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Original article

# Curcuminoid analogs with potent activity against *Trypanosoma* and *Leishmania* species

Chatchawan Changtam [a], Harry P. de Koning [b], Hasan Ibrahim [b], M. Sohail Sajid [b], Matthew K. Gould [b], Apichart Suksamrarn [a,*]

[a] Department of Chemistry, Faculty of Science, Ramkhamhaeng University, Ramkhamhaeng Road, Bangkok 10240, Thailand
[b] Institute of Biomedical and Life Sciences, Division of Infection and Immunity, University of Glasgow, Glasgow G12 8TA, UK

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ABSTRACT

The natural curcuminoids curcumin (**1**), demethoxycurcumin (**2**) and bisdemethoxycurcumin (**3**) have been chemically modified to give 46 analogs and 8 pairs of 1:1 mixture of curcuminoid analogs and these parent curcuminoids and their analogs were assessed against protozoa of the *Trypanosoma* and *Leishmania* species. The parent curcuminoids exhibited low antitrypanosomal activity ($EC_{50}$ for our drug-sensitive *Trypanosoma brucei brucei* line (WT) of compounds **1**, **2** and **3** are 2.5, 4.6 and 7.7 μM, respectively). Among 43 curcuminoid analogs and 8 pairs of 1:1 mixture of curcuminoid analogs tested, 8 pure analogs and 5 isomeric mixtures of analogs exhibited high antitrypanosomal activity in sub-micromolar order of magnitude. Among these highly active analogs, 1,7-bis(4-hydroxy-3-methoxyphenyl)hept-4-en-3-one (**40**) was the most active compound, with an $EC_{50}$ value of $0.053 \pm 0.007$ μM; it was about 2-fold more active than the standard veterinary drug diminazene aceturate ($EC_{50}$ $0.12 \pm 0.01$ μM). Using a previously characterized diminazene-resistant *T. b. brucei* (TbAT1-KO) and a derived multi-drug resistant line (B48), no cross-resistance of curcuminoids was observed to the diamidine and melaminophenyl arsenical drugs that are the current treatments. Indeed, curcuminoids carrying a conjugated keto (enone) motif, including **40**, were significantly more active against *T. b. brucei* B48. This enone motif was found to contribute to particularly high trypanocidal activity against all *Trypanosoma* species and strains tested. The parent curcuminoids showed low antileishmanial activity ($EC_{50}$ values of compounds **1** and **2** for *Leishmania mexicana* amastigotes are $16 \pm 3$ and $37 \pm 6$ μM, respectively) while the control drug, pentamidine, displayed an $EC_{50}$ of $16 \pm 2$ μM. Among the active curcuminoid analogs, four compounds exhibited $EC_{50}$ values of less than 5 μM against *Leishmania major* promastigotes and four against *L. mexicana* amastigotes. No significant difference in sensitivity to curcuminoids between *L. major* promastigotes and *L. mexicana* amastigotes was observed. The parent curcuminoids and most of their analogs were also tested for their toxicity against human embryonic kidney (HEK) cells. All the curcuminoids exhibited lower toxicity to HEK cells than to *T. b. brucei* bloodstream forms and only one of the tested compounds showed significantly higher activity against HEK cells than curcumin (**1**). The selectivity index for *T. b. brucei* ranged from 3-fold to 1500-fold. The selectivity index for the most active analog, the enone **40**, was 453-fold.



## 1. Introduction

Trypanosomiasis and leishmaniasis are among the most neglected diseases in the world [1,2]. The diseases are caused by related protozoan parasites, *Trypanosoma* and *Leishmania* species, respectively, both of the order Kinetoplastida. Therapeutic options for both diseases are severely limited and first line treatment still depends on decades-old preparations based on toxic heavy metals: arsenic in melarsoprol for trypanosomiasis and antimony in meglumine antimoniate (Glucantime) and sodium stibogluconate (Pentostam) for leishmaniasis [3,4]. Any newer options such as difluoromethyl ornithine (DFMO) for late stage human trypanosomiasis or lipid formulations of amphotericin B (AmBisome) suffer from serious drawbacks such as very high cost and/or extreme dosage schemes [3,5,6]. Yet, human African trypanosomiasis (HAT, or sleeping sickness) reached epidemic proportions during the 1990s [7], at the same time that treatment failure with the traditional treatment, melarsoprol, reached alarming rates of up to 30%

* Corresponding author. Tel.: +662 3190931; fax: +662 3108404.
*E-mail addresses:* s_apichart@ru.ac.th, asuksamrarn@yahoo.com (A. Suksamrarn).

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[3,8]. In addition, the impact of trypanosomiasis on livestock farming and development in sub-Saharan Africa is enormous, responsible for losses topping 1 billion US dollars [9]. *Trypanosoma evansi* is further responsible for infections in camels, buffalo and other livestock in South Asia [10] and *Trypanosoma vivax* affects livestock in South America [11]. Similarly, various forms of leishmaniasis, ranging from cutaneous and muco-cutaneous to visceral leishmaniasis or kala-azar, is a severe disease throughout Africa, South Asia, Mid and South America, with antimony resistance particularly acute in India. The estimated mortality of these tropical diseases is exceeded only by malaria and tuberculosis [2]. Thus, new, affordable drugs are urgently required, preferably with a broad activity against the kinetoplastid parasites.

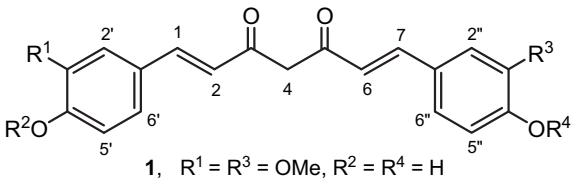
Curcuminoids are the major constituents of turmeric (*Curcuma longa* L.) and some other *Curcuma* species, including a number of other plant species. It has been known for some time that curcuminoids have been used as a natural food additive. The main curcuminoid isolated from *C. longa* is curcumin (**1**). The minor constituents include demethoxycurcumin (**2**) and bisdemethoxycurcumin (**3**). Curcuminoids exhibited many interesting biological activities [12], for example, antioxidant activity [13,14], anti-inflammatory activity [15,16], anticancer activity [13,17,18], anti-protozoal activity [19] and anti-HIV activity [20]. In recent years, it has been reported that curcumin (**1**) displays moderate-to-low activity against *Trypanosoma brucei brucei* [21] and against promastigotes of *Leishmania major* [22] but no effort to systematically screen for a curcuminoid analog with enhanced anti-kinetoplastid activity has been reported. Here we describe the synthesis and antiparasitic screening of a series of curcuminoid analogs. Compared to the parent compound, curcumin (**1**), many of the new compounds displayed a much-enhanced activity against kinetoplastid parasites that cause human and veterinary disease, while displaying limited if any toxicity against a human cell line. We propose that analogs of curcuminoids are promising new lead compounds against leishmaniasis and African trypanosomiasis.

## 2. Chemistry

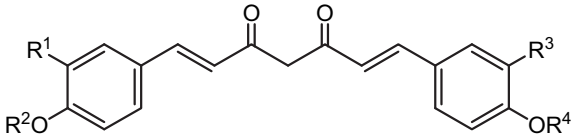
In order to investigate the antitrypanosomal and antileishmanial activities of curcuminoid analogs, the parent curcuminoids curcumin (**1**), demethoxycurcumin (**2**) and bisdemethoxycurcumin (**3**), which were obtained from the rhizomes of *C. longa* in a ratio of 76.0:16.2:3.8, were subjected to structural modification.

### 2.1. Demethylated and methylated analogs

To study the effects of free hydroxyl group and hydrogen bonding of the oxygen function at the aromatic rings, the parent curcuminoids **1**–**3** were subjected to demethylation and methylation to the appropriate demethylated and methylated analogs (see Scheme 1). Thus, starting from the parent compound **1**, demethylation to the corresponding mono-*O*-demethyl analog **4** and di-*O*-demethyl analog **5** were accomplished in 43 and 33% yields, respectively, by treatment of **1** with boron tribromide in dry dichloromethane. Demethylation of the curcuminoid **2** was similarly achieved to the corresponding *O*-demethyl analog **6** in 64% yield. The spectroscopic (IR, $^1$H NMR and mass spectra) data of **4**, **5** and **6** were consistent with the reported values [23]. Methylation of compounds **1**, **2** and **3** was achieved by reacting with methyl iodide in acetone in the presence of potassium carbonate to give the corresponding ether analogs **7**, **8**, **9** and **10** in 80, 20, 54 and 27% yields, respectively. The spectroscopic data of **7** were in agreement with the reported values [24], and those of the ether analogs **8** and **10** were consistent with those reported previously [25]. The IR, $^1$H NMR and MS data of the ether **9** were consistent with their structures (see Section 5).




**1**, $R^1 = R^3 = OMe$, $R^2 = R^4 = H$
**2**, $R^1 = R^2 = R^4 = H$, $R^3 = OMe$
**3**, $R^1 = R^2 = R^3 = R^4 = H$




| | | Reagent |
|---|---|---|
| **4**, | $R^1 = OH$, $R^2 = R^4 = H$, $R^3 = OMe$ | *a* |
| **5**, | $R^1 = R^3 = OH$, $R^2 = R^4 = H$ | *a* |
| **6**, | $R^1 = R^2 = R^4 = H$, $R^3 = OH$ | *a* |
| **7**, | $R^1 = R^3 = OMe$, $R^2 = R^4 = Me$ | *b* |
| **8**, | $R^1 = R^4 = H$, $R^3 = OMe$, $R^2 = Me$ | *b* |
| **9**, | $R^1 = H$, $R^2 = R^4 = Me$, $R^3 = OMe$ | *b* |
| **10**, | $R^1 = R^3 = R^4 = H$, $R^2 = Me$ | *b* |
| **11**, | $R^1 = R^3 = OMe$, $R^2 = R^4 =$  | *c* |
| **12**, | $R^1 = R^3 = OMe$, $R^2 =$  , $R^4 = H$ | *d* |
| **13**, | $R^1 = R^3 = OMe$, $R^2 = R^4 =$  | *d* |
| **14**, | $R^1 = R^3 = OMe$, $R^2 =$  , $R^4 = H$ | *e* |
| **15**, | $R^1 = R^3 = OMe$, $R^2 = R^4 =$  | *e* |
| **16**, | $R^1 = H$, $R^2 = R^4 =$  , $R^3 = OMe$ | *e* |
| **17**, | $R^1 = R^3 = OMe$, $R^2 =$  , $R^4 = H$ | *f* |
| **18**, | $R^1 = R^3 = OMe$, $R^2 = R^4 =$  | *f* |
| **19a**, | $R^1 = H$, $R^2 =$  , $R^3 = OMe$, $R^4 = H$ | *f* |
| **19b**, | $R^1 = R^2 = H$, $R^3 = OMe$, $R^4 =$  | *f* |

**Scheme 1.** Demethylation and alkylation of curcuminoids **1**-**3**. Reagents and conditions: (a) $BBr_3$, $CH_2Cl_2$, 0 °C, then ambient temp.; (b) $CH_3I$, $K_2CO_3$, acetone, reflux; (c) *n*-propyl iodide, $K_2CO_3$, acetone, reflux; (d) allyl bromide, $K_2CO_3$, acetone, reflux; (e) 3,3-dimethylallyl bromide, $K_2CO_3$, acetone, reflux; (f) 2-bromoethanol, $K_2CO_3$, acetone, reflux.

### 2.2. Higher alkyl ether analogs

A number of higher alkyl ether analogs of curcumin (**1**) were prepared to study the effect of different nature of the alkyl groups on antiprotozoal activities. By employing a similar procedure for the preparation of the foregoing methyl ethers, but using different alkyl bromide or iodide (*n*-propyl iodide, allyl bromide and 3,3-dimethylallyl bromide), the corresponding alkyl ethers, di-*O*-*n*-propylcurcumin (**11**) (85%), mono-*O*-allylcurcumin (**12**) (33%), di-*O*-allylcurcumin (**13**) (46%), mono-*O*-(3,3-dimethylallyl)curcumin (**14**) (43%) and di-*O*-(3,3-dimethylallyl)curcumin (**15**) (40%), were prepared (see Scheme 1). The *O*-3,3-dimethylallyl analog of compound **2**, di-*O*-(3,3-dimethylallyl)demethoxycurcumin (**16**), was also prepared in 43% yield. Compounds **12**, **13** and **15** have previously been prepared and have been used in some biological evaluations [26,27]. By comparison with compound **15**, the spectroscopic data of compounds **14** and **16** (see Section 5) were

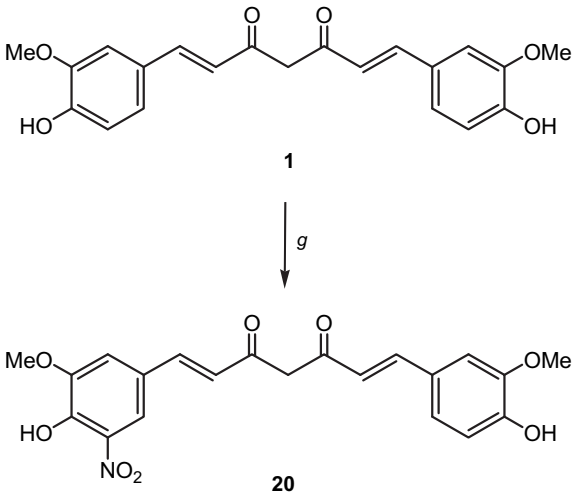
compatible with their structures. The structure of **11** was confirmed by spectroscopic data (see Section 5). Moreover, by using 2-bromoethanol as the alkylating agent, the 2-hydroxyethyl ether analogs, mono-*O*-(2-hydroxyethyl)curcumin (**17**) and di-*O*-(2-hydroxyethyl)curcumin (**18**), were also prepared in 47 and 32% yields. The presence of the 2-hydroxyethoxyl moiety was evident from the two-proton signals at $\delta$ 4.11 and 3.92 for the mono-*O*-(2-hydroxyethyl) ether (**17**), and the four-proton signals at $\delta$ 4.07 and 3.90 for the di-*O*-(2-hydroxyethyl) ether (**18**). The IR and mass spectral data also confirmed the structures of these two compounds. A mixture of 4″-*O*-(2-hydroxyethyl)demethoxycurcumin (**19a**) and 4′-*O*-(2-hydroxyethyl)demethoxycurcumin (**19b**) was similarly obtained from compound **2**. Pure **19a** was obtained in 28% yield after careful repeated column chromatography followed by recrystallization. However, compound **19b** could not be purified in similar manner to that of compound **19a**. The structure of **19a** was confirmed by spectroscopic data (see Section 5).

### 2.3. Nitro analog

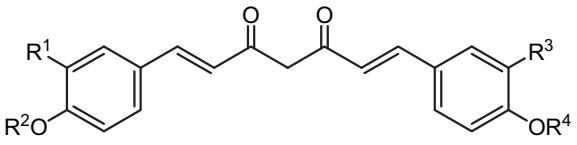
To see the influence of an extra substituent on the aromatic ring of curcumin (**1**), the nitro analog **20** was prepared in 27% yield as described in the Section 5 (Scheme 2). The presence of a nitro group at the 5′-position was evident from the proton resonance of H-6′, which has changed from a broad doublet signal ($J = 8.1$ Hz) at $\delta$ 7.10 in compound **1** to a singlet signal at $\delta$ 7.87 in compound **20**. The mass spectral data also confirmed the structure of the nitro compound **20**.

### 2.4. Acetate and benzoate esters

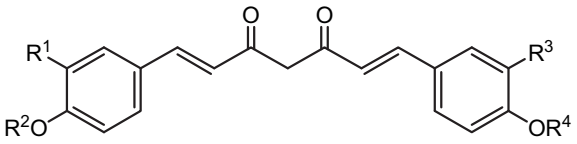
The ester derivatives chosen for this study were the acetate and benzoate esters. Acetylation of the parent curcuminoids **1**, **2** and **3** by conventional method furnished the di-*O*-acetyl analogs **21** [28], **22** [29] and **23** [30] in good yields (Scheme 3). Benzoylation of **1** with benzoic anhydride in pyridine furnished the monobenzoate **24** and the dibenzoate **25** [31] in 47 and 33% yields, respectively. The curcuminoid **2** was similarly benzoylated to the monobenzoates **26** and **27**, and the dibenzoate **28** in 34, 29 and 21% yields, respectively. The curcuminoid **3** was also benzoylated to the monobenzoates **29** and the dibenzoate **30** in 53 and 31% yields, respectively (Scheme 3). The spectroscopic data of the synthesized benzoates (see Section 5) were in agreement with the structures.




