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ScienceDirect
Bioorganic & Medicinal Chemistry 16 (2008) 2894–2902
Bioorganic & Medicinal Chemistry

Synthesis and exploration of novel curcumin analogues as anti-malarial agents

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Received 15 November 2007; revised 27 December 2007; accepted 28 December 2007
Available online 1 January 2008

Abstract—Curcumin, a major yellow pigment and active component of turmeric, has been shown to possess anti-inflammatory and anti-cancer activities. Recent studies have indicated that curcumin inhibits chloroquine-sensitive (CQ-S) and chloroquine-resistant (CQ-R) *Plasmodium falciparum* growth in culture with an IC_{50} of ~3.25 μM (MIC = 13.2 μM) and IC_{50} 4.21 μM (MIC = 14.4 μM), respectively. In order to expand their potential as anti-malarials a series of novel curcumin derivatives were synthesized and evaluated for their ability to inhibit *P. falciparum* growth in culture. Several curcumin analogues examined show more effective inhibition of *P. falciparum*growth than curcumin. The most potent curcumin compounds **3**, **6**, and **11** were inhibitory for CQ-S *P. falciparum* at IC_{50} of 0.48, 0.87, 0.92 μM and CQ-R *P. falciparum* at IC_{50} of 0.45 μM, 0.89, 0.75 μM, respectively. Pyrazole analogue of curcumin (**3**) exhibited sevenfold higher anti-malarial potency against CQ-S and ninefold higher anti-malarial potency against CQ-R. Curcumin analogues described here represent a novel class of highly selective *P. falciparum* inhibitors and promising candidates for the design of novel anti-malarial agents.

1. Introduction

Malaria is caused by the protozoa of the genus *Plasmodium*. Because of its prevalence, virulence, and drug resistance, it is the most serious and widespread parasitic disease. It has an overwhelming impact on public health in developing regions of the world.[1–3] Attempts to control mosquito vector and the use of anti-malarial drugs notwithstanding, there are yet some 2.5–3 million fatalities, mostly children and women annually from malaria.[4] Not clearly evident in the mortality statistics are the vast range of problems associated with the millions of cases suffering from morbidity derived from malaria infection. In part, the extent of the economic and public health problems relevant to malaria pertains to difficulties in the chemotherapy of this protozoan disease.[5] As a result, there is an urgent need for new efficient anti-malarial agents.[6,7] Thus, identification of an anti-malarial agent that is easy to synthesize/isolate, is inexpensive, and demonstrates little toxicity across a diverse population represents the ideal choice for treating malaria.

Curcumin is a β-diketone constituent of the turmeric that is obtained from the powdered root of *Curcuma longa Linn*. It is used as a spice to give a specific flavor and yellow color to curry, which is consumed in trace quantities daily by nearly a billion people. Curcumin has been used by traditional medicine for liver disease (jaundice), indigestion, urinary tract diseases, rheumatoid arthritis, and insect bites. This phytochemical has also been demonstrated to possess both anti-cancer and anti-angiogenic properties. Its anti-tumor properties include growth inhibition and apoptosis induction in a variety of cancer cell lines in vitro, as well as the ability to inhibit tumorigenesis in vivo.[8–14] Moreover, pyrazole analogues of curcumin were synthesized and investigated for lipoxygenase inhibitory activity,[15] cytotoxic activity[16,17], and anti-oxidant activity.[18] Recently, curcumin has been shown to have synergistic effects with artemisinin against *Plasmodium berghei* in vivo.[19,20] Curcumin is also potent against both chloroquine (CQ)-susceptible (CQ-S) and -resistant (CQ-R)

Keywords: *Plasmodium falciparum*; Curcumin; Antimalarial; Pyrazole.
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doi:10.1016/j.bmc.2007.12.054

Plasmodium falciparum strains, and the parasiticidal effect is at least partially due to the generation of reactive oxygen species (ROS), and down-regulation of the *Pf* GCN5 HAT activity.[21] The *P. falciparum* general control non-derepressed 5 (PfGCN5) is a HAT that preferentially acetylates K9 and K14 of histone H3. Knoevenagel condensates of curcumin analogues were found to be capable of inhibiting NF-κβ activation.[22] In addition, there are no reports on development of resistance to curcumin against anti-cancer activity.[23] In view of the above, we have synthesized curcumin analogues to improve its potency as an anti-malarial agent. Isoxazole derivative of curcumin (**2**), pyrazole derivatives of curcumin (**3**–**8**), Knoevenagel condensate derivatives of curcumin (**9**–**11**), di-*O*-acetyl curcumin (**12**), and di-*O*-methylcurcumin (**13**), thus synthesized exhibit anti-malarial activity against both CQ-R and CQ-S *P. falciparum*.

2. Chemistry

The isoxazole derivative of curcumin was prepared as depicted in Scheme 1. Isoxazole derivative (**2**) of curcumin was synthesized by treatment of curcumin with hydroxylamine hydrochloride in acetic acid at 85 °C for 6 h.[18] Pyrazole derivatives of curcumin were synthesized as reported previously (Scheme 1).[15] Pyrazole derivatives (**3**–**8**) were prepared by heating curcumin (**1**) for 8 h with hydrazine hydrate, phenylhydrazine, 2-fluorophenylhydrazine hydrochloride, 3-nitrophenylhydrazine hydrochloride, 2, 4-dichlorophenylhydrazine hydrochloride, 4-methoxyphenylhydrazine hydrochloride in acetic acid in good yield. Compounds **9**–**11** reported in Table 1 were synthesized according to Scheme 2. Knoevenagel condensates of curcumin (**9**–**11**) were prepared by treatment of curcumin with aromatic aldehyde (benzaldehyde, 4-hydroxybenzaldehyde, 4-hydroxy-3-methoxybenzaldehyde) in presence of triphenylphosphane (as catalyst) to yield respective compounds. Di-*O*-acetyl and di-*O*-methyl curcumin were synthesized as previously reported.[16,24] Acetylation of curcumin (**1**) with acetic anhydride in presence of pyridine gave di-*O*-acetyl derivative (**12**). Di-*O*-methyl curcumin derivative **13** was synthesized by methylation of curcumin (**1**) with dimethyl sulfate (Scheme 3). All the synthesized derivatives were characterized by ^{1}H NMR, ^{13}C NMR, and Electrospray–mass spectrometry [ESI–MS].

