3</b> | Initial parasitized count/ fields before used | Total number of parasitized and non-parasitized fields | Concentrations (mg/ml) | Final count of dead parasites/ fields at the end of incubation | Mean of dead parasites at end of incubation 72 h | % elimination at end of incubation |
|----------|---|--|------------------------|--|--|------------------------------------|
| <b>a</b> | 88  | 116  | 10, 5, 2.5, 1.25       | 35+25+22+ 14   | 24   | 55                                 |
| <b>b</b> | 88  | 116  | 10, 5, 2.5, 1.25       | 47+38+25+ 19   | 32.25  | 48                                 |
| <b>c</b> | 88  | 116  | 10, 5, 2.5, 1.25       | 40+36+22+ 16   | 28.5   | 51                                 |
| <b>d</b> | 88  | 116  | 10, 5, 2.5, 1.25       | 35+22+17 +12   | 21.5   | 57                                 |
| <b>e</b> | 88  | 116  | 10, 5, 2.5, 1.25       | 52+48+33 +21   | 38.5   | 42.6                               |

### 3.3. Docking studies

*In silico* exploration studies of the PfDXR active-site [20] revealed additional, adjacent binding pockets that could be explored. The presence of this additional binding pocket identified has prompted the *de novo* design of *N*-benzylated phosphonate esters as potential antimalarials [21].

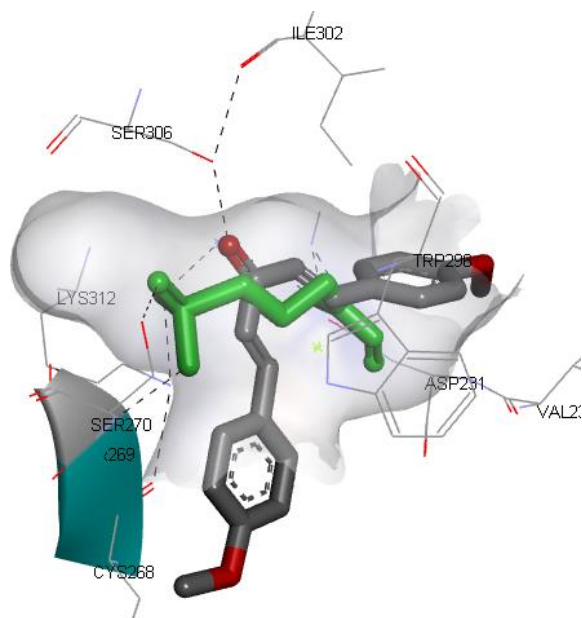


Figure 2. Docked conformations of **3d** in the PfDXR receptor cavity (3AU9) [22] illustrating the close alignment of the ligand with fosmidomycin. The crystal structure conformation of fosmidomycin is shown in stick format coloured green, and the ligand in stick format coloured by atom type. Protein active-site amino acid residues are shown in wire-frame coloured by atom type. Hydrogen bonds are shown as black dashed lines.

Molecular docking plays a vital role in the rational design of drugs, by elucidating the interactions between the proposed ligands and the active-site. The different types of interactions that occur between the ligand and the active-site, can be explored and exploited to improve binding. The PfDXR crystal structure used in these modelling studies was accessed from the Research Collaboratory for Structural Bioinformatics (RCSB) protein data bank, using Discovery Studio Visualizer 4.0 [22]. Thus modelling studies were carried out in order to establish an understanding of the similarities between the synthesized compounds and fosmidomycin (a known antimalarial drug) at the PfDXR receptor cavity. To validate the reliability of the bioassay results, the protein

active-site and surrounding residues were also mapped using Discovery Studio Visualizer [22]. In each case, the ligand was docked, using Autodock tools Version 4.2, into the DXR active-site after removing the water molecules present, but leaving the fosmidomycin in the PfDXR X-ray structure to note the overlap with the ligands; non-polar hydrogens were added and merged. For visual analysis, the docked ligands were overlaid with the crystal structure containing fosmidomycin within the active site.

The binding of compounds **3a-e** to the PfDXR active-site revealed that all the synthesized compounds fit into the PfDXR active-site receptor cavity, exhibit potential hydrogen-bonding interactions with the proximal amino acid residues and align similar to fosmidomycin. The intrinsic feature of fosmidomycin is that its hydrophobic patch aligns parallel with the indole ring of Trp296, and each of these ligands also aligns with the indole ring. The binding also permits hydrogen-bonding interactions between the carbonyl oxygen of the ligands and Ser360 and Ile302 residues. It is noteworthy that all these compounds fill the adjacent vacant pocket (previously described) of the PfDXR active-site.

### 4. Conclusion

Five monocarbonyl curcumin analogues were synthesized and their structures identified by IR and NMR spectroscopic analysis. Their antimalarial activities were examined by measuring the percent elimination of the chloroquine-resistant parasitemia of *P. falciparum*. Although the synthesis of these compounds along with the antimalarial activities of two of them has previously been reported, in the present study the same activity for the remaining analogues is explored for the first time. All the five compounds showed some potency compared to the known antimalarial drug, artemether. Furthermore, a molecular docking study suggests that the nature and position of the substituent play a vital role in the antimalarial activities of the compounds.

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### Conflict of interest

Authors declare no conflict of interest.

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