**Scheme 2.** Nitration of curcumin (**1**). Reagents and conditions: (g) $NaNO_2$, AcOH, $CH_2Cl_2$.




**1**, $R^1 = R^3 = OMe$, $R^2 = R^4 = H$
**2**, $R^1 = R^2 = R^4 = H$, $R^3 = OMe$
**3**, $R^1 = R^2 = R^3 = R^4 = H$




Reagent

| | | |
|---|---|---|
| **21**, | $R^1 = R^3 = OMe$, $R^2 = R^4 = Ac$ | *h* |
| **22**, | $R^1 = H$, $R^2 = R^4 = Ac$, $R^3 = OMe$ | *h* |
| **23**, | $R^1 = R^3 = H$, $R^2 = R^4 = Ac$ | *h* |
| **24**, | $R^1 = R^3 = OMe$, $R^2 = PhCO$, $R^4 = H$ | *i* |
| **25**, | $R^1 = R^3 = OMe$, $R^2 = R^4 = PhCO$ | *i* |
| **26**, | $R^1 = R^4 = H$, $R^2 = PhCO$, $R^3 = OMe$ | *i* |
| **27**, | $R^1 = R^2 = H$, $R^3 = OMe$, $R^4 = PhCO$ | *i* |
| **28**, | $R^1 = H$, $R^2 = R^4 = PhCO$, $R^3 = OMe$ | *i* |
| **29**, | $R^1 = R^3 = R^4 = H$, $R^2 = PhCO$ | *i* |
| **30**, | $R^1 = R^3 = H$, $R^2 = R^4 = PhCO$ | *i* |

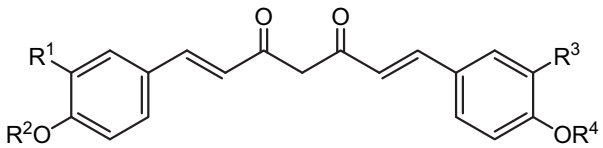
**Scheme 3.** Acetylation and benzoylation of curcuminoids **1**–**3**. Reagents and conditions: (h) $Ac_2O$, pyridine; (i) $(PhCO)_2O$, pyridine.

### 2.5. Reduced curcuminoid analogs

A number of non-conjugated analogs of curcuminoids **1**, **2** and **3** were prepared for biological evaluation (Scheme 4). Catalytic hydrogenation of compound **1**, with palladium on charcoal as a catalyst, furnished tetrahydrocurcumin (**31**), hexahydrocurcumin (**32**) and octahydrocurcumin (**33**) in 64, 20 and 15% yields, respectively. The spectroscopic data of compounds **31**–**33** were consistent with the reported values [32,33]. From the curcuminoids **2** and **3**, the tetrahydro analogs **34** and **35** were similarly prepared in 72 and 75% yields, respectively. The spectroscopic data of compounds **34** and **35** were consistent with the reported values [34]. In addition, the reduced analogs **36**, **37a**/**37b** mixture, **38** and **39** were similarly prepared from the curcuminoid analogs **4**, **5** and **7** in 40, 37, 65 and 62% yields, respectively. The spectroscopic data of compound **36** were in agreement with the structure (see Section 5). The spectroscopic data of compounds **38** and **39** were consistent with the reported values [26,34,35]. The products **37a** and **37b** are a 1:1 inseparable mixture. One of them, **37a**, has been isolated from *Zingiber officinale* [35]. At this stage, no further investigation for the separation of these two compounds and other mixture was attempted.

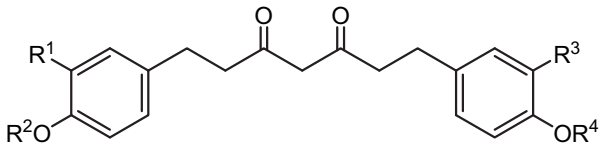
### 2.6. Monoketo analogs

In order to prepare monoketo analogs of the curcuminoid **1**, compound **32** was subjected to dehydration, with *p*-toluenesulfonic acid as a catalyst, to afford the enone **40** in quantitative yield (Scheme 4). The presence of the α,β-unsaturated keto system was



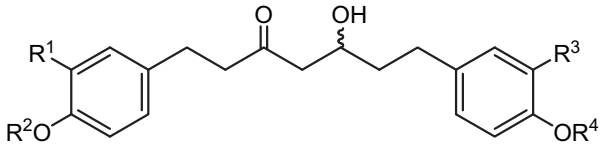
**1**, $R^1 = R^3 = OMe$, $R^2 = R^4 = H$
**2**, $R^1 = R^2 = R^4 = H$, $R^3 = OMe$
**3**, $R^1 = R^2 = R^3 = R^4 = H$
**4**, $R^1 = OH$, $R^2 = R^4 = H$, $R^3 = OMe$
**5**, $R^1 = R^3 = OH$, $R^2 = R^4 = H$
**7**, $R^1 = R^3 = OMe$, $R^2 = R^4 = Me$

*j*



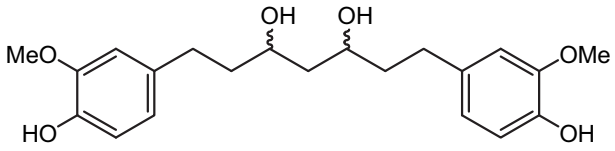
**31**, $R^1 = R^3 = OMe$, $R^2 = R^4 = H$
**34**, $R^1 = R^2 = R^4 = H$, $R^3 = OMe$
**35**, $R^1 = R^2 = R^3 = R^4 = H$
**36**, $R^1 = OH$, $R^2 = R^4 = H$, $R^3 = OMe$
**38**, $R^1 = R^3 = OH$, $R^2 = R^4 = H$

+



**32**, $R^1 = R^3 = OMe$, $R^2 = R^4 = H$
**37a**, $R^1 = OMe$, $R^2 = R^4 = H$, $R^3 = OH$
**37b**, $R^1 = OH$, $R^2 = R^4 = H$, $R^3 = OMe$
**39**, $R^1 = R^3 = OMe$, $R^2 = R^4 = Me$

+



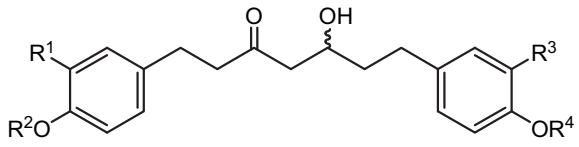
**33**

**Scheme 4.** Catalytic hydrogenation of curcuminoids **1**–**7**. Reagents and conditions: (j) $H_2$/Pd-C, EtOH.

evident from the IR absorption band at 1660 $cm^{-1}$ and the broad doublet signal ($J = 15.9$ Hz, H-4) at $\delta$ 6.08 and a partially overlapping signal at $\delta$ 6.77–6.84 (H-5) in the $^1$H NMR spectrum. Other proton resonances and mass spectral data (see Section 5) confirmed the structure of compound **40**. The structure of this compound was further confirmed by comparison with the reported $^1$H NMR data of gingerenone, which was isolated from *Z. officinale* [36]. Dehydration of the isomeric mixture of the hexahydro analogs **37a** and **37b**, the corresponding inseparable isomeric enone **41a** and **41b** were obtained in 88% yield (Scheme 5). The $^1$H NMR data of compounds **41a** and **41b** mixture (see Section 5) were consistent with the structures and with those reported values of **41a** [37]. The enone **42** was prepared by sodium borohydride reduction of the saturated diketone **38** followed by dehydration of the corresponding hexahydro analog to the required enone **42** in 65% (Scheme 6). The spectroscopic data of compound **42** were consistent with the reported values [38]. Methylation of compound **40** furnished the mono-*O*-methyl analogs **43a** and **43b** (1:1 mixture) and di-*O*-methyl analogs **29** in 30 and 57% yields, respectively. The $^1$H NMR spectrum of **43** was similar to that of **40**, except for the presence of additional methoxyl signal in that of compounds **43** and **43b** mixture. The $^1$H NMR data of **44** were consistent with those reported previously [39]. Following the method for the preparation of the enone **42** from the dione **38**, a 1:1 mixture of compounds **45a** and **45b** was prepared from the diketo compound **34** in 62% overall yield (Scheme 6). The spectroscopic data of the products **45a** and **45b** were consistent with the reported values [36,40]. Compound **46** was similarly prepared in 60% overall yield from compound **35**. The spectroscopic (IR, $^1$H NMR and mass spectra) data of compound **46** were consistent with the reported values [41].

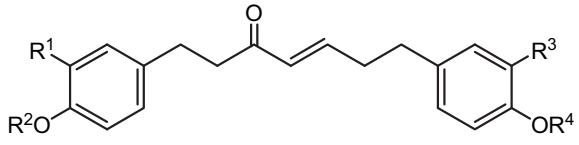
### 2.7. Dienone and trienone analogs

In order to see whether higher degree of unsaturation would affect the biological activities, the enone **40** was subjected to dehydrogenation with DDQ in THF to give the dienones **47** [42] and **48**, and the trienone **49** [43] in 28, 23 and 32% yields, respectively (Scheme 7). The $^1$H NMR spectrum of **47** showed the characteristic α,β,γ,δ-unsaturated keto system at $\delta$ 6.22 (d, $J = 15.4$ Hz, H-4), 7.28 (dd, $J = 15.4$, 10.7 Hz, H-5), 6.64–6.73 (obscured signal, H-6) and 6.83 (d, $J = 15.4$ Hz, H-7). Compound **48** also exhibited two characteristic



**32**, $R^1 = R^3 = OMe$, $R^2 = R^4 = H$
**37a**, $R^1 = OMe$, $R^2 = R^4 = H$, $R^3 = OH$
**37b**, $R^1 = OH$, $R^2 = R^4 = H$, $R^3 = OMe$

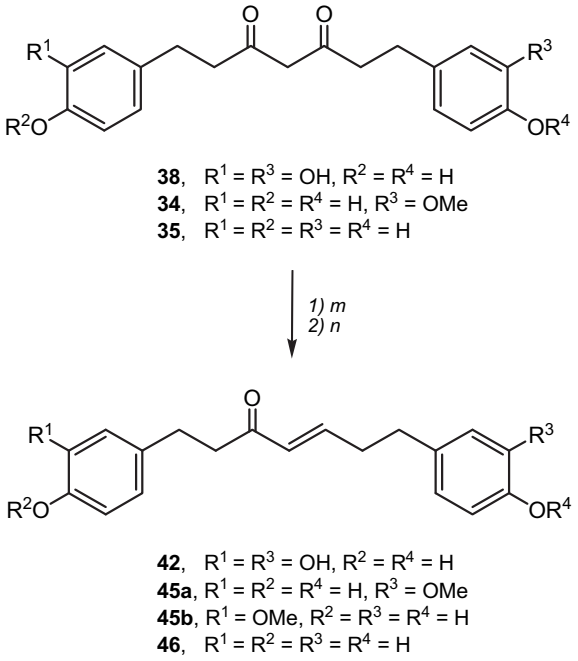
*k*



**40**, $R^1 = R^3 = OMe$, $R^2 = R^4 = H$
**41a**, $R^1 = OMe$, $R^2 = R^4 = H$, $R^3 = OH$
**41b**, $R^1 = OH$, $R^2 = R^4 = H$, $R^3 = OMe$
**43a**, $R^1 = R^3 = OMe$, $R^2 = H$, $R^4 = Me$
**43b**, $R^1 = R^3 = OMe$, $R^2 = Me$, $R^4 = H$
**44**, $R^1 = R^3 = OMe$, $R^2 = R^4 = Me$

*l*

**Scheme 5.** Dehydration of hexahydrocurcuminoid analogues **37a** and **37b**, and methylation of the enone **40**. Reagents and conditions: (k) *p*-TsOH, $C_6H_6$, reflux; (l) $CH_3I$, $K_2CO_3$, acetone, reflux.




**Scheme 6.** Preparation of the enones **42**, **45a**, **45b** and **46** from the diketones **38**, **34** and **35**. Reagents and conditions: (m) $NaBH_4$, EtOH; (n) *p*-TsOH, $C_6H_6$, reflux.

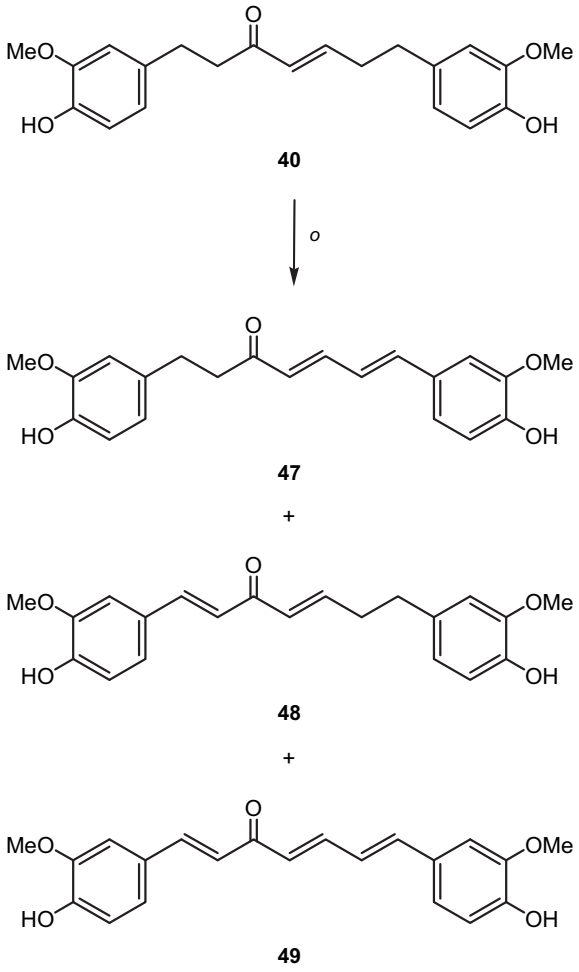
α,β-unsaturated keto systems at δ 7.52 (d, $J = 15.9$ Hz, H-1), 6.76 (d, $J = 15.9$ Hz, H-2), 6.42 (d, $J = 15.6$ Hz, H-4) and 6.98 (m, H-5). The spectroscopic data of compound **49** were in agreement with the structure and were consistent with the reported values [43].

### 2.8. Alkyl ethers and acetate derivatives of enone

A number of alkyl derivatives of the enone **40** were prepared (see Scheme 8). Thus pentylation of **40** with *n*-pentyl iodide afforded the mono-*O*-*n*-pentyl analogs **50a** and **50b** (1:1 mixture) and di-*O*-*n*-pentyl analog **51** in 53 and 27%, respectively. Each of the proton resonances of these two isomeric analogs **50a** and **50b** (see Section 5) were superimposed, except for the methoxy resonances which appeared at δ 3.81, 3.82, 3.83 and 3.84 and were the diagnostic signals for the existence of two isomers. The mono-*O*-(2-hydroxyethyl) analogs **52a** and **52b** (1:1 mixture) were prepared in 27% from **40** in the same manner as that of compound **17**. Like the analogs **50a** and **50b** mixture, each of the proton resonances of the two isomeric analogs **52a** and **52b** (see Section 5) were also superimposed, except for the methoxy resonances at δ 3.80 (s, 3H), 3.81 (2 × 3H) and 3.82 (3H) that revealed the existence of two isomers. Acetylation of the enone **40** gave a 1:1 mixture of the monoacetates **53a** and **53b** and the diacetate **54** (Scheme 8). For the isomeric mixture **53a** and **53b**, the existence of two isomers was evident from the two AcO signals at δ 2.27 and 2.28, and four MeO signals at δ 3.78, 3.79, 3.84 and 3.85. The spectroscopic data of the diacetate **54** were consistent with the reported values [44].

### 2.9. Saturated keto analogs and enol analog

To see whether the olefinic function in the conjugated enone system is important for biological activity, compounds **40**, and **45a** and **45b** mixture were subjected to catalytic hydrogenation to the corresponding dihydro analogs **55**, and **56a** and **56b** (1:1 mixture) in 86 and 86% yields (Scheme 9). The spectroscopic data of compound **55** (see Section 5) were in agreement with the structure and were consistent with the reported values [33]. Compounds **56a** and **56b** mixture showed almost identical $^1$H NMR data. The only difference was the resonance positions of the methoxyl groups of these two isomers, which appeared at δ 3.83 and 3.84. Finally, in order to see the influence of the keto function in biological activity, the keto group in the enone **40** was reduced with sodium borohydride to the corresponding alcoholic group in **57** in 96% yield (Scheme 9). The spectroscopic data (see Section 5) were consistent with the structure of **57**.