3. Results and discussion

While curcumin itself has recently been shown to exhibit anti-malarial activity, prior to these studies compounds designed around the scaffold of curcumin have not been explored systematically for their anti-malarial activity against both CQ-S and CQ-R *P. falciparum*. A correlation between their structure and anti-malarial activity has not been attempted so far. Moreover, this study attempts for the first time a structure–activity relation for the anti-malarial activity of curcumin scaffold.

3.1. Anti-malarial activity and structure–activity relationship of curcumin and its analogues against chloroquine sensitive (CQ-S) and chloroquine resistant (CQ-R) *P. falciparum*

Based on the above-mentioned curcumin properties, we used its scaffold to develop novel anti-malarial agents. All the synthesized curcumin analogues were checked on *P. falciparum* cultures to determine the IC_{50} values for inhibitors. Each test was performed in triplicate and the IC_{50} reported represent the result of at least two repetitions. Curcumin inhibits CQ-S *P. falciparum* at IC_{50} of 3.25 ± 0.6 μM and CQ-R *P. falciparum* at IC_{50} of 4.21 ± 0.8, in a similar range as reported earlier for chloroquine-resistant *P. falciparum* cultures in a dose dependent manner with an IC_{50} of ~5 μM;[19] while its minimum inhibitory concentration (MIC) against CQ-S and CQ-R was found to be 13.2 ± 1.3 and 14.4 ± 1.6 μM. MIC was read as the lowest concentration that resulted in no malarial parasite growth. The slight difference in IC_{50} between our results and others could be due to variations in culture conditions, genetic background of the parasite, developmental stages, drug purity, solvents for dissolving the drug, etc.

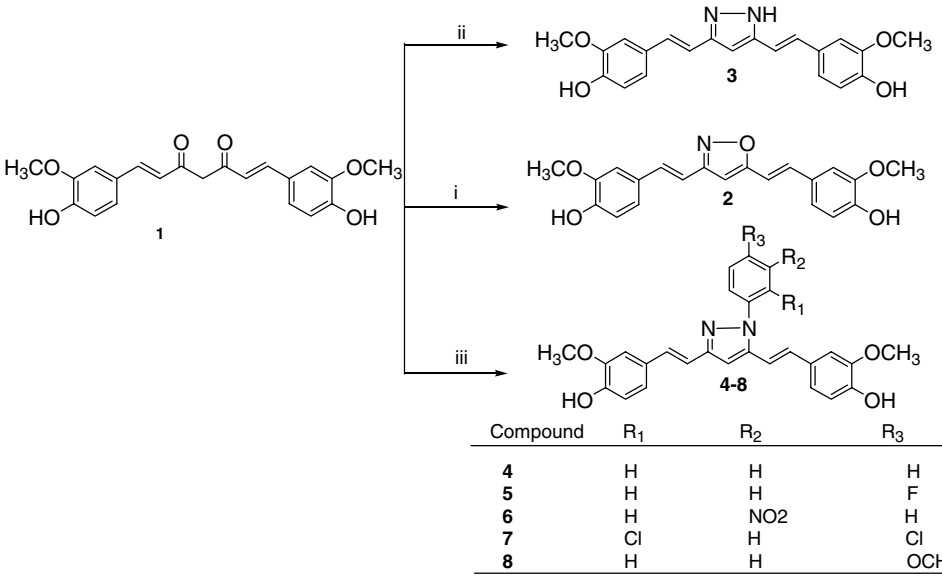
All the compounds mentioned in the manuscript excepting curcumin used here as the reference compound have been reported for the first time for their anti-malarial activity. While, compounds (**4**–**11**) are novel and have been synthesized and characterized by us for the first time, compounds (**2**, **3**, **12**, and **13**)[15–18] have been synthesized previously, but have been checked for their anti-malarial activity for the first time in this study. Starting from curcumin structure, we have investigated the effect of different substituents on *N*-phenylpyrazole and Knoevenagel condensates of curcumin.

3.2. Pyrazole versus isoxazole

When isoxazole group in isoxazole derivative of curcumin (**2**) was replaced by a pyrazole group, it led to a moderate increase of anti-malarial potency against CQ-S and CQ-R. Thus converting the keto-enol moiety (**1**) to the corresponding pyrazole (**3**) led to increased anti-malarial potency against *P. falciparum* in culture (Table 1) and replacing –NH group of pyrazole derivative of curcumin (**3**) by –O isoxazole derivative (**2**) results in decrease of potency. It therefore implies that the presence of –NH group in pyrazole derivative (**3**) is necessary for its potency. Pyrazole derivative of curcumin (**3**) inhibited the CQ-S and CQ-R *P. falciparum* cultures at nanomolar concentrations with IC_{50} of 0.48 and 0.45 μM, respectively.

3.3. Effect of electron withdrawing group on *N*-phenyl curcumin pyrazole

Electron withdrawing substituents at *N*-phenyl curcumin pyrazole system affect the anti-malarial potency of corresponding compounds. Previous reports show that electron withdrawing groups enhance the biological activity[25,26] while electron donating groups show marked decrease in activity. Similarly, in our case elec-



Scheme 1. Synthesis of pyrazole, isoxazole, and *N*-(substituted) phenylpyrazole analogues of curcumin. Reagents and conditions: (i) NH_2-OH·HCl, CH_3COOH/85 °C 6 h; (ii and iii) hydrazinehydrate, phenylhydrazine, 2-fluorophenylhydrazine hydrochloride, 3-nitrophenylhydrazine hydrochloride, 2, 4-dichlorophenylhydrazine hydrochloride, 4-methoxyphenylhydrazine hydrochloride, CH_3COOH/8 h, reflux.

tron withdrawing substituted compounds (**5**–**7**) have shown improved activity while compounds with electron donating group substitutions show drastic reduction in anti-malarial activity as is evident from the activities of unsubstituted phenylpyrazole curcumin (**4**) and substituted phenylpyrazole (**5**–**8**).

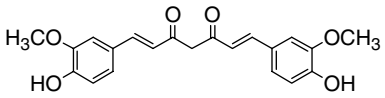
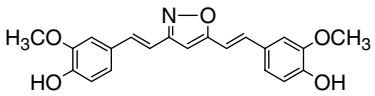
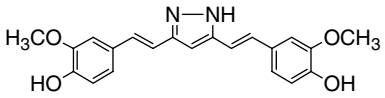
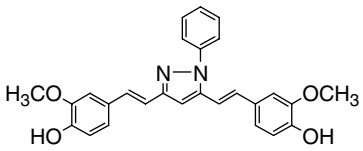
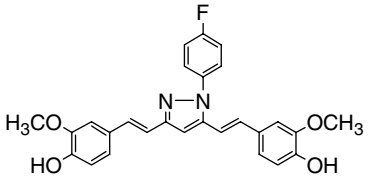
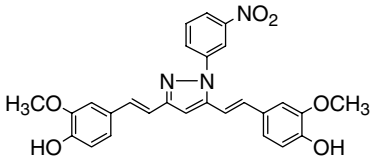
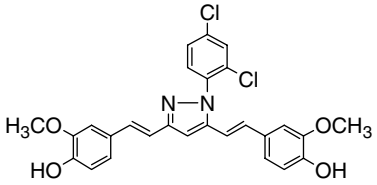
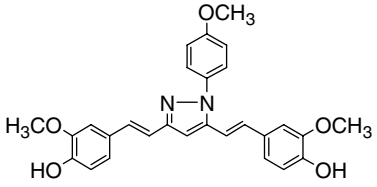
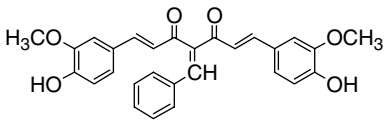
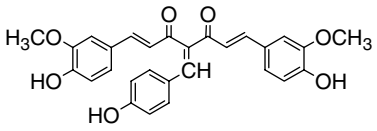
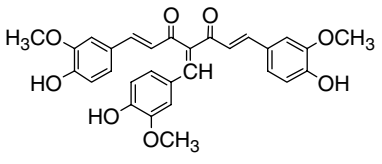
While a dramatic decrease in potency was observed when the unsubstituted phenylpyrazole derivative was replaced by electron donating 4-methoxyphenyl group (**4** vs **8**), activity was enhanced in both CQ-S and CQ-R strains when 3-nitrophenyl substitution was performed (**4** vs **6**), The most active 3-nitrophenylpyrazole derivative of curcumin (Table 1 and Fig. 1) exhibits tenfold more potency as compared with *N*-phenylpyrazole curcumin and four to fivefold more potency as compared to curcumin against CQ-S and CQ-R strains, respectively. We also explored the effect of the introduction of other electron withdrawing groups viz. 4-fluoro and 2, 4-dichloro at *N*-phenylpyrazole system, in order to increase the anti-malarial potency of *N*-phenylpyrazole. When 4-fluoro and 2, 4-dichloro substituents were introduced, the resulting compounds **5** and **7** display two to fourfold more potencies, respectively, relative to the parent *N*-phenyl curcumin pyrazole against CQ-S and CQ-R. Although 4-fluoro (**5**) and 2, 4-di-chloro (**7**) substituted *N*-phenyl pyrazole derivative have also electron withdrawing properties, they are weaker than the nitro substituted derivative. Consequently compounds **5** and **7** are less potent than nitro substituted *N*-phenylpyrazole curcumin (**6**). Also compound **5** has identical potency to curcumin and **7** has lower potency compared to that of curcumin (Table 1). Lower inhibition by compound (**7**) was perhaps due to steric hindrance caused by the presence of two chlorine atoms. Comparisons between **5** and **8** suggest the effect of electron withdrawing and electron donating groups on unsubstituted phenylpyrazole curcumin (**4**).

We investigated the role played by various electron withdrawing and electron donating groups on phenylpyrazole curcumin analogues. No significant correlation between Hammett σ and the IC_{50} values was found for the substituted phenylpyrazole compounds. From the above outcome it is concluded that nature of substitutions on *N*-phenylpyrazole derivative affects the potency of compounds. 3-Nitro phenylpyrazole curcumins (**6**) showed better inhibition as compared to curcumin and other *N*-phenylpyrazole curcumin against both CQS and CQR (Table 1). Thus, the ring substituents affect the potency of the phenylpyrazole derivatives.

3.4. Substitution on Knoevenagel condensates of curcumin

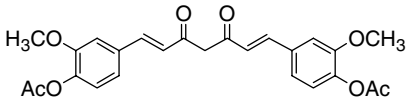
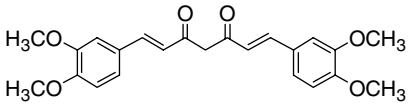
Prior to this study some Knoevenagel condensates of curcumin were synthesized and found to be capable of inhibiting NF-κβ activation.[22] In the present communication we have synthesized a number of novel Knoevenagel condensates of curcumin and evaluated their anti-malarial activity against CQ-S and CQ-R. Compounds **9**, **10**, and **11** are Knoevenagel condensate derivatives of curcumin and inhibit CQ-S cultures at IC_{50} = 3.89 μM (MIC 16.5 μM), IC_{50} = 5.85 μM (MIC = 23.7), and IC_{50} = 0.920 μM (MIC 5.2 μM), respectively. Their inhibitory potencies for CQ-R cultures also lie in a similar range (Table 1). The most active compound amongst Knoevenagel condensates of curcumin was found to be **11** (4-hydroxy-3-methoxybenzylidene derivative of curcumin) suggesting an aryl hydroxy pharmacophore at the methylene center (C_4) containing methoxy group at meta position is contributing significantly to the enhanced anti-malarial potency (Table 1). Compounds **9** (benzylidene derivative of curcumin) and **10** (4-hydroxy-benzylidene derivative of curcumin) were as effective as curcumin. Therefore, the presence of methoxy group (electron donor group) at

Table 1. IC_{50} and minimum inhibitory concentration (MIC) values of curcumin and its derivatives on cultures of *Plasmodium falciparum*

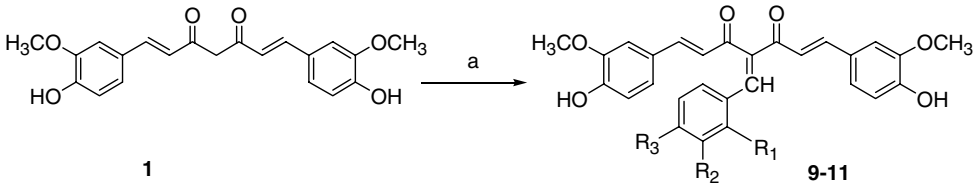
Compound ID	Structure	*P. falciparum*[a,b] IC_{50} (μM)	*P. falciparum*[a,b] MIC (μM)
1		3.25 ± 0.6 (4.21 ± 0.8)	13.2 ± 1.3 (14.4 ± 1.6)
2		8.44 ± 2.1 (7.92 ± 2.4)	27.3 ± 6.7 (25.3 ± 4.5)
3		0.48 ± 0.04 (0.45 ± 0.07)	3.9 ± 0.8 (3.6 ± 0.63)
4		8.48 ± 0.8 (9.10 ± 0.9)	22.4 ± 6.1 (25.0 ± 6.6)
5		2.42 ± 0.4 (2.10 ± 0.36)	14.3 ± 1.4 (12.9 ± 1.6)
6		0.87 ± 0.07 (0.89 ± 0.10)	4.9 ± 0.7 (5.1 ± 0.81)
7		4.65 ± 0.7 (4.80 ± 0.9)	21.81 ± 5.7 (21.4 ± 5.4)
8		22.60 ± 2.7 (24.56 ± 3.8)	35.80 ± 3.2 (41.20 ± 4.6)
9		3.89 ± 0.7 (4.12 ± 0.6)	16.5 ± 1.7 (17.20 ± 1.9)
10		5.85 ± 0.9 (5.36 ± 1.0)	23.7 ± 7.3 (23.10 ± 6.1)
11		0.92 ± 0.09 (0.75 ± 0.06)	5.2 ± 0.9 (4.80 ± 0.63)