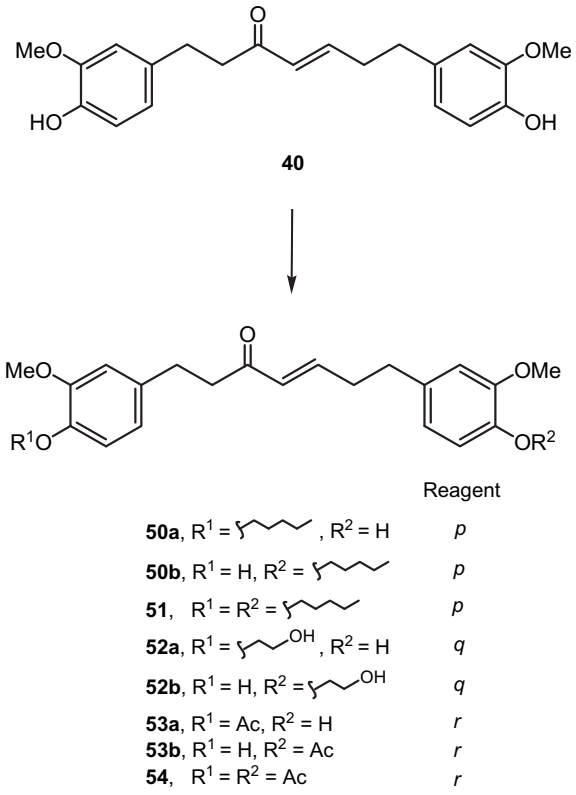

**Scheme 7.** Dehydrogenation of the enone **40**. Reagent and condition: (o) DDQ, THF.

## 3. Results and discussion

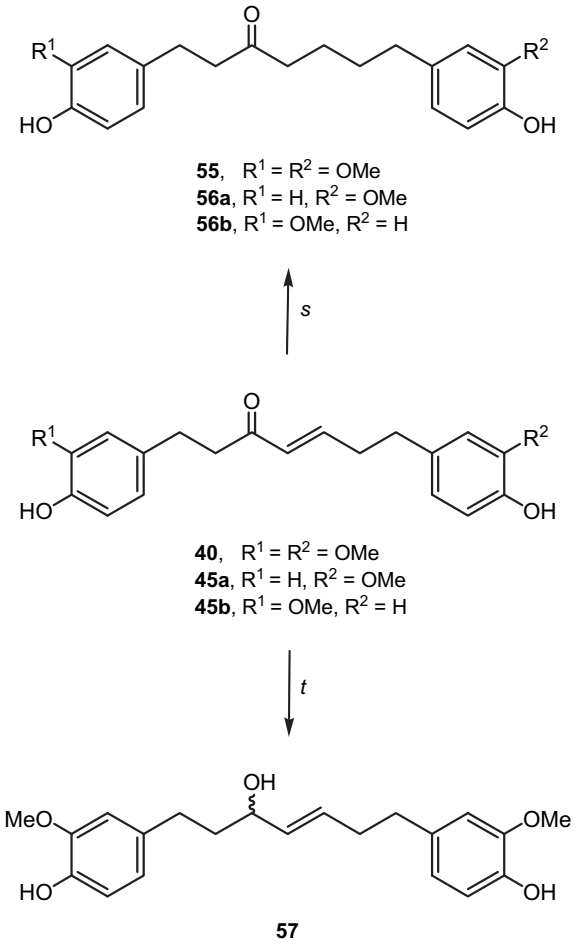
### 3.1. Trypanocidal effects of curcuminoid analogs

#### 3.1.1. Activity against a standard T. brucei strain 427

The three parent curcuminoids (compounds **1**–**3**), 43 curcuminoid analogs (compounds **4**–**24**, **26**, **27**, **29**, **31**–**36**, **38**–**40**, **42**, **44**, **46**–**49**, **51**, **54**, **55** and **57**) and 8 pairs of 1:1 isomeric mixtures of curcuminoid analogs (compounds **37a**/**37b**, **41a**/**41b**, **43a**/**43b**, **45a**/**45b**, **50a**/**50b**, **52a**/**52b**, **53a**/**53b** and **56a**/**56b**) were assessed for *in vitro* trypanocidal properties on the standard drug-sensitive laboratory strain 427 (Table 1). It has previously been reported that curcumin (**1**) has distinct trypanocidal properties, with an *in vitro* $EC_{50}$ value of 4.8 ± 0.9 μM for bloodstream forms of the polymorphic strain GUTat 3.1 [21]. Our own value for compound **1** against bloodstream forms of the monomorphic strain 427 was similar at 2.5 ± 0.4 μM ($n = 3$). The two other parent curcuminoids,




**Scheme 8.** Alkylation and acetylation of the enone **40**. Reagents and conditions: (p) *n*-pentyl iodide, $K_2CO_3$, acetone, reflux; (q) 2-bromoethanol, $K_2CO_3$, acetone, reflux; (r) $Ac_2O$, pyridine.




**Scheme 9.** Catalytic hydrogenation and sodium borohydride reduction of the ketone **40**. Reagents and conditions: (s) $H_2$/Pd-C, EtOH; (t) $NaBH_4$, EtOH.

demethoxycurcumin (**2**) and bisdemethoxycurcumin (**3**) were approximately 2 and 3-fold less active than compound **1**. To see whether the polarity contributed from the oxygen functions on the aromatic ring exert any effect on the biological activity of curcuminoids, the demethylated analogs **4**, **5** and **6**, and the methylated analogs **7**, **8**, **9** and **10** were evaluated for antitrypanosomal activity. It was found that the mono-*O*-demethylated curcuminoid **4** exhibited approximately 3-fold higher activity than the parent compound, curcumin (**1**). Further demethylation to the di-*O*-demethylated analog **5**, however, resulted in about 2-fold decrease in activity, though it still was more active than compound **1**. Compound **6**, the demethylated analog of the parent curcuminoid **2**, was 7-fold more active than the parent compound. At this point it seemed that increase in polarity in the molecule resulted in increase in antitrypanosomal activity. We therefore assessed whether increase in lipophilicity of the parent compounds caused decrease in activity. Rather surprisingly, compound **7**, the di-*O*-methyl analog of compound **1**, was almost 6-fold more active than its parent compound **1**, whereas the mono- and di-*O*-methylated analogs **8** and **9** were almost 3-fold more active than their parent compound **2**. However, the *O*-methylated analog **10** was slightly less active than the parent curcuminoid **3**. In order to see the effect of higher alkyl ether analogs on the biological activity of curcuminoid analogs, the *n*-propyl ether **11**, allyl ethers **12** and **13**, 3,3-dimethylallyl ethers **14**, **15** and **16**, and 2-hydroxyethyl ethers **17**, **18** and **19a** were evaluated for antitrypanosomal activity. The assay results have demonstrated that the activity of these alkyl ether analogs was 1 to 4-fold more active than their respective parent curcuminoids, except for compound **19a** that the activity was 6.5-fold more active than the parent compound **2**.

To test for the effect of an additional polar group on the aromatic ring of curcuminoid, the nitro analog **20** was synthesized and evaluated for antitrypanosomal activity and it turned out that the presence of a nitro substituent resulted in a significant (6-fold) decrease in activity.

In addition to the ether derivatives, the ester analogs, i.e. the acetates **21**, **22** and **23**, and the benzoates **24**–**30**, were subjected to biological evaluation. The results demonstrated that the acetate analogs were about 1.5 to 3-fold more active than their parent curcuminoids (see Table 1). However, it was worth noting that their $EC_{50}$ values were still in excess of 1 μM. For the benzoate analogs, some displayed 2 to 4-fold higher activity (compounds **29** and **27**) while others displayed almost identical activity (compounds **24** and **26**) to those of the parent curcuminoids. It should be mentioned that compounds **25**, **28** and **30** were insoluble under the condition used for biological evaluation and this was due to the hydrophobic nature of the two benzoyl groups in the molecule.

It should be noted that a decrease in oxygenated (hydroxyl and alkyl) substituents in the aromatic rings of curcuminoids tended to generally decrease antitrypanosomal activity of the curminoids and analogs. However, the existing information did not permit a definitive conclusion for these observations.

The reduced analogs of curcuminoids were next tested against *T. b. brucei* s427. For the tetrahydro analogs **31**, **34**–**36**, and **38**, the first two analogs (i.e. **31** and **34**) were approximately 8 and 5-fold less active than their respective parent compounds. However, the number and nature of the oxygen functions on the aromatic rings strongly determined the antitrypanosomal activity of the tetrahydro analogs. Thus, the analog **35** was 3-fold more active than its

**Table 1**
Antitrypanosomal, antileishmanial and cytotoxic activities of curcuminoids and analogs.

| Compound | *T. brucei* strain 427 | *T. brucei* ΔTbat1 | *T. brucei* clone B48 | *L. major* promastigotes | *L. mexicana* amastigotes | HEK |
|---|---|---|---|---|---|---|
| **1** | 2.5 ± 0.4 | 4.7 ± 0.3 | 2.9 ± 1.1 | 33 ± 4 | 16 ± 3 | 37 ± 6 |
| **2** | 4.6 ± 0.8 | 5.9 ± 0.5 | 2.2 ± 0.24 | 37 ± 1 | 37 ± 4 | 40 ± 5 |
| **3** | 7.7 ± 1.1 | 9.5 ± 0.3 | 4.5 ± 0.46 | 72 ± 3 | 63 ± 3 | 200 ± 40 |
| **4** | 0.87 ± 0.06 | 1.1 ± 0.1 | 2.7 ± 0.5[b] | 4.3 ± 0.5 | 3.2 ± 0.5 | 36 ± 4 |
| **5** | 1.8 ± 0.1 | 2.5 ± 0.2 | 3.4 ± 0.1 | 26 ± 0.1 | 12 ± 3 | 39 ± 7 |
| **6** | 0.65 ± 0.02 | 1.8 ± 0.1 | 1.0 ± 0.1 | 22 ± 2 | 21 ± 2 | 30 ± 3 |
| **7** | 0.45 ± 0.07 | 0.62 ± 0.07 | 0.77 ± 0.02 | 2.8 ± 0.4 | 10 ± 1 | 200 ± 50 |
| **8** | 1.7 ± 0.7 | 2.0 ± 0.2 | 2.1 ± 0.1 | 23 ± 3 | 38 ± 6 | 79 ± 8 |
| **9** | 1.6 ± 0.4 | 1.9 ± 0.2 | 1.9 ± 0.1 | 23 ± 6 | 61 ± 3 | 690 ± 230 |
| **10** | 8.9 ± 1.4 | 8.8 ± 0.2 | 2.9 ± 0.4[b] | 87 ± 5 | 32 ± 2 | 400 ± 0 |
| **11** | 1.3 ± 0.4 | 1.8 ± 0.1 | 1.6 ± 0.1 | 28 ± 2 | 34 ± 0.2 | >500 |
| **12** | 2.4 ± 0.1 | 2.2 ± 0.1 | 12 ± 1.6[c] | 5.7 ± 0.7 | 12 ± 4 | 270 ± 130 |
| **13** | 1.6 ± 0.1 | 1.9 ± 0.1 | 5.8 ± 0.6[c] | 25 ± 9 | >100 | >500 |
| **14** | 1.4 ± 0.2 | 1.7 ± 0.1 | 2.0 ± 0.2 | 7.6 ± 0.7 | 21 ± 4 | >500 |
| **15** | 1.2 ± 0.2 | 1.6 ± 0.2 | 1.3 ± 0.1 | >100 | 27 ± 0.8 | >1000 |
| **16** | 1.9 ± 0.3 | 2.9 ± 0.3 | 3.1 ± 0.1 | 48 ± 9 | 27 ± 7 | >500 |
| **17** | 2.9 ± 1.1 | 2.9 ± 0.3 | 1.8 ± 0.4 | 22 ± 4 | 30 ± 4 | 25 ± 3 |
| **18** | 1.8 ± 0.8 | 2.3 ± 0.1 | 1.6 ± 0.5 | 33 ± 8 | 37 ± 5 | 20 ± 1 |
| **19a** | 0.7 ± 0.3 | 0.85 ± 0.13 | 1.1 ± 0.1 | 9.7 ± 1.5 | 32 ± 0.7 | 46 ± 3 |
| **20** | 15 ± 2 | 18 ± 0.2 | 25 ± 8 | 28 ± 3 | 14 ± 4 | 82 ± 5 |
| **21** | 1.6 ± 0.5 | 2.2 ± 0.2 | 1.2 ± 0.2 | 73 ± 18 | 34 ± 4 | 80 ± 18 |
| **22** | 1.5 ± 0.3 | 2.0 ± 0.1 | 0.92 ± 0.08 | >100 | 43 ± 7 | 49 ± 6 |
| **23** | 3.2 ± 0.4 | 3.9 ± 0.1 | 3.2 ± 0.3 | 66 ± 10 | 7.1 ± 1 | >500 |
| **24** | 3.1 ± 0.7 | 3.8 ± 0.2 | 2.1 ± 0.5 | 9.3 ± 1.1 | 76 ± 10 | 94 ± 11 |
| **25** | INS | INS | INS | INS | INS | INS |
| **26** | 3.7 ± 0.4 | 7.8 ± 0.8 | 7.8 ± 0.8 | 20 ± 1 | >100 | >500 |
| **27** | 1.2 ± 0.3 | 1.6 ± 0.16 | 4.7 ± 0.4 | 42 ± 12 | >100 | 370 ± 110 |
| **28** | INS | INS | INS | INS | INS | INS |
| **29** | 4.1 ± 1.7 | 10 ± 2 | 12 ± 2 | 60 ± 10 | >100 | 270[a] |
| **30** | INS | INS | INS | INS | INS | INS |
| **31** | 21 ± 11 | 37 ± 7 | 12 ± 2 | 90 ± 17 | 43 ± 9 | 120 ± 10 |
| **32** | 17 ± 3 | 38 ± 3 | 5.9 ± 0.9[b] | >100 | >100 | 370 ± 60 |
| **33** | 78 ± 5 | 61 ± 5 | >100 | >100 | >100 | ND |
| **34** | 22 ± 8 | 34 ± 1 | 18 ± 1 | >100 | >50[a] | ND |
| **35** | 2.5 ± 0.4 | 4.0 ± 0.3 | 5.1 ± 0.9 | 80 ± 12 | 64 ± 14 | 350 ± 90 |
| **36** | 0.50 ± 0.10 | 0.52 ± 0.10 | 0.43 ± 0.01 | 11 ± 1 | 16 ± 3 | 130 ± 19 |
| **37a + 37b** | 3.0 ± 0.7 | 2.9 ± 0.5 | 2.5 ± 0.4 | >100 | 40 ± 5 | 77 ± 2 |
| **38** | 1.9 ± 0.6 | 1.5 ± 0.2 | 1.7 ± 0.1 | >100 | 53 ± 3 | 150 ± 8 |
| **39** | 33 ± 13 | 22 ± 5 | 19 ± 4 | 41 ± 12 | >100 | 370 ± 15 |
| **40** | 0.053 ± 0.007 | 0.082 ± 0.005 | 0.023 ± 0.004[b] | 8.9 ± 0.8 | 17 ± 2 | 24 ± 2 |
| **41a + 41b** | 0.75 ± 0.13 | 0.73 ± 0.08 | 0.30 ± 0.06[b] | 10 ± 2 | 7.4 ± 1 | 63 ± 9 |
| **42** | 2.5 ± 0.5 | 3.2 ± 0.1 | ND | >100 | 17 ± 5 | 220 ± 60 |
| **43a + 43b** | 0.14 ± 0.05 | 0.29 ± 0.10 | 0.032 ± 0.004 | 13 ± 1 | 28 ± 6 | 20 ± 2 |
| **44** | 1.9 ± 0.2 | 1.6 ± 0.1 | 0.023 ± 0.003[d] | 12 ± 1 | 28 ± 8 | 26 ± 2 |
| **45a + 45b** | 3.0 ± 0.3 | 2.1 ± 0.2 | 0.049 ± 0.008[d] | 15 ± 2 | 23 ± 4 | 40 ± 5 |
| **46** | 2.6 ± 0.5 | 1.9 ± 0.1 | 0.12 ± 0.02[b] | 9.9 ± 1.2 | 21 ± 8 | 45 ± 3 |
| **47** | 4.1 ± 1.6 | 9.7 ± 1.7 | 2.2 ± 0.4 | 30 ± 2 | 22 ± 7 | 76 ± 3 |
| **48** | 0.22 ± 0.09 | 0.22 ± 0.02 | 0.10 ± 0.03 | 2.7 ± 0.7 | 4.6 ± 0.7 | 8.1 ± 1.0 |
| **49** | 1.1 ± 0.3 | 1.4 ± 0.2 | 0.58 ± 0.08 | 7.4 ± 0.1 | 4.8 ± 0.6 | 22 ± 1 |
| **50a + 50b** | 0.86 ± 0.3 | 0.62 ± 0.1 | 0.89 ± 0.2 | 4.0 ± 1.3 | 19 ± 4 | 30 ± 0.3 |
| **51** | 1.1 ± 0.3 | 1.2 ± 0.2 | 1.8 ± 0.1 | 6.6 ± 1.0 | 53 ± 8 | 43 ± 2 |
| **52a + 52b** | 0.27 ± 0.06 | 0.27 ± 0.07 | 0.11 ± 0.04 | 15 ± 0.1 | 41 ± 4 | 26 ± 1 |
| **53a + 53b** | 0.089 ± 0.027 | 0.079 ± 0.024 | 0.045 ± 0.015 | 7.5 ± 0.8 | 29 ± 0.8 | 25 ± 1 |
| **54** | 0.052 ± 0.015 | 0.042 ± 0.017 | 0.021 ± 0.007 | 7.8 ± 1.0 | 35 ± 1 | 30 ± 2 |
| **55** | 32 ± 0.4 | 28 ± 1 | 27 ± 2 | 37 ± 3 | >50 | 140 ± 13 |
| **56a + 56b** | 45 ± 1 | 48 ± 2 | 9.0 ± 2.8[d] | 55 ± 3 | >100 | 200 ± 2 |
| **57** | 40 ± 3 | 42 ± 4 | 6.3 ± 0.6[c] | 50 ± 4 | 64 ± 15 | 130 ± 2 |
| Diminazene | 0.12 ± 0.01 | 2.4 ± 0.3 | 1.0 ± 0.2 | | | |
| Pentamidine | | | 0.67 ± 0.06 | 3.8 ± 0.4 | 16 ± 2 | |

Data are $EC_{50}$ values in μM ± standard errors and are the average and S.E.M. of 3–6 independent experiments except where indicated. ND = Not done. INS = Insoluble.
[a] $n = 1$.
[b] $P < 0.05$ relative to strain 427.
[c] $P < 0.01$ relative to strain 427.
[d] $P < 0.001$ relative to strain 427.

parent curcuminoid **3** and was almost 9-fold more active than compound **34**, the 3″-*O*-methoxy analog. Compound **36**, the mono-*O*-demethyl analog of **31**, was even 42-fold more active than the methylated analog **31** (0.50 ± 0.10 μM). The hexahydro analogs **32** and **39** exhibited very low activity, being 7 and 73-fold less active than the conjugated compounds **1** and **7**, respectively. The 1:1 mixture **37a**/**37b** was 3.5-fold less active than the corresponding conjugated compound **4**. The octahydro analog **33** exhibited extremely low activity; it was 31-fold less active than the parent conjugated compound **1** (Table 1).

We next explored mono-oxygenated analogs of the heptyl linker chain. We first synthesized the monoketo analog **40**, which was obtained by catalytic dehydration of compound **32**. We discovered that the enone **40** exhibited very high antitrypanosomal activity of

$0.053 \pm 0.007$ μM, which was 47-fold more active than the parent compound **1**, or 321-fold more active than its immediate precursor, the hexahydro analog **32**. The activity of **40** was superior to the activity of the standard veterinary trypanocide diminazene aceturate, which displayed an activity of $0.12 \pm 0.01$ μM against s427.

In order to study more about structure–activity relationship and to find any possible additional potent analogs by using the monoketo analog **40** as the lead structure, we further explored different analogs. It was found that the mono-demethylated analog mixture **41a**/**41b**, the completely demethylated analog **42**, the methylated analogs **43a**/**43b** (1:1 mixture), **44**, **45a**/**45b** (1:1 mixture), and the enone **46** were all more active than their respective parent curcuminoids **1**–**3**, except for compound **42** whose activity was about the same as that of its parent compound **1**. However, none of them showed higher activity than the enone **40**.

With the framework of the most active compound **40**, we investigated whether additional olefinic function would give rise to an increase in biological activity. The dienones **47** and **48**, and the trienone **49** were synthesized and assessed for antitrypanosomal activity. The results revealed that compounds **48** and **49** were approximately 2 to 11-fold more active than the parent curcuminoid **1**, whereas compound **47** displayed activity similar to **1**. We then investigated the alkyl ether and the acetate analogs of the enone **40** (i.e. compounds **50**–**54**) and it was found that all the analogs exhibited high activity. The monoacetates **53a** and **53b** mixture and the diacetate **54** displayed the highest activity, at $0.089 \pm 0.027$ and $0.052 \pm 0.015$ μM, or 28 and 48-fold more active than the natural parent curcuminoid **1**. These two compounds thus displayed activity statistically identical to the enone **40** ($P > 0.05$, Student's *T*-test) and superior to that of diminazene aceturate.

In order to see the contribution of the olefinic group in the enone **40** to the antitrypanosomal activity, the corresponding dihydro analog **55** was prepared and it was found that removal of the conjugated olefinic function resulted in almost complete loss of activity. The activity of **55** was 607-fold less active than that of **40**. Similar result was also observed for the 1:1 mixture of the saturated keto analogs **56a**/**56b** (see Table 1). To prove that the keto function was also essential for activity, the enol **57** was prepared; this change resulted in an almost complete loss of activity. We conclude that the most essential structural feature for a curcuminoid to exhibit strong antitrypanosomal activity is the presence of an α,β-keto system at the heptyl chain.

A logical next step would be to identify the mode of action of **40** on the trypanosome, and optimize further by studying the interaction with the cellular target. This work, although in progress, falls outside the remit of the current report. It is relevant to reiterate that a great many hypotheses have been proposed for the mechanism of action of curcumin (**1**) and its analogs, and in many different cell types. Indeed, the activity of curcuminoids is likely to be the result of multiple cellular interactions [12,45]. Thus, in the absence of an optimization strategy based on target structure, the structure–activity analysis offered here, based on overall antiparasitic effects, is the most direct approach to new and improved lead compounds for the target diseases.

### 3.1.2. ΔTbat1 and B48 strains

As it is vital that any new drug developed against African trypanosomiasis is not cross-resistant with the diamidine and arsenic-based drugs currently in use, we tested the activity of all the analogs on two additional clonal lines, derived from *T. b. brucei* strain 427. The first line, ΔTbat1, was derived by the disruption of both alleles coding for the TbAT1/P2 transporter [46]. The TbAT1/P2 transporter is the main transport protein mediating uptake of the diamidine and melaminophenyl arsenical classes of trypanocides [47,48] and the genetic deletion of the encoding gene thus creates a mutant line that is highly resistant to diminazene aceturate as well as slightly resistant to pentamidine and melaminophenyl arsenicals. The second line, B48, was derived from ΔTbat1 by incremental exposure to pentamidine *in vitro*, and having lost or mutated a second drug transporter, HAPT1, is highly resistant to most diamidines including diminazene, pentamidine and to the melaminophenyl arsenicals [49,50]. Whereas ΔTbat1 was 20-fold resistant to diminazene aceturate (positive control) in this series of experiments ($0.12 \pm 0.01$ ($n = 13$) *vs.* $2.4 \pm 0.3$ μM ($n = 14$); $P < 0.001$, Student *T*-test), there was no significant resistance to curcuminoid analogs in this line (Wilcoxon's signed ranks test for two groups, paired observations; $P > 0.05$). The curcuminoids and analogs exhibited antitrypanosomal activity against these two clonal lines in similar manner to that of the wild-type strain (Table 1). The activity of most of the compounds appeared to be within a 2-fold difference in sensitivity against the two lines and at most 2.7-fold for compound **6**. It should be emphasized that no resistance was observed for any of the highly active analogs, such as **40**, **48** and **54** and the **53a**/**53b** mixture.

B48 is a multi-drug resistant line, with *in vitro* resistance to pentamidine and melarsen oxide of 128 and 11-fold, respectively [49]. Out of 57 compounds tested (Table 1), only three displayed significantly less activity against this line: compounds **4** (3.1-fold), **12** (5-fold) and **13** (3.6-fold). All are close analogs of **1**, with conjugated diketo linkers, differing only in the substitutions at the aromatic rings, in particular allyl ether substitutions (**12**, **13**). Unexpectedly, the B48 line proved to be significantly more sensitive to some of the most active curcuminoids (Table 1), including compounds **40**, **41a**/**41b**, **44**, **45a**/**45b** and **46**. These compounds all share the enone motif of the most active compound for WT trypanosomes, **40**; compounds **44** and **45a**/**45b** displayed a highly significant ($P < 0.001$, Student's *T*-test) 83 and 60-fold higher activity against the B48 strain.

The tests with the transporter mutant lines thus lead us to conclude that there is little or no risk of cross-resistance between curcumin (analogs) and existing therapies of the diamidine and melaminophenyl arsenical categories, which form the mainstay of trypanosomiasis treatment [1,2]. It is clear that the deletion of the *TbAT1* gene does not diminish uptake of the curcuminoids significantly. This strongly suggests that curcuminoids are not a substrate for this transporter, in line with our understanding of the TbAT1/P2 recognition motif [51]. The result with the B48 line was unexpected, as this line displays hypersensitivity to some of the most promising curcuminoids. This would appear to be highly advantageous in dealing with the current resistance problems in the field. Further investigations are required to fully understand this phenomenon, and whether it extends to other pentamidine-resistant lines. One possible explanation is a mutation in the HAPT transporter gene, creating an altered transporter with lower affinity for diamidines but higher affinity for some curcuminoids, specifically those with the enone motif.

A sub-set of the analogs was also tested against *T. evansi*. Most of the analogs were equally or more potent against this species than against our *T. b. brucei* reference strain 427 (Table 2), but there was no statistical significant difference in overall curcuminoid sensitivity between the two species ($P > 0.05$; Wilcoxon's signed ranks test for two groups, paired observations). Although the *T. evansi* strain was significantly more sensitive to diminazene than *T. b. brucei* WT ($0.018 \pm 0.003$ μM, $n = 5$; $P < 0.001$; unpaired Student's *T*-test) and compound **12** ($P < 0.05$), it was less sensitive to compound **40** ($P < 0.02$) and compound **13** ($P < 0.01$). Fig. 1 illustrates the results obtained for *T. b. brucei* WT and *T. evansi*. Importantly, these experiments establish that the curcuminoids have highly similar activity against multiple species of African trypanosomes. Whereas human African trypanosomiasis is caused only by

**Table 2**
Comparison of the activity of curcuminoids on *T. b. brucei* WT and *T. evansi*.

| Compound | *T. b. brucei* WT | *T. evansi* | Student's *T*-test |
|---|---|---|---|
| **1** | 2.5 ± 0.4 | 2.0 ± 0.3 | NS |
| **4** | 0.87 ± 0.06 | 1.1 ± 0.2 | NS |
| **12** | 2.4 ± 0.1 | 1.6 ± 0.2 | $P < 0.05$ |
| **13** | 1.6 ± 0.1 | 5.6 ± 0.7 | $P < 0.01$ |
| **23** | 3.2 ± 0.4 | 2.5 ± 0.6 | NS |
| **24** | 3.1 ± 0.7 | 1.5 ± 0.1 | NS |
| **26** | 3.7 ± 0.4 | 2.9 ± 0.2 | NS |
| **40** | 0.053 ± 0.007 | 0.17 ± 0.02 | $P < 0.02$ |
| **41** | 0.75 ± 0.13 | 0.99 ± 0.19 | NS |
| **43** | 0.14 ± 0.05 | 0.29 ± 0.04 | NS |
| **48** | 0.22 ± 0.09 | 0.21 ± 0.02 | NS |
| Diminazene | 0.12 ± 0.01 | 0.018 ± 0.003 | $P < 0.001$ |

Data are the average of 3–4 independent experiments, given as $EC_{50}$ values in μM ± S.E.M. NS = not significant.

*T. brucei* subspecies [6], the infection in domestic and wild animals is caused by several related species, including *T. evansi*.

### 3.2. Antileishmanial activity of curcuminoid analogs

The same curcuminoids and analogs were tested on promastigotes of *L. major* (Table 1). The published $EC_{50}$ value for curcumin against these cells is 37.6 ± 3.5 μM [22]. Once again, our own value at 33 ± 4 μM ($n = 3$) is in very good agreement with this value. Most analogs displayed higher activity than curcumin (**1**) against the promastigotes. However, the activity was generally below that displayed for *T. b. brucei* or *T. evansi*. For *L. major* promastigotes, four curcuminoid analogs, **4**, **7**, **48** and **50a**/**50b**, exhibited antileishmanial activity with $EC_{50}$ less than 5 μM, whereas ten curcuminoid analogs, **12**, **14**, **19a**, **24**, **40**, **46**, **49**, **51**, **53a**/**53b**, and **54**, showed activity within the $EC_{50}$ range of 5–10 μM. The remaining compounds displayed $IC_{50}$ values higher than 10 μM. The most active compounds, **7** and **48**, exhibited activity of 2.8 ± 0.4 and 2.7 ± 0.7 μM, respectively (Table 1). The compounds were also assessed against axenic *Leishmania mexicana* amastigotes and the same analogs showed highest antileishmanial activity (**4**, **48**, **49**) and had also displayed submicromolar activity against *T. b. brucei* (see above). This suggests that these compounds act on a well-conserved target in the kinetoplastids. However, the best performing compound against *T. b. brucei*, the enone **40**, performed relatively poorly against *L. mexicana* amastigotes, with an $EC_{50}$ value of 17 ± 2 μM. The general trend was that the curcuminoid analogs were, on average, very slightly less active against the axenic amastigotes than against promastigotes, but this was not statistically significant ($n = 39$, $P < 0.2$, Wilcoxon's signed ranks test for two groups, paired observations). Eight compounds, **4**, **5**, **7**, **12**, **20**, **23**, **48** and **49**, and one mixture, **41a** + **41b**, displayed *in vitro* activity against the amastigote stage that was superior to the clinically used drug pentamidine ($EC_{50} = 16 \pm 2$ μM; $n = 6$) whereas compound **36** and curcumin (**1**) were as active as pentamidine (see Table 1).

It seemed that influence of polarity from the oxygen function on the aromatic ring(s) has varying effects on antileishmanial activity. This was exemplified in the case of the parent curcuminoid **1** which exhibited antileishmanial activity (against promastigotes) of 33 ± 4 μM, whereas its mono-*O*-demethylated analog **4** was almost 8-fold more active. However, going from **4** to the more polar analog **5**, the di-*O*-demethylated analog of **1**, resulted in a 6-fold decrease in activity. The increase in lipophilicity in going from **1** to the di-*O*-methylated analog **7** resulted in ~12-fold increase in activity (see Table 1). A larger *O*-alkyl group which increased the lipophilicity of the molecule also improved the antileishmanial activity of the analogs. For example, an increase of 5.7 and 4.3-fold in activity was noted for the mono-*O*-alkyl analogs **12** and **14**, respectively, compared to the parent curcuminoid **1**. However, addition of the second alkyl group to the analogs **12** and **14** to give the corresponding dialkyl analogs **13** and **15** caused sharp decrease in activity.

The conjugated keto system is also required for a curcuminoid analog to exhibit high antileishmanial activity. However, analogs with a conjugated diketo system (e.g. **4**, **7**), as well as analogs with the conjugated monoketo system (enones, **48**–**54**), displayed similarly high antileishmanial activity.

### 3.3. Effect of curcuminoid analogs on a human cell line

To assess whether the antiprotozoal activity described above should be attributed to general toxicity, rather than specific antiprotozoal activity, the analogs were also tested for their effect on human embryonic kidney (HEK) cells (see Table 1). Without exception, the toxicity to HEK cells was lower than to *T. b. brucei* WT bloodstream forms. Out of **46** analogs and 8 pairs of two isomers, only one compound, **48**, had significantly higher activity against HEK cells than curcumin (**1**) itself ($EC_{50} = 37 \pm 6$ μM) and was the only compound that displayed an $EC_{50}$ value below 20 μM. The ratio of $EC_{50}$(HEK)/$EC_{50}$(*T. b. brucei*) could be described as the *in vitro* selectivity index and ranged from ~3-fold for compound **57** to >1000-fold for compound **15** and 453-fold for the most active analog, **40**.