(*continued on next page*)

Table 1 (*continued*)

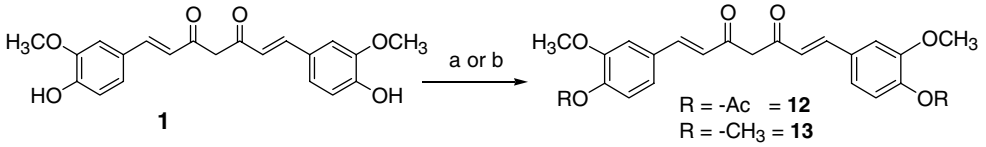
Compound ID	Structure	*P. falciparum*[a,b] IC_{50} (μM)	*P. falciparum*[a,b] MIC (μM)
12		2.34 ± 0.4 (2.51 ± 0.5)	14.9 ± 1.1 (15.30 ± 1.0)
13		7.86 ± 1.1 (8.4 ± 1.2)	29.6 ± 8.7 29.1 ± 8.1

[a] Data are expressed as means ± SD from at least three different experiments in duplicate.
[b] Values in parantheses indicate the activities of these derivatives against a CQ resistant strain MP-14 of *P. falciparum*.



Compound	R1	R2	R3
9	H	H	H
10	H	H	OH
11	H	OCH3	OH

Scheme 2. Synthesis of curcumin Knoevenagel condensates (**9**–**11**). Reagents and conditions: (a) benzaldehyde, 4-hydroxybenzaldehyde, 4-hydroxy-3-methoxy-benzaldehyde (vanillin), triphenylphosphine (catalytic amount), 75–80 °C/ 3.5 h.



Scheme 3. Synthesis of di-acetyl and di-methyl curcumin. Reagents and conditions: (a) acetyl chloride/pyridine, rt, overnight; (b) di-methyl sulfate, K_2CO_3in benzene, reflux.

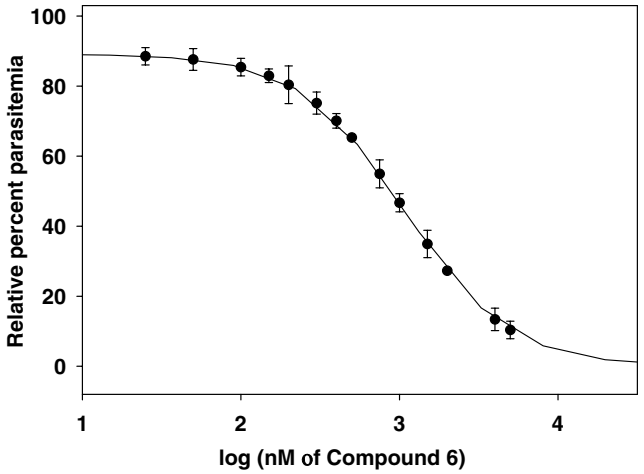


Figure 1. Inhibition of the growth of the parasite in red blood cell cultures. 3-Nitro-phenylpyrazole curcumin (Compound **6**) was assayed for parasite culture growth inhibition of *P. falciparum* by incubating the cultures with different concentrations of the inhibitors in DMSO (final concentration, 0.05%). The cultures were checked for growth inhibition by microscopic examination by assessing the parasitemia. The average of three data sets has been plotted, and the error bars are shown.

the meta position of 4-hydroxy-3-methoxy-benzylidene derivative of curcumin appears to play an important role for the potency of this class of compounds.

To understand the significance of phenolic OH group of curcumin in its anti-malarial activity, di-*O*-acetyl (**12**) and di-*O*-methyl (**13**) derivatives of curcumin have also been tested for their anti-malarial activity. The di-*O*-acetyl curcumin shows almost same potency as curcumin against *P. falciparum* culture. Reason may be that acetyl groups are attached to curcumin by ester bonds, which are biodegradable, and are apparently easily cleaved by esterases in the parasite to release the parent molecule curcumin. Hence the potency of compound **12** was equal to that of the parent molecule. Whereas methylation of the –OH resulted in the loss of potency because alkyl groups are not cleavable. From the above results (Table 1) it is apparent that two unsubstituted phenolic groups are necessary for curcumin's anti-malarial activity.

Among all the derivatives tested, pyrazole derivative of curcumin (**3**) showed the highest potency against both CQ-S and CQ-R *P. falciparum* with an IC_{50} of 0.48

and 0.45 μM, respectively (Table 1). The inhibitory potency of compound **3** was six to sevenfold higher against CQ-S and ninefold higher against CQ-R than that observed for curcumin. 3-nitrophenylpyrazole curcumin (**6**) and 4-(4-hydroxy-3-methoxy-benzylidene) derivative (**11**) of curcumin also showed an enhanced (three to five-folds) anti-malarial potency in *P. falciparum* cultures. It was found that curcumin analogues inhibited both CQ-R and CQ-S *P. falciparum* cultures in a similar fashion, indicating that curcumin and its analogues are able to bypass the mechanisms that the parasite has evolved toward CQ resistance. Given the structural differences between chloroquine and curcumin derivatives, these findings can therefore easily be rationalized.

Although the specific mechanism by which curcumin and its derivatives enact their activity against the human malaria parasite is currently under study,[15–17] their efficacy in doing so, coupled with their relatively straightforward chemical structure and amenability to chemical substitutions, make them attractive lead compounds for structure modification and drug developmental studies.

4. Conclusion

This work attempts to rationalize the structure–activity correlation for curcumin scaffold as an anti-malarial agent. The design strategy was focused on the development of curcumin analogue with high anti-malarial activity especially against CQ-R strains. Prior to these studies compounds designed around the scaffold of curcumin, excepting the usage of curcumin by itself, have not been evaluated for their anti-malarial activity against *P. falciparum* culture. We have now evaluated the anti anti-malarial activity of curcumin and its derivatives against this parasite.