As the leishmanicidal activity of most analogs was less than their trypanocidal activity, the therapeutic index calculated from

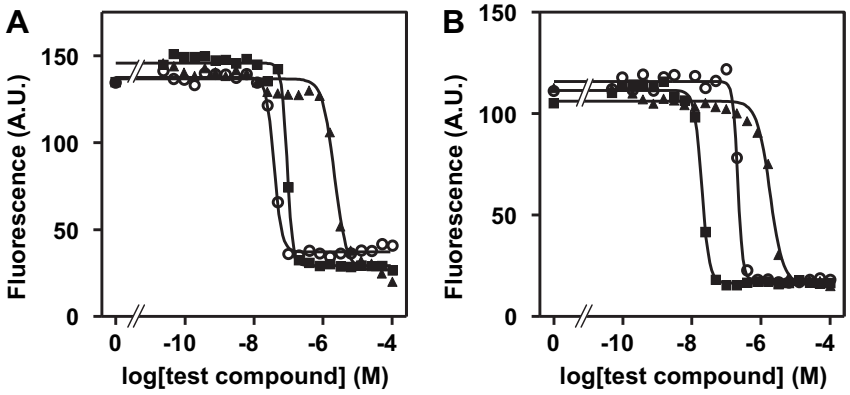



**Fig. 1.** Effect of some curcuminoid analogs on *Trypanosoma brucei* and *Trypanosoma evansi*. Curcuminoid analogs and a control drug (diminazene aceturate) were tested for effect against the two trypanosome species *in vitro*, using the Alamar Blue protocol described in the Experimental section. A: *T. b. brucei* WT. B: *T. evansi*. (■) diminazene aceturate; (○) compound 40; (▲) compound 1. Data are representative of 3–4 independent experiments.

the *L. major* $EC_{50}$ values was correspondingly lower. The most promising compound, by this measure was **7**, with an index of just over 70-fold.

## 4. Conclusion

The natural curcuminoids curcumin (**1**), demethoxycurcumin (**2**) and bisdemethoxycurcumin (**3**) isolated from *C. longa* L. were assessed against *Trypanosoma* and *Leishmania* species. *Trypanosoma* species cause human African trypanosomiasis or sleeping sickness, as well as the corresponding veterinary infections known as nagana and surra. In this study we used three isogenic clonal lines of increasing drug resistance. The *T. b. brucei* clonal line 427 was chosen as drug-sensitive standard ('WT'). From this line, a diminazene-resistant clone was derived by deletion of the *TbAT1* gene that encodes the P2 aminopurine transporter ('KO') [46]. *In vitro* exposure to increasing concentrations of pentamidine yielded a new clonal line [49] highly resistant to the diamidine and melaminophenyl arsenical drugs that currently form the mainstay of trypanosomiasis treatment [3]. This allowed us to not only assess activity against a well-characterized line of this parasite, but also to determine any issues about cross-resistance from the outset. For the *Leishmania* species, *L. major* promastigotes and *L. mexicana* amastigotes, have been used.

The natural curcuminoids exhibited fairly low antitrypanosomal activity when compared with the drug diminazene aceturate. They similarly showed somewhat lower antileishmanial activity than the reference drug pentamidine. The natural curcuminoids **1**–**3** were thus subjected to a series of chemical modifications to the corresponding demethylated, methylated and higher alkylated analogs, acetate and benzoate esters, tetrahydro and hexahydro analogs, conjugated and non-conjugated monoketo analogs including their demethylated, methylated and higher alkylated analogs, and acetate derivatives. Out of 43 curcuminoid analogs and 8 pairs of 1:1 mixture of isomeric curcuminoid analogs tested, thirteen analogs exhibited submicromolar antitrypanosomal activity, including three with $EC_{50}$ values below 100 nM (compounds **40**, **53a**/**53b** and **54**). These last three share an enone motif that, although not present in all the compounds with submicromolar activity (e.g. **6**, **7**, **36**), may be one structural motif associated with particularly high trypanocidal activity. While the three most active compounds displayed identical activity on HEK cells and thus a similar *in vitro* selectivity index (250–500), we consider **40** as the most promising lead compound because of its better solubility and simpler synthetic route.

It should be noted that, although in many cases trypanocidal and antileishmanial activities were linked, the association is not absolute and of the four compounds displaying $EC_{50}$ values below 5 μM against promastigotes, two were diketones (**4** and **7**) and two were enones (**48** and **50a**/**50b**). The situation with amastigotes was similar. Nor were the conjugated ketones inherently more toxic to human embryonic kidney (HEK) cells (e.g. **42**). Some of the analogs displayed no detectable effect on HEK until the limit of solubility and compound **40** displayed a selectivity index of 453 using the $EC_{50}$ against WT trypanosomes and >1000 against B48. While none of the curcuminoids displayed submicromolar activity against the *Leishmania* promastigotes or amastigotes, 11 of them displayed activity better or equal to the reference drug pentamidine, which is in common clinical use [4].

Importantly, the activities against the WT and KO lines of *T. b. brucei* were highly similar. This clearly shows that the activity of this class of compounds is not dependent on the TbAT1/P2 aminopurine transporter that has been widely associated with resistance to some diamidines particularly diminazene [47] and furamidine [52] and melaminophenyl arsenicals [3,50]. For most of the curcuminoids, this was also observed for the B48 strain, which is much more highly resistant to these drugs and has lost an additional drug transporter, HAPT [49]. Unexpectedly, some of the curcumin analogs, bearing the enone motif, were much more active against the B48 line. The reason for this is unclear at this moment, but it can be speculated that the loss of both the P2/TbAT1 and HAPT transporters could induce, in compensation, a higher level of expression for another transport mechanism, capable of taking up curcuminoids with this particular structural motif. Thus, a curcumin-based trypanocide would be at least equally effective against strains sensitive and resistant to current trypanocides. In addition, it appears that the analogs have similar activities for different *Trypanosoma* and *Leishmania* species (compare *T. b. brucei* with *T. evansi* and *L. major* with *L. mexicana*). This feature is of great practical importance for the treatment of leishmaniasis or veterinary trypanosomiasis, as it is often unknown which species has caused the infection.

In conclusion, we have identified a class of curcuminoids with potential applications against trypanosomiasis and/or leishmaniasis, based on their strong antiparasitic activity and low toxicity. The compounds compare, at least *in vitro*, favorably with existing medications. *In vivo* experimentation, as well as further *in vitro* testing of additional analogs, is expected to identify serious drug candidates against these diseases.

## 5. Experimental

### 5.1. General

Melting points were determined on an Electrothermal melting point apparatus and are uncorrected. IR spectra were recorded in KBr on a Perkin–Elmer FT-IR Spectrum BX spectrophotometer. $^1$H NMR spectra were recorded on a Bruker AVANCE 400 spectrometer. Electron impact (EI) and electrospray (ES) mass spectra were obtained using a Finnigan Polaris Q and a Finnigan LC-Q mass spectrometers, respectively. High resolution mass spectra were obtained using a Bruker micrOTOF mass spectrometer. Column chromatography and TLC were carried out using Merck silica gel 60 (<0.063 mm) and precoated silica gel 60 $F_{254}$ plates, respectively. Spots on TLC were detected under UV light (254 nm) and by spraying with anisaldehyde–$H_2SO_4$ reagent followed by heating.

### 5.2. Isolation and identification of curcuminoids

The crude curcuminoids from Thai-China Flavours and Fragrances (TCFF) were subjected to column chromatography using silica gel as the adsorbent ($CH_2Cl_2$–MeOH, gradient elution) resulted in the isolation of curcumin (**1**), demethoxycurcumin (**2**) and bisdemethoxycurcumin (**3**) in 60.8, 16.2 and 3.0% yields, respectively. The ratios of the curcuminoids **1**, **2** and **3** are 76.0, 20.2 and 3.8 respectively. The spectroscopic (IR, $^1$H NMR and mass spectra) data were consistent with the reported values [23].

### 5.3. Chemical modifications of curcuminoids

#### 5.3.1. Demethylation of curcumin (*1*)

A solution of compound **1** (200 mg, 0.54 mmol) in dry $CH_2Cl_2$ (45 ml) was stirred at 0 °C for 5 min and $BBr_3$ (1.5 ml) was slowly added. The reaction mixture was allowed to warm up to ambient temperature and stirring was continued for 10 h. Water was added and the mixture was extracted with EtOAc. The combined organic phase was washed with $H_2O$, dried over anhydrous $Na_2SO_4$; the solvent was evaporated and the residue chromatographed using $CH_2Cl_2$–MeOH–AcOH (50:5:2) to yield mono-*O*-demethylcurcumin (**4**) (80 mg, 43%) as orange amorphous solid, m.p. 168–170 °C (lit.

[23] m.p. 164–166 °C) and di-*O*-demethylcurcumin (**5**) (60 mg, 33%) as orange plates, m.p. 301–303 °C (lit. [23] m.p. 302–304 °C). The spectroscopic (IR, $^1$H NMR and mass spectra) data were consistent with the reported values [23].

*5.3.2. Demethylation of demethoxycurcumin (**2**)*

Compound **2** was subjected to demethylation in similar manner to that of compound **1** to give *O*-demethyldemethoxycurcumin (**6**) (64%) as orange amorphous solid, m.p. 216–218 °C (lit. [23] 218–220 °C). The spectroscopic (IR, $^1$H NMR and mass spectra) data were consistent with the reported values [23].

*5.3.3. Methylation of curcumin (**1**)*

Compound **1** (50 mg, 0.14 mmol) was dissolved in dry acetone (2 ml) and anhydrous $K_2CO_3$ (40 mg) and MeI (0.6 ml) was added. The reaction mixture was reflux for 5 h. Water was added and the mixture was extracted with $CH_2Cl_2$. The combined organic phase was washed with $H_2O$, dried over anhydrous $Na_2SO_4$ and the solvent removed under vacuum. The crude products were purified by column chromatography using $CH_2Cl_2$ to yield di-*O*-methylcurcumin (**7**) (45 mg, 80%) as orange powder, m.p. 128–130 °C (lit. [24] m.p. 129–130 °C). The spectroscopic (IR, $^1$H NMR and mass spectra) data were consistent with the reported values [24].

*5.3.4. Methylation of demethoxycurcumin (**2**)*

Compound **2** was subjected to methylation in similar manner to that of compound **1** to give 4″-*O*-methyldemethoxycurcumin (**8**) (20%) and di-*O*-methyldemethoxycurcumin (**9**) (54%).

*Compound **8***: Orange foam; IR $\nu_{max}$: 3420, 1640, 1585, 1508, 1263, 1136, 1020, 963, 832 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 3.89 and 3.91 (each s, 2 × 3H, 2 × OMe), 5.78 (s, 1H, H-4), 6.46 and 6.47 (each d, $J$ = 15.7 Hz, 2 × 1H, H-2 and H-6), 6.84 (br d, $J$ = 8.2 Hz, 2H, H-3′ and H-5′), 6.85 (d, $J$ = 8.3 Hz, 1H, H-5″), 7.05 (br s, 1H, H-2″), 7.11 (br d, $J$ = 8.3 Hz, 1H, H-6″), 7.43 (d, $J$ = 8.3 Hz, 2H, H-2′ and H-6′), 7.58 (d, $J$ = 15.7 Hz, 2H, H-1 and H-7); ESMS (+ve): *m*/*z* (% rel. abund.) 727 $[2M + Na]^+$ (100); HR-TOFMS (APCI$^+$): *m*/*z* 353.1373 $[M + H]^+$; calcd for $C_{23}H_{24}O_7$ + H, 353.1384.

*Compound **9***: Yellow needles (from *n*-hexane–$CH_2Cl_2$), m.p. 159–161 °C; IR $\nu_{max}$: 2937, 1624, 1601, 1515, 1458, 1256, 1138, 1027, 968, 840 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 3.83, 3.90 and 3.91 (each s, 3 × 3H, 3 × OMe), 5.78 (s, 1H, H-4), 6.48 (d, $J$ = 15.7 Hz, 2H, H-2 and H-6), 6.86 (d, $J$ = 8.2 Hz, 1H, H-5″), 6.90 (br d, $J$ = 8.5 Hz, 2H, H-3′ and H-5′), 7.06 (br s, 1H, H-2″), 7.12 (br d, $J$ = 8.2 Hz, 1H, H-6″), 7.49 (d, $J$ = 8.5 Hz, 2H, H-2′ and H-6′), 7.58 and 7.60 (each d, $J$ = 15.7 Hz, 2H, H-1 and H-7); ESMS (+ve): *m*/*z* (% rel. abund.) 367 $[M + H]^+$ (100).

*5.3.5. Methylation of bisdemethoxycurcumin (**3**)*

Compound **3** was subjected to methylation in similar manner to that of compound **1** to give mono-*O*-methyldemethoxycurcumin (**10**) (27%) as orange solid, m.p. 188–189 °C. The spectroscopic ($^1$H NMR and mass spectra) data of the later product were consistent with the reported values [25].

*5.3.6. Propylation of curcumin (**1**)*

Compound **1** was subjected to *n*-propylation in similar manner to that of methylation of compound **1**, but using *n*-propyl iodide in place of methyl iodide, to give di-*O*-*n*-propylcurcumin (**11**) (85%) as yellow powder, m.p. 127 °C.

*Compound **11***: IR $\nu_{max}$: 2939, 2877, 1620, 1595, 1578, 1511, 1468, 1423, 1334, 1273, 1234, 1131, 1011, 974 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 1.03 (t, $J$ = 7.3 Hz, 2 × 3H, H-3‴ and H-3⁗), 1.86 (sextet, $J$ = 7.1 Hz, 2 × 2H, H-2‴ and H-2⁗), 3.89 (s, 6H, 2 × OMe), 4.00 (t, $J$ = 6.7 Hz, 4H, H-1‴ and H-1⁗), 5.79 (s, 1H, H-4), 6.46 (d, $J$ = 15.7 Hz, 2H, H-2 and H-6), 6.85 (d, $J$ = 8.4 Hz, 2 × 1H, H-5′ and H-5″), 7.06 (br s, 2H, H-2′ and H-2″), 7.14 (br d $J$ = 8.4 Hz, 2H, H-6′ and H-6″), 7.58 (d, $J$ = 15.7 Hz, 2H, H-1 and H-7); ESMS (+ve): *m*/*z* (% rel. abund.) 453 $[M + H]^+$ (100).

*5.3.7. Allylation of curcumin (**1**)*

Curcumin (**1**) was subjected to allylation in similar manner to that of methylation of compound **1**, but using allyl bromide in place of methyl iodide, to afford mono-*O*-allylcurcumin (**12**) in 33% and di-*O*-allylcurcumin (**13**) [27] in 46%.

*Compound **12***: Orange amorphous solid, m.p. 132–133 °C; IR $\nu_{max}$: 3293, 2927, 1623, 1587, 1512, 1256, 1135, 967, 840 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 3.90 and 3.92 (each s, 2 × 3H, 2 × OMe), 4.63 (br d, $J$ = 5.4 Hz, 2H, H-1‴), 5.30 (br dd, $J$ = 10.5, 1.3 Hz, 1H, H-3a‴), 5.40 (br dd, $J$ = 17.2 Hz, 1.3, 1H, H-3b‴), 5.78 (s, 1H, H-4), 5.85 (s, 1H, OH), 6.05 (m, 1H, H-2‴), 6.45 and 6.47 (each d, $J$ = 15.7 Hz, 2 × 1H, H-2 and H-6), 6.86 (d, $J$ = 8.2 Hz, 1H, H-5″), 6.91 (d, $J$ = 8.2 Hz, 1H, H-5′), 7.02 and 7.06 (each d, $J$ = 1.6 Hz, 2 × 1H, H-2′ and H-2″), 7.09 and 7.11 (each dd, $J$ = 8.2 Hz, 1.6, 2 × 1H, H-6′ and H-6″), 7.58 (d, $J$ = 15.7 Hz, 2 × 1H, H-1 and H-7); EIMS: *m*/*z* (% rel. abund.) 408 $[M]^+$ (12), 390 (27), 367 (5), 178 (69), 177 (61), 139 (100).

*Compound **13***: Yellow needles (from *n*-hexane–$CH_2Cl_2$), m.p. 117–118 °C; IR $\nu_{max}$: 2928, 1621, 1595, 1578, 1510, 1458, 1423, 1336, 1254, 1232, 1132, 1011, 976, 845 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 3.91 (s, 6H, 2 × OMe), 4.64 (br d, $J$ = 5.3 Hz, 4H, H-1‴ and H-1⁗), 5.30 (br dd, $J$ = 10.5, 1.3 Hz, 2H, H-3a‴ and H-3a⁗), 5.40 (br dd, $J$ = 17.2, 1.3 Hz, 2H, H-3b‴ and H-3b⁗), 5.79 (s, 1H, H-4), 6.06 (m, 2H, H-2‴ and H-2⁗), 6.47 (d, $J$ = 15.7 Hz, 2H, H-2 and H-6), 6.86 (d, $J$ = 8.2 Hz, 2H, H-5′ and H5″), 7.06 (d, $J$ = 1.7 Hz, 2H, H-2′ and H-2″), 7.08 (dd, $J$ = 8.2, 1.7 Hz, 2H, H-6′ and H-6″), 7.57 (d, $J$ = 15.7 Hz, 2H, H-1 and H-7); EIMS: *m*/*z* (% rel. abund.) 448 $[M]^+$ (25), 430 (80), 407 (12), 389 (100), 230 (56), 217 (63), 189 (46), 177 (80).

*5.3.8. Dimethylallylation of curcumin (**1**)*

Compound **1** was subjected to alkylation in similar manner to that of methylation of compound **1**, but using 3,3-dimethylallyl bromide in place of methyl iodide, to afford mono-*O*-(3,3-dimethylallyl)curcumin (**14**) in 43% as orange gum, and di-*O*-(3,3-dimethylallyl)curcumin (**15**) in 40% as yellow solid, m.p. 123–124 °C (lit. [26] m.p. 124–125 °C). The spectroscopic (IR, $^1$H NMR and mass spectra) data of the latter product were consistent with the reported values [26].

*Compound **14***: IR $\nu_{max}$: 3413, 2927, 1624, 1582, 1508, 1261, 1134, 968 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 1.72 and 1.76 (each s, 2 × 3H, 2 × Me) 3.89 and 3.92 (each s, 2 × 3H, 2 × OMe), 4.60 (br d, $J$ = 6.4 Hz, 2H, H-1‴), 5.49 (m, 1H, H-2‴), 5.78 (s, 1H, H-4), 5.85 (brs, 1H, OH), 6.45 and 6.46 (each d, $J$ = 15.7 Hz, 2 × 1H, H-2 and H-6), 6.85 (d, $J$ = 8.2 Hz, 1H, H-5″), 6.91 (d, $J$ = 8.2 Hz, 1H, H-5′), 7.02 and 7.05 (each m, 2 × 1H, H-2′ and H-2″), 7.09 (br d, $J$ = 8.2 Hz, 2 × 1H, H-6′ and H-6″), 7.56 and 7.58 (each d, $J$ = 15.7 Hz, 2 × 1H, H-1 and H-7); ESMS (+ve): *m*/*z* (% rel. abund.) 437 $[M + H]^+$ (100); HR-TOFMS (ES$^+$): *m*/*z* 437.1982 $[M + H]^+$; calcd for $C_{26}H_{28}O_6$ + H, 437.1964.

*5.3.9. Dimethylallylation of demethoxycurcumin (**2**)*

Compound **2** was subjected to alkylation in similar manner to that of methylation of compound **1**, but using 3,3-dimethylallyl bromide in place of methyl iodide, to afford di-*O*-(3,3-dimethylallyl)demethoxycurcumin (**16**) in 43% as yellow aggregated needles (from *n*-hexane–$CH_2Cl_2$), m.p. 107–109 °C.

*Compound **16***: IR $\nu_{max}$: 2920, 1623, 1600, 1509, 1254, 1170, 1134, 971, 829 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 1.72, 1.76, 1.78, 1.79 (each s, 4 × 3H, 4 × Me), 3.89 (s, 3H, OMe), 4.52 (br d, $J$ = 6.4 Hz, 2H) and 4.60 (br d, $J$ = 6.4 Hz, 2H) (H-1‴ and H-1⁗), 5.47 (m, 2H, H-2‴ and H-2⁗), 5.77 (s, 1H, H-4), 6.47 (d, $J$ = 15.7 Hz, 2H, H-2 and H-6), 6.83 (d, $J$ = 7.9 Hz, 1H, H-5″), 6.90 (br d, $J$ = 8.5 Hz, 2H, H-3′ and H-5′), 7.05 (br s, 1H, H-2″), 7.11 (d, $J$ = 7.9, 1H, H-6″), 7.47 (br d, $J$ = 8.5 Hz, 2H, H-2′ and H-6′), 7.57 and 7.59 (each d, $J$ = 15.7 Hz,

2 × 1H, H-1 and H-7); ESMS (−ve): *m/z* (% rel. abund.) 473 $[M-H]^-$ (100); HR-TOFMS ($ES^+$): *m/z* 497.2307 $[M+Na]^+$; calcd for $C_{30}H_{34}O_5 + Na$, 497.2304.

*5.3.10. Hydroxyethylation of curcumin (**1**)*

Compound **1** was subjected to hydroxyethylation in similar manner to that of methylation of compound **1**, but using 2-bromoethanol in place of methyl iodide, to afford mono-*O*-(2-hydroxyethyl)curcumin (**17**) and di-*O*-(2-hydroxyethyl)curcumin (**18**) in 47 and 32%.

*Compound **17***: Yellow amorphous solid, m.p. 154–156 °C; IR $\nu_{max}$: 3449, 2927, 1623, 1508, 1271, 1135, 1028, 960, 847 $cm^{-1}$; $^1H$ NMR ($CDCl_3$ + 1 drop of $CD_3OD$, 400 MHz): $\delta$ 3.92 (br s, 6H, 2 × OMe), 3.92 (obscured signal, 2H, H-2‴), 4.11 (br s, 2H, H-1‴), 5.78 (br s, 1H, H-4), 6.45 and 6.47 (each d, *J* = 15.3 Hz, 2H, H-2 and H-6), 6.89 (m, 2H, H-5′ and H-5″), 7.02–7.10 (m, 4H, H-2′, H-2″, H-6′, H-6″), 7.56 (d, *J* = 15.6 Hz, 2H, H-1 and H-7); ESMS (−ve): *m/z* (% rel. abund.) 411 $[M-H]^-$ (100); HR-TOFMS ($ES^+$): *m/z* 435.1420 $[M+Na]^+$; calcd for $C_{23}H_{24}O_7 + Na$, 435.1418.

*Compound **18***: Orange needles (from $CH_2Cl_2$–MeOH), m.p. 174–175 °C; IR $\nu_{max}$: 3538, 2934, 1623, 1585, 1509, 1455, 1423, 1309, 1261, 1134, 1083, 1031, 973, 838, 805 $cm^{-1}$; $^1H$ NMR ($CDCl_3$ + 2 drops of $CD_3OD$, 400 MHz): $\delta$ 3.90 (br s, 6H, 2 × OMe), 3.90 (obscured signal, 4H, H-2‴ and H-2⁗), 4.07 (br s, 4H, H-1‴ and H-1⁗), 5.78 (s, 1H, H-4), 6.46 (d, *J* = 15.7 Hz, 2H, H-2 and H-6), 6.85 (d, *J* = 7.8 Hz, 2H, H-5′ and H-5″), 7.04 (br s, 2H, H-2′ and H-2″), 7.15 (br d, *J* = 7.8 Hz, 2H, H-6′ and H-6″), 7.54 (d, *J* = 15.7 Hz, 2H, H-1 and H-7); ESMS (−ve): *m/z* (% rel. abund.) 455 $[M-H]^-$ (100); HR-TOFMS ($APCI^+$): *m/z* 457.1843 $[M+H]^+$; calcd for $C_{25}H_{28}O_8 + H$, 457.1857.

*5.3.11. Hydroxyethylation of demethoxycurcumin (**2**)*

Following the same procedure for the preparation of compounds **17** and **18** from compound **1**, the mono-4″-*O*-(2-hydroxyethyl)demethoxycurcumin (**19a**) and its isomeric compound mono-4′-*O*-(2-hydroxyethyl)demethoxycurcumin (**19b**) were obtained. Careful repeated column chromatography followed by recrystallization resulted in the separation and purification of compound **19a** in 28%. However, attempt to purify the contaminated **19b** was unsuccessful.

Compound ***19a***: Yellow solid, m.p. 169–171 °C; IR $\nu_{max}$: 3507, 2934, 1619, 1594, 1559, 1508, 1273, 1136, 970 $cm^{-1}$; $^1H$ NMR ($CDCl_3$ + 4 drops of $CD_3OD$, 400 MHz): $\delta$ 3.90 (s, 3H, OMe), 3.91 (m, 2H, H-2‴), 4.08 (m, 2H, H-1‴), 5.75 (br s, 1H, H-4), 6.43 and 6.46 (each d, *J* = 15.7 Hz, 2 × 1H, H-2 and H-6), 6.80 (d, *J* = 8.3 Hz, 2H, H-3′ and H-5′), 6.85 (d, *J* = 8.1 Hz, 1H, H-5″), 7.05 (br s, 1H, H-2′), 7.10 (d, *J* = 8.1 Hz, 1H, H-6″), 7.40 (d, *J* = 8.3 Hz, H-2′ and H-6′), 7.54 and 7.56 (each d, *J* = 15.7 Hz, 2H, H-1 and H-7); ESMS (−ve): *m/z* (% rel. abund.) 381 $[M-H]^-$ (100); HR-TOFMS ($ES^-$): *m/z* 381.1320 $[M-H]^-$; calcd for $C_{22}H_{22}O_6$–H, 381.1338.

*5.3.12. Nitration of curcumin (**1**)*

Compound **1** (300 mg, 0.81 mmol) was dissolved in $CH_2Cl_2$ and $NaNO_2$ (100 mg) was added. The reaction mixture was stirred at ambient temperature for 5 min, and 3–4 drops of AcOH was added. The reaction mixture was stirred for another 30 min; the reaction was worked up by $H_2O$ and extracted with EtOAc. The organic phase was washed with $H_2O$, dried over anhydrous $Na_2SO_4$ and the solvent was evaporated to dryness. The crude product was purified by column chromatography using $CH_2Cl_2$–MeOH (200:2) to yield 5′-nitrocurcumin (**20**) (100 mg, 27%).

*Compound **20***: Orange amorphous solid, m.p. 198 °C (dec.); IR $\nu_{max}$: 3447, 2924, 1654, 1627, 1601, 1541, 1508, 1458, 1266, 1206, 1137, 1026, 966, 843, 668 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz): $\delta$ 3.92 and 3.98 (each s, 2 × 3H, 2 × OMe), 5.81 (s, 1H, OH), 6.46 (d, *J* = 15.7 Hz, 1H, H-6), 6.52 (d, *J* = 15.7 Hz, 1H, H-2), 6.90 (d, *J* = 8.2, 1H, H-5″), 7.03 (d, *J* = 1.5 Hz, 1H, H-2″), 7.10 (dd, *J* = 8.2, 1.5 Hz, 1H, H-6″), 7.25 (obscured signal, H-2′), 7.50 (d, *J* = 15.7 Hz, 1H, H-7), 7.58 (d, *J* = 15.7 Hz, 1H, H-1), 7.87 (d, *J* = 1.6 Hz, 1H, H-6′); ESMS (−ve): *m/z* (% rel. abund.) 412 $[M-H]^-$ (100); HR-TOFMS ($APCI^+$): *m/z* 414.1168 $[M+H]^+$; calcd for $C_{23}H_{24}O_7 + H$, 414.1183.

*5.3.13. Acetylation of curcumin (**1**)*

$Ac_2O$ (0.5 ml) was added to a solution of compound **1** (100 mg, 0.27 mmol) in pyridine (2 ml) and the reaction mixture was stirred at ambient temperature for 3 h. After the usual work up, the mixture was extracted with $CH_2Cl_2$ and the organic phase was washed with $H_2O$, dried over anhydrous $Na_2SO_4$ and the solvent evaporated to dryness. The crude product was purified by column chromatography eluting with $CH_2Cl_2$ to afford di-*O*-acetylcurcumin (**21**) (95 mg, 85%) as yellow amorphous solid; m.p. 163–165 °C (lit. [28] 160 °C). The spectroscopic (IR, $^1H$ NMR and mass spectra) data of compound **21** were consistent with the reported values [28].

*5.3.14. Acetylation of demethoxycurcumin (**2**)*

Compound **2** was subjected to acetylation in the same manner to that of compound **1** to give di-*O*-acetyldemethoxycurcumin (**22**) in 89% yield. The spectroscopic (IR, $^1H$ NMR and mass spectra) data of compound **22** were consistent with the reported values [29].

*Compound **22***: Yellow aggregated needles (from *n*-hexane–$CH_2Cl_2$), m.p. 140–141 °C; IR $\nu_{max}$: 2942, 1760, 1634, 1597, 1559, 1541, 1508, 1368, 1257, 1201, 1014, 969, 835 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz): $\delta$ 2.29 and 2.31 (each s, 2 × 3H, 2 × OAc), 3.86 (s, 3H, OMe), 5.82 (s, 1H, H-4), 6.54 and 6.55 (each d, *J* = 15.8 Hz, 2 × 1H, H-2 and H-6), 7.04 (d, *J* = 8.1 Hz, 1H, H-5″), 7.11 (d, *J* = 8.5 Hz, 2H, H-3′ and H-5′), 7.13 (partially obscured signal, 1H, H-6″), 7.55 (d, *J* = 8.4 Hz, 2H, H-2′ and H-6′), 7.60 and 7.62 (each d, *J* = 15.8 Hz, 2H, H-1 and H-7); ESMS (+ve): *m/z* (% rel. abund.) 445 $[M+Na]^+$ (34), 423 $[M+H]^+$ (100).

*5.3.15. Acetylation of bisdemethoxycurcumin (**3**)*

Compound **3** was subjected to acetylation in the same manner to that of compound **1** to give di-*O*-acetylbisdemethoxycurcumin (**23**) in 87% yield as yellow needles (from *n*-hexane–$CH_2Cl_2$), m.p. 172–174 °C. The spectroscopic (IR, $^1H$ NMR and mass spectra) data of compound **23** were consistent with the reported values [30].

*5.3.16. Benzoylation of curcumin (**1**)*

A solution of compound **1** (175 mg, 0.48 mmol) in pyridine (5 ml) was treated with benzoic anhydride (90 mg) and the mixture was stirred at ambient temperature for 4 h. The reaction was worked up with $H_2O$ and extracted with EtOAc; the organic phase was washed with $H_2O$, dried over anhydrous $Na_2SO_4$ and the solvent was evaporated to dryness. The crude products were separated and purified by column chromatography using $CH_2Cl_2$–MeOH (200:1) to give mono-*O*-benzoylcurcumin (**24**) (105 mg, 47%) and di-*O*-benzoylcurcumin (**25**) (90 mg, 33%). The spectroscopic (IR, $^1H$ NMR and mass spectra) data of the dibenzoate **25** were consistent with the reported values [31].

*Compound **24***: Yellow needles (from *n*-hexane–$CH_2Cl_2$), m.p. 134–136 °C; IR $\nu_{max}$: 3448, 2920, 1735, 1628, 1560, 1508, 1458, 1265, 1204, 1123, 1024, 967, 843, 707 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz): $\delta$ 3.84 and 3.92 (each s, 2 × 3H, 2 × OMe), 5.82 (s, 1H, H-4), 6.47 and 6.55 (each d, *J* = 15.7 Hz, 2 × 1H, H-2 and H-6) 6.91 (d, *J* = 8.1 Hz, 1H, H-5″), 7.03 (br s, 1H, H-2″), 7.10 (br d, *J* = 8.1 Hz, 1H, H-6″), 7.14–7.19 (m, 3H, H-2′, H-5′, H-6′), 7.49 (t, *J* = 7.4 Hz, 2H, *m*-ArH), 7.57–7.64 (m, 3H, H-1, H-7, *p*-ArH), 8.20 (d, *J* = 7.4 Hz, 2H, *o*-ArH); ESMS (+ve): *m/z* (% rel. abund.) 473 $[M+H]^+$ (100); HR-TOFMS ($ES^+$): *m/z* 495.1436 $[M+Na]^+$; calcd for $C_{28}H_{24}O_7 + Na$, 495.1420.