From the results shown in Table 1 one can say that pyrazole curcumin (**3**), 3-nitrophenylpyrazole curcumin (**6**), and 4-hydroxy-3-methoxy-benzylidene derivative (**11**) of curcumin were more potent than curcumin (**1**) itself. These compounds (**3**, **6** and **11**) inhibited at nanomolar concentration. Their enhanced potencies are probably due to their increased cellular uptake and/or retention by the parasite in addition to their greater efficacies of inhibiting the target itself. The high potencies of some of the curcumin derivatives reported here together with the possibility of synthesizing a number of derivatives around 'curcumin' scaffold open up new opportunities for anti-malarial therapy.

5. Experimental

Reagents were purchased from Aldrich and were used as received. Reaction progress was monitored by TLC using Merck silica gel 60 F_{254} with detection by UV. Column chromatography was performed using Merck silica gel 100–200 mesh. Melting points were determined in Pyrex capillary tube using Büchi Melting Point B-540 apparatus. ^{1}H NMR spectra was recorded on 300 and 400 MHz Bruker NMR spectrometers using tetramethylsilane as internal standard and the chemical shifts are reported in (δ) units. Multiplicities are reported as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broadened). Coupling constants are reported as a J value in Hertz (Hz).The sample concentration in each case was approximately 10 mg in chloroform-d/methanol-d (0.6 mL). Mass spectra were recorded on an Electrospray–MS (Bruker Daltonis, esquire 3000^{plus}) instrument. All solvents used were of spectral grade or distilled prior to use.

5.1. Isoxazole analogue of curcumin (2)

The isoxazole analogue was prepared by treating curcumin 0.736 g (2 mmol) with hydroxylamine hydrochloride, 0.139 g (2.2 mmol), in acetic acid at 85 °C for 6 h (Scheme 1). The reaction mixture was evaporated to dryness; residue was dissolved in dichloromethane and washed with water. The crude product was chromatographed on a silica gel column, eluted with hexane/ethyl acetate, 40:60. $R_f = 0.75$ (DCM/MeOH 9:1), 0.65 (DCM/MeOH 9:1) mp 162 °C (lit. 162–163 °C). Yield: 0.556 g (76%), ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.90 (s, 6H, OCH_3), 6.68 (s, 1H, C_4-H), 6.86–6.92 (m, 4H), 6.99–7.11 (m, 4H, *Ar-H*), 7.14 (d, 1H, $J = 12.2$ Hz), 7.2 (d. 2H, $J = 12.2$ Hz). ^{13}C NMR (300 MHz, $CDCl_3$): 169.6, 163.2, 151.5, 151.1, 149.3, 149.2, 138.2, 135.6, 130.1, 129.8, 122.8, 121.6, 120.5, 120.1, 117.4, 116.2, 115.9, 115.3, 98.7, 56.7. ESI–MS m/z $[M+H]^+$366.1.

5.2. General procedure for the preparation of *N*-(substituted)phenylcurcumin pyrazole compounds (3–8)

Curcumin (1.2 mmol) was dissolved in glacial acetic acid (5 mL), and different hydrazine derivatives (1.5 mmol) (hydrazinehydrate, phenylhydrazine, 4-fluorophenylhydrazine hydrochloride, 3-nitrophenylhydrazine hydrochloride, 2, 4-dichlorophenylhydrazine hydrochloride, 4-methoxyphenylhydrazine hydrochloride) were added to the solution. The solution was refluxed for 8 h, and then the solvent was removed in vacuo. Residue was dissolved in ethyl acetate and washed with water. Organic portion was collected, dried over sodium sulfate, and concentrated in vacuo. Crude product was further purified by column chromatography. All the products (**3–8**) were prepared by using the same procedure.

5.2.1. Curcumin pyrazole (3). The silica gel eluent was hexane/ethyl acetate, 60:40. $R_f = 0.50$ (DCM/MeOH 9:1), yield: 71%, mp 214 °C (lit. 211–214). ^{1}H NMR (400 MHz, CD_3OD): δ 3.85 (s, 6H, $2 \times OCH_3$), 6.65 (s, 1H, C_4-H), 6.91 (d, 2H, $J = 15.8$ Hz, C_2-H and, C_6-H), 7.04 (d, 2H, $J = 15.8$ Hz, C_1-H & C_7-H), 7.16–7.21 (m, 6H, *Ar-H*). ^{13}C NMR (400 MHz, CD_3OD): 151.8, 151.4, 148.8, 148.2, 136.9, 136.4, 131.0, 129.8, 124.6, 124.2, 122.7, 122.6, 117.1, 116.7, 113.8, 110.9, 99.2, 56.5. ESI–MS m/z $[M+H]^+$365.20.

5.2.2. *N*-Phenylpyrazole curcumin (4). The silica gel eluent was hexane/ethyl acetate, 70:30. $R_f = 0.72$ (DCM/MeOH 9:1), yield: 65%, mp 89 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.89 (s, 6H, $2 \times OCH_3$), 6.79 (s,

1H, C_4-*H*), 6.91 (d, 2H, J = 15.8 Hz, C_2-*H* and, C_6-*H*), 7.04 (d, 2H, J = 15.8 Hz, C_1-*H* and C_7-*H*), 7.15–25 (m, 6H, *Ar-H*), 7.48–7.54(m, 5H, *Ar-H*). ^{13}C NMR(300 MHz, $CDCl_3$): 155.6, 151.9, 151.4, 147.5, 146.5, 142.1, 138.4, 133.4, 131.5, 129.6, 129.1, 127.6, 125.4, 125.1, 121.0, 120.6, 120.1, 114.5, 113.7, 108.2, 107.1, 100.1, 55.6, 55.4. ESI–MS m/z $[M+H]^+$441.2.

5.2.3. *N*-(4-Fluorophenylpyrazole) curcumin (5). The silica gel eluent was hexane/ethyl acetate, 70:30. R_f = 0.75 (DCM/MeOH 9:1), yield: 62%. mp 65 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.89 (s, 6H, $2 \times OCH_3$), 6.79 (s, 1H, C_4-*H*), 6.98 (d, 2H, J = 15.6 Hz, C_2-*H* and, C_6-*H*), 7.06 (d, 2H, J = 15.6 Hz, C_1-*H* and C_7-*H*), 7.10–7.22 (m, 6H, *Ar-H*), 7.45–7.69 (m, 4H, *Ar-H*). ^{13}C NMR (300 MHz, $CDCl_3$): 157.8, 154.1, 148.7, 148.2, 145.8, 145.2, 139.5, 135.8, 132.3, 129.8, 129.2, 124.7, 122.1, 117.9, 117.7, 113.5, 110.2, 110.6, 107.6, 56.8. ESI–MS m/z $[M+H]^+$459.10.

5.2.4. *N*-(3-Nitrophenylpyrazole) curcumin (6). The silica gel eluent was hexane/ethyl acetate, 70:30. R_f = 0.65 (DCM/MeOH 9:1), yield: 46%, mp 93 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.90 (s, 6H, OCH_3), 6.80 (s, 1H, C_4-*H*), 7.02 (d, 2H, J = 14.8 Hz, C_2-*H* and, C_6-*H*), 7.10 (d, 2H, J = 14.8 Hz, C_1-*H* and C_7-*H*), 7.15–7.32 (m, 6H, *Ar-H*), 7.65–7.71 (m, 1H, *Ar-H*), 7.98–8.12 (m, 1H, *Ar-H*), 8.22–8.26 (m, 1 H, *Ar-H*), 8.43 (d, 1H, J = 8.2 Hz, *Ar-H*). ^{13}C NMR(300 MHz, $CDCl_3$): 157.3, 153.2, 152.1, 150.8, 147.4, 146.7, 142.5, 139.0, 134.2, 132.8, 130.4, 130.1, 126.1, 124.8, 124.5, 122.0, 121.3, 120.7, 116.9, 116.5, 115.4, 113.1, 112.7, 105.2, 56.8, 56.6. ESI–MS m/z $[M-H]^+$(484.1), $[M+H]^+$486.1.

5.2.5. *N*-(2,4-Dichlorophenylpyrazole) curcumin (7). The silica gel eluent was hexane/ethyl acetate, 70:30. R_f = 0.70 (DCM/MeOH 9:1), yield: 54%, mp 142 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.89 (s, 6H, $2 \times OCH_3$), 6.81 (s, 1H, C_4-*H*), 7.01 (d, 2HJ = 15.6 Hz, C_2-*H* and, C_6-*H*), 7.08 (d, 2H, J = 15.6 Hz, C_1-*H* and C_7-*H*), 7.18–7.31 (m, 6H, *Ar-H*), 7.45 (m, 1H, *Ar-H*), 7.58 (m, 1H, *Ar-H*), 7.78 (m, 1H, *Ar-H*). ^{13}C NMR (300 MHz, $CDCl_3$): 156.6, 154.1, 147.7, 147.0, 144.2, 142.8, 135.8, 132.3, 129.8, 129.2, 124.7, 122.1, 117.9, 117.7, 113.5, 110.2, 110.6, 107.6, 56.8, 56.4. ESI–MS m/z $[M]^+$509.38.

5.2.6. *N*-(4-Methoxyphenylpyrazole) curcumin (8). The silica gel eluent was hexane/ethyl acetate, 80:20. R_f = 0.65 (DCM/MeOH 9:1), yield: 35%, mp 106 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.89 (s, 6H, $2 \times$ OCH_3), 3.90(s, 3H, OCH_3), 6.79 (s, 1H, C_4-*H*), 6.98 (d, 2H, J = 16.2 Hz, C_2-*H* and, C_6-*H*), 7.04 (d, 2H, J = 16.2 Hz, C_1-*H* and C_7-*H*), 7.09–7.20(m, 6H, *Ar-H*), 7.28–7.48 (m, 4H, *Ar-H*). ^{13}C NMR(400 MHz, $CDCl_3$): 158.6, 154.3, 148.5, 148.0, 146.2, 146.4, 137.8, 132.3, 130.5, 129.8, 129.2, 124.7, 122.1, 117.9, 117.7, 113.5, 110.2, 110.6, 107.6, 56.8, 55.4. ESI–MS m/z $[M+H]^+$471.10.

5.3. General procedure for preparation of various curcumin Knoevenagel condensates (9–11)

A mixture of aromatic aldehyde (benzaldehyde, 4-hydroxy-benzaldehyde, 4-hydroxy-3-methoxy-benzaldehyde) (5 mmol), curcumin (6.5 mmol), and triphenylphosphine (20 mol%]) was stirred at 75–80 °C for 3.5 h. The progress of the reaction was monitored by TLC. After complete conversion, as indicated by TLC, the reaction mixture was diluted with water and extracted with ethyl acetate (3×10 mL). The combined organic extracts were concentrated in vacuo and the resulting product was directly charged onto a small silica gel column and eluted with a mixture of ethyl acetate/hexane to afford pure product. All the products (**9**–**11**) were prepared using the same procedure.

5.3.1. 4-Benzylidene curcumin (9). The silica gel eluent was ethyl acetate/ hexane, 15:85. R_f = 0.62 (DCM/MeOH 9:1), yield: 58%, mp 120 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.89 (s, 6H, $2 \times OCH_3$), 6.68–7.15 (m, 6H, *Ar-H*), 7.18 (d, 2H, J = 15.0 Hz, C_2-*H* and, C_6-*H*), 7.85(d, 2H, J = 15.0 Hz, C_1-*H* and C_7-*H*), 7.35–7.48 (m, 5H, *Ar-H*), 8.16 (s, 1H, =*CH–Ar*). ^{13}C NMR(300 MHz, $CDCl_3$): 184.7, 165.9, 149.8, 147.5, 145.8, 142.1, 131.2, 129.0, 128.4, 128.1, 127.2, 124.4 123.1, 117.2, 111.9, 56.5. ESI–MS: m/z $[M+H]^+$457.23.

5.3.2. 4-(4-Hydroxybenzylidene) curcumin (10). The silica gel eluent was ethyl acetate/hexane, 20:80. R_f = 0.55 (DCM/MeOH 9:1), yield: 60%, mp 93 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.89 (s, 6H, $2 \times OCH_3$), 6.77–7.15 (m, 8H, *Ar-H*), 7.22 (d, 2H, J = 15.2 Hz, C_2-*H* and, C_6-*H*), 7.68 (d, 2H, J = 8.6 Hz), 7.84(d, 2H, J = 15.2 Hz, C_1-*H* and C_7-*H*), 8.06 (s, 1H, =*CH-Ar*). ^{13}C NMR (300 MHz, $CDCl_3$): 184.3, 165.7, 156.8, 149.4, 1467.9, 146.8, 142.1, 130.5, 127.9, 126.5, 125.6, 123.1, 117.2, 115.9, 111.9, 56.1. ESI–MS m/z $[M-H]^+$471.2.