*Compound **25***: Yellow amorphous solid, m.p. 228–230 °C; IR $\nu_{max}$: 2920, 1734, 1653, 1626, 1598, 1541, 1514, 1456, 1302, 1269, 1122,

1082, 1027, 974, 854, 709 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz): $\delta$ 3.85 (s, 6H, 2 × OMe), 5.87 (s, 1H, H-4), 6.59 (d, $J$ = 15.8 Hz, 2H, H-2 and H-6), 7.15–7.22 (m, 6H, H-2′, H-5′, H-6′, H-2″, H-5″, H-6″), 7.50 (t, $J$ = 7.6, 4H, *m*-ArH), 7.63 (t, $J$ = 7.6, 2H, *p*-ArH), 7.64 (d, $J$ = 15.8 Hz, 2H, H-1 and H-7), 8.20 (d, $J$ = 7.6 Hz, 4H, *o*-ArH); ESMS (+ve): *m/z* (% rel. abund.) 599 $[M + Na]^+$ (10), 577 $[M + H]^+$ (10).

*5.3.17. Benzoylation of demethoxycurcumin (**2**)*

Compound **2** was subjected to benzoylation in similar manner to that of compound **1** to give the benzoates **26**, **27**and **28** in 34%, 29% and 21% yields respectively.

*Compound **26***: Yellow amorphous solid, m.p. 183–184 °C; IR $\nu_{max}$: 3411, 1721, 1635, 1593, 1559, 1522, 1419, 1373, 1166, 1123, 1064, 972, 820, 709 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz): $\delta$ 3.93 (s, 3H, OMe), 5.81 (s, 1H, H-4), 5.87 (br s, 1H, OH), 6.48 and 6.57 (each d, $J$ = 15.8 Hz, 2 × 1H, H-2 and H-6), 6.91 (d, $J$ = 8.1 Hz, 1H, H-5″), 7.04 (br s, 1H, H-2″), 7.11 (br d, $J$ = 8.1 Hz, 1H, H-6″), 7.25 (d, $J$ = 8.4 Hz, 2H, H-3′ and H-5′), 7.50 (t, $J$ = 7.6 Hz, 2H, *m*-ArH), 7.57–7.66 (m, 5H, H-1, H-7, H-2′, H-6′, *p*-ArH), 8.19 (d, $J$ = 7.6 Hz, 2H, *o*-ArH); ESMS (+ve): *m/z* (% rel. abund.) 443 $[M + H]^+$ (100); HR-TOFMS ($ES^+$): *m/z* 465.1319 $[M + Na]^+$; calcd for $C_{27}H_{22}O_6$ + Na, 465.1314.

*Compound **27***: Yellow needles (from *n*-hexane–$CH_2Cl_2$), m.p. 175–176 °C; IR $\nu_{max}$: 3449, 2934, 1728, 1627, 1601, 1512, 1451, 1267, 1169, 1124, 1063, 967, 833, 707 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz): $\delta$ 3.84 (s, 3H, OMe), 5.81 (s, 1H, H-4), 6.48 and 6.55 (each d, $J$ = 15.8 Hz, 2x1H, H-2 and H-6), 6.82 (d, $J$ = 8.4 Hz, 2H, H-3′ and H-5′), 7.12–7.23 (m, 3H, H-2″, H-5″, H-6″), 7.44 (d, $J$ = 8.4 Hz, 2H, H-2′ and H-6′), 7.50 (t, $J$ = 7.4 Hz, 1H, *m*-ArH), 7.61 (d, $J$ = 15.8 Hz, 2H, H-1 and H-7), 7.63 (obscured signal, *p*-ArH), 8.20 (d, $J$ = 7.4, 2H, *o*-ArH); ESMS (+ve): *m/z* (% rel. abund.) 465 $[M + Na]^+$ (43), 443 $[M + H]^+$ (100); HR-TOFMS ($ES^+$): *m/z* 465.1316 $[M + Na]^+$; calcd for $C_{27}H_{22}O_6$ + Na, 465.1314.

*Compound **28***: Yellow needles (from *n*-hexane–$CH_2Cl_2$), m.p. 177–179 °C; IR $\nu_{max}$: 2934, 1734, 1629, 1597, 1559, 1508, 1209, 1165, 1060, 1025, 969, 808, 706 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz): $\delta$ 3.85 (s, 3H, OMe), 5.86 (s, 1H, H-4), 6.59 and 6.60 (each d, $J$ = 15.8 Hz, 2 × 1H, H-2 and H-6), 7.16–7.27 (m, 5H, H-3′, H-5′, H-2″, H-5″, and H-6″), 7.50 (m, 2H, *m*-ArH), 7.60–7.69 (m, 6H, H-1, H-7, H-2′, H-6′ and *p*-ArH), 8.19 (m, 4H, *o*-ArH); ESMS (+ve): *m/z* (% rel. abund.) 569 $[M + Na]^+$ (100), 547 $[M + H]^+$ (55); HR-TOFMS ($APCI^+$): *m/z* 547.1744 $[M + H]^+$; calcd for $C_{34}H_{26}O_7$ + H, 547.1751.

*5.3.18. Benzoylation of bisdemethoxycurcumin (**3**)*

Compound **3** was subjected to benzoylation in similar manner to that of compound **1** to give the benzoates **29** and **30** in 53 and 31% yields respectively.

*Compound **29***: Yellow needles (from $CH_2Cl_2$–hexane), m.p. 228–230 °C; IR $\nu_{max}$: 3440, 1723, 1635, 1601, 1541, 1438, 1276, 1221, 1168, 1141, 1066, 972, 837, 707 $cm^{-1}$; $^1H$ NMR ($CDCl_3$ + 2 drops of $CD_3OD$, 400 MHz): $\delta$ 5.78 (s, 1H, H-4), 6.42 and 6.52 (each d, $J$ = 15.7 Hz, 2 × 1H, H-2 and H-6), 6.81 (br d, $J$ = 8.4 Hz, 2H, H-3″ and H-5″), 7.25 (obscured by $CHCl_3$ signal, H-3′ and H-5′), 7.42 (d, $J$ = 8.4 Hz, 2H, H-2″ and H-6″), 7.49 (m, 2H, *m*-ArH), 7.57–7.66 (m, 5H, H-1, H-7, H-2′, H-6′ and *p*-ArH), 8.17 (d, $J$ = 7.6 Hz, 2H, *o*-ArH); ESMS (+ve): *m/z* (% rel. abund.) 413 $[M + H]^+$ (100); HR-TOFMS ($ES^+$): *m/z* 435.1208 $[M + Na]^+$; calcd for $C_{26}H_{20}O_5$ + Na, 435.1208.

*Compound **30***: Yellow amorphous solid, m.p. 223–225 °C; IR $\nu_{max}$: 1739, 1654, 1636, 1595, 1560, 1508, 1458, 1270, 1213, 1167, 1060, 970, 821, 704 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz): $\delta$ 5.84 (s, 1H, H-4), 6.60 (d, $J$ = 15.8 Hz, 2H, H-2 and H-6), 7.25 (d, $J$ = 8.5 Hz, 4H, H-3′, H-5′, H-3″ and H-5″), 7.51 (t, $J$ = 7.5 Hz, 4H, *m*-ArH), 7.60–7.65 (m, 6H, H-2′, H-6′, H-2″, H-6″ and *p*-ArH), 7.67 (d, $J$ = 15.8 Hz, 2H, H-1 and H-7), 8.19 (d, $J$ = 7.5 Hz, 2H, *o*-ArH); ESMS (+ve): *m/z* (% rel. abund.) 517 $[M + H]^+$ (100); HR-TOFMS ($ES^+$): *m/z* 539.1472 $[M + Na]^+$; calcd for $C_{33}H_{24}O_6$ + Na, 539.1471.

*5.3.19. Catalytic hydrogenation of curcumin (**1**)*

To a solution of compound **1** (100 mg, 0.27 mmol) in EtOH (10 ml) was added 10% Pd-C (50 mg). After degassing, the mixture was hydrogenated at room temperature and at atmospheric pressure for 4 h. The mixture was filtered through a column of Celite and the solvent was evaporated. The residue was purified by column chromatography using $CH_2Cl_2$–MeOH (gradient elution) to afford tetrahydrocurcumin (**31**) (65 mg, 64%) as crystals from *n*-hexane–$CH_2Cl_2$, m.p. 93–94 °C (lit. [24] m.p. 92–93 °C), hexahydrocurcumin (**32**) (20 mg, 20%) as white amorphous solid, m.p. 81–82 °C (lit. [33] m.p. 80–82 °C), and octahydrocurcumin (**33**) (15 mg, 15%) as colorless sticky solid. The spectroscopic (IR, $^1H$ NMR and mass spectra) data of compounds **31**–**33** were consistent with the reported values [24,33].

*5.3.20. Catalytic hydrogenation of demethoxycurcumin (**2**)*

Demethoxycurcumin was subjected to catalytic hydrogenation in similar manner to that of compound **1** to afford tetrahydrodemethoxycurcumin (**34**) (72%) as colorless sticky solid and a small quantity of a product, which was supposed to be the hexahydro analog. The spectroscopic (IR, $^1H$ NMR and mass spectra) data of compound **34** were consistent with the reported values [34].

*5.3.21. Catalytic hydrogenation of bisdemethoxycurcumin (**3**)*

Compound **3** was subjected to catalytic hydrogenation in similar manner to that of compound **1** to give tetrahydrobisdemethoxycurcumin (**35**) (75%) as colorless crystal (from acetone–benzene), m.p. 110–111 °C (lit. [34] m.p. 101–102 °C). The spectroscopic (IR, $^1H$ NMR and mass spectra) data of compound **35** were consistent with the reported values [34].

*5.3.22. Catalytic hydrogenation of mono-O-demethylcurcumin (**4**)*

Compound **4** was subjected to catalytic hydrogenation in similar manner to that of compound **1** to give mono-*O*-demethyltetrahydrocurcumin (**36**) (40%) as colorless sticky solid and a mixture of mono-*O*-demethylhexahydrocurcumin (**37a** and **37b**) (37%). The identity of compounds **36,** and **37a**/**37b** mixture was confirmed by comparison of the spectroscopic (IR, $^1H$ NMR and mass spectra) data with the reported values [34,35].

*Compound **36***: $^1H$ NMR ($CDCl_3$ + 1 drop of $CD_3OD$, 400 MHz): $\delta$ 2.33 (m), 2.82 (t, $J$ = 7.0 Hz) and 2.46–2.82 (m) (8H, H-1, H-2, H-6, H-7), 3.80 (s 3H, OMe), 6.51 (m, 1H, H-6″), 6.59–6.63 (m, 3H, H-2′, H-2″, H-6′), 6.70 (d, $J$ = 8.0 Hz, 1H, H-5′), 6.71 (d, $J$ = 7.9 Hz, 1H, H-5″); ESMS (−ve): *m/z* (% rel. abund.) 357 $[M - H]^-$ (100).

*Compounds **37a** and **37b** (1:1 mixture)*: $^1H$ NMR ($CDCl_3$ + 3 drops of $CD_3OD$, 400 MHz): $\delta$ 1.57 and 1.68 (each m, 2 × 2H, H-6a and H-6b), 2.46–2.77 (m, 2 × 8H, H-1, H-2, H-4, H-7), 3.79 (s, 3H, OMe), 3.80 (s, 3H, OMe), 3.96 (m, 2 × 1H, H-5), 6.47 (br dd, $J$ = 7.9, 1.8 Hz, 1H, H-6″), 6.49 (br dd, $J$ = 7.9, 1.8 Hz, 1H, H-6″), 6.57–6.64 (m, 2 × 3H, H-2′, H-2″, H-6′), 6.69 (d, $J$ = 8.0 Hz, 2 × 1H, H-5′), 6.75 (d, $J$ = 7.9 Hz, 1H, H-5″); 6.76 (d, $J$ = 7.9 Hz, 1H, H-5″); ESMS (−ve): *m/z* (% rel. abund.) 359 $[M - H]^-$ (100).

*5.3.23. Catalytic hydrogenation of didemethylcurcumin (**5**)*

Compound **5** was subjected to catalytic hydrogenation in similar manner to that of compound **1** to give didemethyltetrahydrocurcumin (**38**) (65%) as colorless sticky solid. The spectroscopic data of compound **38** were consistent with the reported values [34,35].

*5.3.24. Preparation of di-O-methylhexahydrocurcumin (**39**)*

Compound **7** was subjected to catalytic hydrogenation in similar manner to that of compound **1** to give di-*O*-methyltetrahydrocurcumin, which was directly subjected to sodium borohydride reduction in ethanol to the corresponding hexahydro analog **39**

(62% overall yield from compound **7**) as white solid, m.p. 92–93 °C (lit. [26] m.p. 93–94 °C). The spectroscopic (IR, $^1$H NMR and mass spectra) data of compound **38** were consistent with the reported values [26].

*5.3.25. Dehydration of hexahydrocurcumin*

Hexahydrocurcumin (**32**) (185 mg, 0.49 mmol) was dissolved in benzene (45 ml) and *p*-toluenesulfonic acid monohydrate (50 mg) was added. The reaction mixture was reflux for 3 h; $H_2O$ was added and the mixture was extracted with EtOAc. The combined organic phase was washed with $H_2O$ and dried over anhydrous $Na_2SO_4$; the solvent was evaporated and the residue chromatographed using hexane–EtOAc (3:2) to yield compound **40** (175 mg, quantitative yield) as white amorphous solid. The spectroscopic (IR, $^1$H NMR and mass spectra) data of compounds **40** was consistent with the reported values [36].

*Compound **40***: IR $\nu_{max}$: 3421, 2935, 2840, 1660, 1624, 1541, 1515, 1457, 1362, 1268, 1238, 1154, 1037, 974, 816, 796 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 2.47 (m, 2H), 2.67 (t, $J = 7.6$ Hz, 2H) and 2.80 (m, 4H) (H-1, H-2, H-6, H-7), 3.84 (s, 3H, OMe), 3.85 (s, 3H, OMe), 5.47 (s, 1H, OH), 5.48 (s, 1H, OH), 6.08 (br d, $J = 15.9$ Hz, 1H, H-4), 6.62–6.68 (m, 4H), 6.77–6.84 (m, 3H) (H-5, H-2′, H-5′, H-6′, H-2″, H-5″ and H-6″); EIMS: *m*/*z* (% rel. abund.) 356 $[M]^+$ (60), 219 (17), 206 (49), 205 (14), 137 (100).

*5.3.26. Dehydration of a mixture of **37a** and **37b***

A mixture of compounds **37a** and **37b** was subjected to dehydration in similar manner to that of compound **31** to give a mixture of products **41a** and **41b** (88%). The $^1$H NMR data of compound **41a**/**41b** mixture were consistent with those reported values of **41a** [37].

*Compounds **41a** and **41b** (1:1 mixture)*: Colorless sticky solid; IR $\nu_{max}$: 3384, 3019, 2936, 2853, 1655, 1516, 1450, 1366, 1273, 1152, 1113, 1034, 814, 754 cm$^{-1}$; $^1$H NMR ($CDCl_3$ + 2 drops of $CD_3OD$, 400 MHz): $\delta$ 2.44 (m, 2 × 2H, H-6), 2.60 (br t, $J = 7.3$ Hz, 2 × 1H), 2.66 (m, 2 × 1H), 2.74 (m) and 2.78 (m) (2 × 4H, H-1, H-2, H-7), 3.82 (s, 3H, OMe), 3.83 (s, 3H, OMe), 6.04 (d, $J = 15.8$ Hz, 2 × 1H, H-4), 6.51 (m, 2 × 1H), 6.62 (m, 2 × 3H) and 6.70–6.80 (m, 2 × 3H) (H-5, H-2′, H-2″, H-5′, H-5″, H-6′, H-6″); ESMS (−ve): *m*/*z* (% rel. abund.) 341 $[M - H]^-$ (100).

*5.3.27. Preparation of compound **42***

Compound **38** (50 mg, 0.15 mmol) in methanol (2 ml) was treated with sodium borohydride (5 mg, 0.13 mmol) to give the corresponding hexahydro analog which was directly dehydrated without purification to afford the product **42** (32 mg, 65% overall yield from compound **38**) as colorless sticky solid. The spectroscopic (IR, $^1$H NMR and mass spectra) data of compound **42** were consistent with the reported values [38].