5.3.3. 4-(4-Hydroxy-3-methoxybenzylidene) curcumin (11). The silica gel eluent was ethyl acetate/hexane, 20:80. R_f = 0.60 (DCM/MeOH 9:1), yield: 65%, mp 96 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.87 (s, 3H, OCH_3), 3.89 (s, 6H, $2 \times OCH_3$), 6.77–7.13 (m, 9H, *Ar-H*), 7.21 (d, 2H, J = 15.0 Hz, C_2-*H* and, C_6-*H*), 7.83(d, 2H, J = 15.0 Hz, C_1-*H* and C_7-*H*), 8.01 (s, 1H, =*CH–Ar*). ^{13}C NMR (300 MHz, $CDCl_3$): 184.3, 165.7, 149.8, 149.3, 1467.9, 147.6, 146.8, 146.5, 142.1, 130.5, 128.6, 128.1, 126.4, 125.6, 123.1, 117.1, 116.1, 111.6, 56.1, 55.9. ESI–MS m/z $[M+H]^+$503.2.

5.4. Di-*O*-acetylcurcumin (12)

A solution of curcumin 0.736 g (2 mmol) in anhydrous pyridine was treated with anhydrous Ac_2O (0.28 mL; 3 mmol) and stirred overnight. It was then poured in crushed ice, extracted with carbon tetrachloride, and the organic layer was concentrated in vacuo. Organic layer was washed with 1% $NaHCO_3$until effervescence ceased. The organic layer was dried over Na_2SO_4 and crystallized from absolute ethanol. Compound **12** was identified on the basis of ^{1}H NMR data comparison with literature values, yield 85% (0.768 g), R_f = 0.90 (DCM/MeOH 9:1), mp 170 °C (lit. 170–172 °C). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.3 (s, 6H, acetyl), 3.89 (s, 6H, $2 \times OCH_3$), 4.71 (s, 2H, C_4-*H*), 6.9 (d, 2H, J = 6.2 Hz, C_2-*H* and C_6-*H*), 7.15–7.34 (m, 6H, *Ar-H*),

7. 58 (d, 2H, $J = 6.0$ Hz, C_1-*H* and C_7-*H*). ^{13}C NMR (300 MHz, $CDCl_3$): δ 184.6, 183.4, 169.4, 150.4, 150.0, 149.8, 141.3, 140.3, 129.8, 128.7, 123.5, 122.6, 121.8, 111.6, 111.2, 101.4, 65.9, 55.9, 55.8. ESI–MS *m*/*z* $[M+H]^+$453.1, $[M+Na]^+$ 475.0.

5.5. Di-*O*-methylcurcumin (13)

Curcumin 0.736 g (2 mmol) in dry benzene (100 mL) was refluxed with di-methyl sulfate (10 mL) over anhydrous potassium carbonate (1 g) for 24 h, with stirring. After evaporation of benzene, the residue was stirred with warm aqueous sodium hydrogen carbonate. The mixture was extracted with chloroform, and the extracts were washed with water, dried, and evaporated. The residue was purified by column chromatography (ethyl acetate/hexane, 80:20). $R_f = 0.85$ (DCM/MeOH 9:1), yield 72% (0.570 g), mp 129–130 °C (lit. 128–130 °C). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.90 (s, 6H, $2 \times OCH_3$), 3.92 (s, 6H, $2 \times OCH_3$), 4.65 (s, 2H, C_4-H), 6.98 (d, 2H, $J = 12.4$ Hz, C_2-*H* and C_6-*H*), 7.05–7.32 (m, 6H, *Ar*-*H*), 7.61 (d, 2H, $J = 12.4$ Hz, C_1-*H* and C_7-*H*). ^{13}C NMR (300 MHz, CDCl3): δ 183.2, 151.0, 149.2, 140.4, 128.1, 122.6, 122.0, 111.1, 109.8, 101.3, 56.0. ESI–MS *m*/*z* $[M+H]^+$ 397.2.

5.6. Assessment of inhibition of growth of *P. falciparum* and determination of IC_{50} of the compounds

The experiments were performed using two strains of *P. falciparum*, FCK2 strain (chloroquine-sensitive, IC_{50}, 18 nM), and MP-14 (chloroquine-resistant, IC_{50}, 300 nM for chloroquine), isolates from Karnataka and Maharashtra states of India. *P. falciparum* was cultured using standard techniques[27,28] and synchronized using 5% sorbitol[29] at 4% hematocrit in RPMI 1640 medium (Invitrogen, CA, USA) supplemented with 10% human serum, 0.225% sodium bicarbonate, and 0.01 mg/mL gentamicin. Growth inhibition was monitored using microscopic examination of the parasites by standard Giemsa staining. Typically uninfected or infected (1–2% parasitemia, ring stage) red blood cells (2% hematocrit) were added to the culture medium in the wells of a 96-well plate (Nunc, Roskilde, Denmark), and different concentrations of inhibitor in DMSO did not exceed 0.05%. The experiment started with the synchronized parasite culture in the early trophozoite stage and inhibitors were added up to fourth day. DMSO (0.05%) was used as the solvent control. The IC_{50} was calculated from a plot of relative percent parasitemia versus log concentration of the inhibitor by fitting it to non-linear regression analysis using Sigma Plot 2000 software (Systat Software Inc., CA, USA). The MIC of the curcumin derivatives is defined as the minimum concentration at which more than 99% of the parasites, relative to the control, were inhibited from developing to schizonts.

5.7. Assessment of inhibition of growth of *P. falciparum* and determination of IC_{50} of the compounds by [^{3}H]-hypoxanthine uptake assay

IC_{50} was also determined by the [^{3}H]-hypoxanthine uptake assay. The parasites were cultured using standard techniques as described above for the microscopic examination. The semi-automated microdilution technique of Desjardins et al., which is based on [^{3}H]-hypoxanthine uptake by parasites, was used to assess the sensitivity of the parasites to various inhibitors.[30] Briefly, synchronized parasites were cultured in 96-well plates (Nunc, Copenhagen, Denmark) at 2–3% hematocrit and at an initial parasitemia of 1–2%, with varying concentrations of the inhibitors, and addition of the inhibitor in fresh medium every 24 h for 48 h. All additions were done in duplicate. Inhibitor stocks were made in sterile DMSO and dilutions made such that the final concentration of DMSO in the parasite culture did not exceed 0.05%. Parasites synchronized at the ring stage were cultured in the presence of varying concentrations of the inhibitors for the first 48 h and then incubated with [^{3}H]-hypoxanthine (1 μCi/well) for the next 36 h and harvested. They were then harvested using a Nunc cell harvester onto glass fiber filters, washed, and subjected to liquid scintillation counting (Hewlett-Packard). IC_{50s} were calculated from plots of relative percent parasitemia versus log concentration of the respective inhibitors, fitted to non-linear regression analysis using Sigma Plot 2000 software. The IC_{50} values using this method were similar to those obtained using microscopic examination.

Acknowledgments

This work was supported by a grant from the Department of Science and Technology (DST, Government of India) under PRDSF to N.S. and also by a grant from the Centre of Excellence, DBT, to A.S. A.S. is J.C. Bose fellow of the Department of Science and Technology. S.M. acknowledges DBT, Government of India, for postdoctoral fellowship. K.K. acknowledges the CSIR, Government of India, for a senior research fellowship. We do not have any competing financial interests with regard to this work.

References and notes

1. National Institute of Allergy and Infectious Diseases (NIAID). Global health research plan for HIV/AIDS, Malaria and Tuberculosis. World Health Organization: Geneva, 2001; p18.
2. Butcher, G. A.; Sinden, R. E.; Curtis, C. *Today* **2000**, *16*, 43.
3. Adams, J. H.; Wu, Y.; Fairfield, A. *Parasitol. Today* **2000**, *16*, 89.
4. News from the WHO Division of Control of Tropical Diseases. *TDR News* **1994**, *46*, 5.
5. Clark, I. A.; Schofield, L. *Parasitol. Today* **2000**, *16*, 451.
6. Greenwood, B.; Mutabingwa, T. *Nature* **2002**, *415*, 670.
7. Ridley, R. G. M. *Nature* **2002**, *415*, 686.
8. Mehta, K.; Pantazis, P.; McQueen, T.; Aggarwal, B. B. *Anticancer Drugs* **1997**, *8*, 470.
9. Kuo, M. L.; Huang, T. S.; Lin, J. K. *Biochim. Biophys. Acta* **1996**, *1317*, 95.
10. Jee, S. H.; Shen, S. C.; Tseng, C. R.; Chiu, H. C.; Kuo, M. L. *J. Invest. Dermatol.* **1998**, *111*, 656.
11. Kawamori, T.; Lubet, R.; Steele, V. E.; Kelloff, G. J.; Kaskey, R. B.; Rao, C. V.; Reddy, B. S. *Cancer Res.* **1999**, 597.

12. Singletary, K.; MacDonald, C.; Iovinelli, M.; Fisher, C.; Wallig, M. *Carcinogenesis* **1998**, *19*, 1039.
13. Ruby, A. J.; Kuttan, G.; Babu, K. D.; Rajasekeharan, K. N.; Kuttan, R. *Cancer Lett.* **1995**, *94*, 79.
14. Kuttan, R.; Bhanumathy, P.; Nirmala, K.; George, M. C. *Cancer Lett.* **1985**, *29*, 197.
15. Flynn, D. L.; Belliotti, T. R.; Boctor, A. M.; Connor, D. T.; Kostlan, C. R.; Nies, D. E.; Ortwine, D. F.; Schirier, D. J.; Sricar, J. C. *J. Med. Chem.* **1991**, *34*, 518.
16. Ishida, J.; Ohtsu, H.; Tachiban, Y.; Nakanishi, Y.; Bastow, K. F.; Nagai, M.; Wang, H. K.; Itokawa, H.; Lee, K. H. *Bioorg. Med. Chem.* **2002**, *10*, 3481.
17. Shim, J. S.; Kim, D. H.; Jung, H. J.; Kim, J. H.; Lim, D.; Lee, S. K.; Kim, K. W.; Ahn, J. W.; Yoo, J. S.; Rho, J. R.; Shin, J.; Kwon, H. J. *Bioorg. Med. Chem.* **2002**, *10*, 2987.
18. Selvam, C.; Jachak, S. M.; Thilagavathi, R.; Chakrabortib, A. K. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1793.
19. Reddy, R. C.; Vatsalab, P. G.; Keshamounia, V. G.; Padmanaban, G.; Rangarajan, P. N. *Biochem. Biophys. Res. Commun.* **2005**, *326*, 472.
20. Nandakumar, D. N.; Nagaraj, V. A.; Vathsala, P. G.; Rangarajan, P.; Padmanaban, G. *Antimicrob. Agents Chemother.* **2006**, 1859.
21. Cui, L.; Miao, J.; Cui, L. *Antimicrob. Agents Chemother.* **2007**, *51*, 488.
22. Zambre, A. P.; Kulkarni, V. M.; Padhye, S.; Sandur, S. K.; Aggarwal, B. B. *Bioorg. Med. Chem.* **2006**, *14*, 7196.
23. Cheng, A.-L.; Hsu, C.-H.; Lin, J.-K.; Hsu, M.-M.; Ho, Y.-F.; Shen, T.-S.; Ko, J.-Y.; Lin, J.-T.; Lin, B.-R.; Wu, M.-S.; Yu, H.-S.; Jee, S.-H.; Chen, G.-S.; Chen, T.-M.; Chen, C.-A.; Lai, M.-K.; Pu, Y.-S.; Pan, M.-H.; Wang, Y.-J.; Tsai, C.-C.; Hsieh, C.-Y. *Anticancer Res.* **2001**, *21*, 2895.
24. Roughley, P. J.; Whiting, D. A. *J. Chem. Soc., Perkin Trans. 1* **1973**, *20*, 2379.
25. Venkatachalam, T. K.; Qazi, S.; Samuel, P.; Uckun, F. M. *Bioorg. Med. Chem.* **2003**, *11*, 1095.
26. Peat, A. J.; Townsend, C.; Worley, J. F.; Allen, S. H.; Garrido, D.; Mertz, R. J.; Pfohl, J. L.; Terry, C. M.; Truax, J. F.; Veasey, R. L.; Thomson, S. A. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2977.
27. Rowe, J. A.; Scragg, I. G.; Kwiatkowski, D.; Ferguson, D. J.; Carucci, D. J.; Newbold, C. I. *Mol. Biochem. Parasitol.* **1998**, *92*, 177.
28. Trager, W.; Jenson, J. B. *Science* **1976**, *193*, 673.
29. Lambros, C.; Vanderberg, J. *J. Parasitol.* **1979**, *65*, 418.
30. Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. *Antimicrob. Agents Chemother.* **1979**, *16*, 710.