*5.3.28. Methylation of compound **40***

Compound **40** (100 mg, 0.28 mmol) was dissolved in dry acetone (3 ml) and anhydrous $K_2CO_3$ (70 mg, 0.50 mmol) and MeI (0.8 ml, 1.27 mmol) were added. The reaction mixture was reflux for 6 h and $H_2O$ was added. The mixture was extracted with $CH_2Cl_2$; the combined organic phase was washed with $H_2O$, dried over anhydrous $Na_2SO_4$ and the solvent removed under vacuum. The crude products were purified by column chromatography using $CH_2Cl_2$ to yield the mono-*O*-methylated products **43a**/**43b** mixture in 29% yield and di-*O*-methylated product **44** in 57% yield, the latter of which was recrystallized from *n*-hexane–$CH_2Cl_2$ to give **44** as needles, m.p. 68 °C. The spectroscopic data of compound **44** were consistent with the reported values [39].

*Compounds **43a** and **43b** (1:1 mixture)*: Colorless sticky solid; IR $\nu_{max}$: 3431, 3017, 2936, 2836, 1667, 1626, 1515, 1262, 1154, 1029, 853, 808, 754 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 2.48 (m, 2 × 2H, H-6), 2.68 (m, 2 × 2H, H-7), 2.81 (m, 2 × 4H, H-1 and H-2), 3.83 (s, 3H, OMe), 3.84 (br s, 4 × 3H, 4 × OMe), 3.85 (s, 3H, OMe), 6.08 (d, $J = 15.9$ Hz, 2 × 1H, H-4), 6.63–6.70 (m, 2 × 4H, H-2′, H-2″, H-5′, H-5″), 6.75–6.85 (m, 2 × 3H, H-5, H-6′, H-6″); ESMS (+ve): *m*/*z* (% rel. abund.) 763 $[2M + Na]^+$ (100); HR-TOFMS ($ES^+$): *m*/*z* 393.1678 $[M + Na]^+$; calcd for $C_{22}H_{26}O_5 + Na$, 393.1678.

*5.3.29. Preparation of enones **45a** and **45b** mixture*

A mixture of the enones **45a** and **45b** was prepared from the diketone **34** in similar manner to the preparation of compound **42** from compound **38** to give a mixture of the enones **45a** and **45b** (67%) as colorless sticky solid. The spectroscopic (IR, $^1$H NMR and mass spectra) data of compounds **45a** and **45b** were consistent with the reported values [36,40].

*5.3.30. Preparation of compound **46***

By using the same procedure for the preparation of compound **42**, the diketone **35** was converted to the corresponding α,β-unsaturated ketone **46** as colorless sticky solid in 60% overall yield from compound **35.** The spectroscopic (IR, $^1$H NMR and mass spectra) data of compound **46** were consistent with the reported values [41].

*5.3.31. Dehydrogenation of compound **40***

Compound **40** (80 mg, 0.22 mmol) was dissolved in THF (6 ml) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (40 mg, 0.18 mmol) was added. The reaction mixture was stirred at ambient temperature for 4 h. The reaction was worked up by $H_2O$ and extracted with EtOAc. The organic phase was washed with $H_2O$, dried over anhydrous $Na_2SO_4$ and the solvent was evaporated to dryness. The crude products were separated and purified by column chromatography using *n*-hexane–EtOAc (2:1) to yield the dienone **47** [42] (22 mg, 28%), dienone **48** (18 mg, 23%) and the trienone **49** [43] (25 mg, 32%).

*Compound **47***: Yellow sticky solid; IR $\nu_{max}$: 3363, 3014, 2927, 1653, 1560, 1514, 1467, 1430, 1339, 1287, 1154, 1032, 858, 798 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 2.87 (br s, 4H, H-1 and H-2), 3.85 (s, 3H, OMe), 3.91 (s, 3H, OMe), 5.45 (s, 1H, OH), 5.77 (s, 1H, OH), 6.22 (d, $J = 15.4$ Hz, 1H, H-4), 6.64–6.73 (m, 3H, H-6, H-5′ or H-5″ and H-2′ or H-2″), 6.82 (d, $J = 7.8$ Hz, 1H, H-5″ or H-5′), 6.83 (d, $J = 15.4$ Hz, 1H, H-7), 6.87 (d, $J = 8.1$ Hz, 1H, H-6′ or H-6″), 6.95 (d, $J = 1.3$ Hz, 1H, H-2″ or H-2′), 6.98 (dd, $J = 8.1$, 1.3 Hz, 1H, H-6″ or H-6′), 7.28 (dd, $J = 15.4$, 10.7 Hz, 1H, H-5); ESMS (+ve): *m*/*z* (% rel. abund.) 377 $[M + Na]^+$ (100).

*Compound **48***: Yellow needles (from *n*-hexane–$CH_2Cl_2$), m.p. 124–126 °C; IR $\nu_{max}$: 3342, 3007, 2934, 1654, 1602, 1568, 1514, 1430, 1376, 1278, 1217, 1168, 1120,1030, 999, 860 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 2.55 (m, 2H, H-6), 2.74 (t, $J = 7.6$ Hz, 2H, H-7), 3.85 (s, 3H, OMe), 3.92 (s, 3H, OMe), 5.47 (s, 1H, OH), 5.87 (s, 1H, OH), 6.42 (d, $J = 15.6$ Hz, 1H, H-4), 6.67 (overlapping signal, 1H, H-2″), 6.68 (overlapping signal, 1H, H-5″), 6.76 (d, $J = 15.9$ Hz, 1H, H-2), 6.83 (d, $J = 7.7$ Hz, 1H, H-5′), 6.91 (d, $J = 8.1$ Hz, H-6″), 6.98 (m, 1H, H-5), 7.04 (d, $J = 1.5$ Hz, 1H, H-2′), 7.10 (dd, $J = 8.1$, 1.5 Hz, 1H, H-6′), 7.52 (d, $J = 15.9$ Hz, 1H, H-1); EIMS: *m*/*z* (% rel. abund.) 354 $[M]^+$ (6), 218 (13), 137 (100); HR-TOFMS ($ES^+$): *m*/*z* 377.1365 $[M + Na]^+$; calcd for $C_{21}H_{22}O_5 + Na$, 377.1365.

*5.3.32. Pentylation of the enone **40***

Compound **40** was subjected to *n*-pentylation in similar manner to that of methylation of compound **1**, but using *n*-pentyl iodide in place of methyl iodide, to give mono-*O*-*n*-pentyl analogs **50a**/**50b** mixture and di-*O*-*n*-pentyl analog **51** in 53 and 27%, respectively.

*Compounds **50a** and **50b** (1:1 mixture)*: Colorless sticky solid; IR $\nu_{max}$: 3438, 2934, 2869, 1667, 1626, 1515, 1464, 1366, 1262, 1153, 1035, 852, 800 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 0.90 (t, $J = 6.5$ Hz,

2 × 3H, H-5‴), 1.38 (m, 2 × 4H, H-3‴ and H-4‴), 1.80 (m, 2 × 2H, H-2‴), 2.48 (m, 2 × 2H, H-6), 2.67 (m, 2 × 2H, H-7), 2.81 (m, 2 × 4H, H-1 and H-2), 3.81 (s, 3H, OMe), 3.82 (s, 3H, OMe), 3.83 (s, 3H, OMe), 3.84 (s, 3H, OMe), 3.95 (t, $J$ = 6.5 Hz, 2 × 2H, H-1‴), 5.51 (br s, 1H, OH), 5.52 (br s, 1H, OH), 6.08 (d, $J$ = 15.8 Hz, 2 × 1H, H-4), 6.63–6.67 (m, 2 × 4H, H-2′, H-2″, H-5′, H-5″), 6.75–6.81 (m, 2 × 3H, H-5, H-6′, H-6″); ESMS (+ve): *m/z* (% rel. abund.) 875 $[2M + Na]^+$ (100); HR-TOFMS ($ES^+$): *m/z* 449.2304 $[M + Na]^+$; calcd for $C_{26}H_{34}O_5$ + Na, 449.2304.

*Compound* ***51***: White amorphous solid, m.p. 65–66 °C; IR $\nu_{max}$: 2958, 2931, 2861, 1670, 1627, 1589, 1517, 1469, 1277, 1241, 1141, 1035, 987, 850, 797 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz): $\delta$ 0.89 (m, 6H, H-5‴ and H-5⁗), 1.38 (m, 8H, H-3‴, H-4‴, H-3⁗ and H-4⁗), 1.80 (m, 4H, H-2‴ and H-2⁗), 2.47 (m, 2H, H-6), 2.68 (m, 2H, H-7), 2.81 (m, 4H, H-1 and H-2), 3.81 (s, 6H, 2 × OMe), 3.94 (m, 4H, H-1‴ and H-1⁗), 6.08 (d, $J$ = 15.8 Hz, 1H, H-4), 6.65 (br s, 2H, H-2′ and H-2″), 6.67 (d, $J$ = 8.2 Hz, 2H, H-5′ and H-5″), 6.76 (br d, $J$ = 8.2 Hz, 2H, H-6′ and H-6″), 6.81 (m, 1H, H-5); ESMS (+ve): *m/z* (% rel. abund.) 497 $[M + H]^+$ (100); HR-TOFMS ($ES^+$): *m/z* 519.3087 $[M + Na]^+$; calcd for $C_{31}H_{44}O_5$ + Na, 519.3086.

### 5.3.33. Hydroxyethylation of the enone *40*

Compound **40** was subjected to hydroxyethylation in similar manner to that of compound **1** to afford mono-*O*-(2-hydroxyethyl) analogs **52a** and **52b** mixture in 27%.

*Compound* ***52a*** *and* ***52b*** (*1:1 mixture*): Colorless sticky solid; IR $\nu_{max}$: 3424, 2937, 1661, 1620, 1515, 1453, 1453, 1367, 1263, 1141, 1033, 899, 810 $cm^{-1}$; $^1H$ NMR ($CDCl_3$ + 4 drops of $CD_3OD$, 400 MHz): $\delta$ 2.47 (m, 2 × 2H, H-6), 2.66 (m, 2 × 2H, H-7), 2.78 (m, 2 × 4H, H-1 and H-2), 3.80 (s, 3H, OMe), 3.81 (s, 2 × 3H, 2 × OMe), 3.82 (s, 3H, OMe), 3.85 (m, 2 × 2H, H-2‴), 4.01 (m, 2 × 2H, H-1‴), 6.06 (d, $J$ = 15.9 Hz, 2 × 1H, H-4), 6.59–6.69 (m, 2 × 4H, H-2′, H-2″, H-5′, H-5″), 6.76 (br d, $J$ = 8.2 Hz, 2 × 2H, H-6′ and H-6″), 6.79 (m, 2 × 1H, H-5); ESMS (−ve): *m/z* (% rel. abund.) 399 $[M - H]^-$ (100); HR-TOFMS ($ES^+$): *m/z* 423.1783 $[M + Na]^+$; calcd for $C_{23}H_{28}O_6$ + Na, 423.1784.

### 5.3.34. Acetylation of the enone *40*

Compound **40** was subjected to acetylation in the same manner to that of compound **1** to give the monoacetates **53a**/**53b** mixture and diacetate **54** in 40 and 42%, respectively. The latter compound has been synthesized previously [44].

*Compound* ***53a*** *and* ***53b*** (*1:1 mixture*): Colorless sticky solid; IR $\nu_{max}$: 3444, 2938, 1763, 1664, 1604, 1514, 1453, 1369, 1272, 1199, 1152, 1122, 1034, 903, 822 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz): $\delta$ 2.27 (s, 3H, OAc), 2.28 (s, 3H, OAc), 2.48 (m, 2 × 2H, H-6), 2.70 (m, 2 × 2H, H-7), 2.81 (m, 2 × 4H, H-1 and H-2), 3.78, (s, 3H, OMe), 3.79, (s, 3H, OMe), 3.84, (s, 3H, OMe), 3.85 (s, 3H, OMe), 6.08 (d, $J$ = 15.7 Hz, 1H, H-4), 6.10 (d, $J$ = 15.7 Hz, 1H, H-4), 6.63–6.92 (m, 2 × 7H, H-5, H-2′, H-2″, H-5′, H-5″, H-6′, H-6″); ESMS (−ve): *m/z* (% rel. abund.) 397 $[M - H]^-$ (100); HR-TOFMS ($ES^+$): *m/z* 421.1627 $[M + Na]^+$; calcd for $C_{23}H_{26}O_6$ + Na, 421.1627.

### 5.3.35. Catalytic hydrogenation of the enone *40*

Compound **40** was subjected to catalytic hydrogenation in similar manner to that of compound **1** to afford the ketone **55** (43 mg, 86%). The spectroscopic (IR, $^1H$ NMR and mass spectra) data of compound **55** were consistent with the reported values [33].

### 5.3.36. Catalytic hydrogenation of a mixture of *56a* and *56b*

A mixture of **45a** and **45b** was subjected to catalytic hydrogenation in similar manner to that of compound **1** to afford a mixture of **56a** and **56b** (86%).

*Compounds* ***56a*** *and* ***56b*** (*1:1 mixture*): Colorless sticky solid; $^1H$ NMR ($CDCl_3$, 400 MHz): $\delta$ 1.54 (m, 2 × 4H), 2.36 (br t, $J$ = 6.0 Hz, 2 × 2H), 2.49 (br t, $J$ = 6.0 Hz, 2 × 2H), 2.65 (br t, $J$ = 7.0 Hz, 2 × 2H) and 2.79 (br t, $J$ = 7.2 Hz, 2 × 2H) (H-1, H-2, H-4, H-5, H-6, H-7), 3.83 (s, 3H, OMe), 3.84 (s, 3H, OMe), 6.63 (m, 2 × 2H, H-2″ and H-5″), 6.71 (d, $J$ = 7.9 Hz, 2 × 2H, H-3′ and H-5′), 6.79 (d, $J$ = 7.7 Hz, 2 × 1H, H-6″), 6.98 and 6.99 (each d, $J$ = 7.9 Hz, 2 × 2H, H-2′ and H-6′); ESMS (−ve): *m/z* (% rel. abund.) 327 $[M - H]^-$ (100).

### 5.3.37. Reduction of the enone *40*

Compound **40** (25 mg, 0.07 mmol) was dissolved in EtOH (2 ml) and $NaBH_4$ (4 mg) was added. The reaction mixture was stirred at ambient temperature for 30 min. The reaction was worked up by $H_2O$ and extracted with EtOAc. The organic phase was washed with $H_2O$, dried over anhydrous $Na_2SO_4$ and the solvent was evaporated to dryness. The crude product was purified by column chromatography using $CH_2Cl_2$ to yield the enol **57** (24 mg, 96%) as colorless sticky solid; IR $\nu_{max}$: 3423, 2935, 2853, 1603, 1515, 1452, 1428, 1364, 1271, 1231, 1152, 1123, 1033, 816 $cm^{-1}$; $^1H$ NMR ($CDCl_3$ + 2 drops of $CD_3OD$, 400 MHz): $\delta$ 1.71 (m, H-2), 2.30 (m, 2H, H-6), 2.49–2.64 (m, 4H, H-1 and H-7), 3.83 (s, 6H, 2 × OMe), 4.01 (m, 1H, H-3), 5.47 (dd, $J$ = 15.3, 5.6 Hz, 1H, H-4), 5.63 (m, 1H, H-5), 6.64 (m, 4H, H-2′, H-2″, H-5′, H-5″), 6.78 (br d, $J$ = 7.6 Hz, 2H, H-6′ and H-6″); ESMS (+ve): *m/z* (% rel. abund.) 359 $[M + H]^+$ (100); HR-TOFMS ($ES^+$): *m/z* 381.1677 $[M + Na]^+$; calcd for $C_{21}H_{26}O_5$ + Na, 381.1678.

## 5.4. Biological activities

### 5.4.1. Parasite strains, human cell line and culture conditions

Bloodstream forms of *T. b. brucei* strain 427 (BS221) were used as drug-sensitive control and the multi-drug resistant clonal line 427ΔTbAT1 was derived from this strain by deletion of the TbAT1 gene that encodes the P2 aminopurine transporter [46] responsible for the uptake of melaminophenyl arsenical and diamidine drugs [48,53]. The clonal line B48, which is highly resistant to melarsoprol and pentamidine, was in turn derived from 427ΔTbAT1 by adaptation to high levels of pentamidine [49]. *T. evansi* strain ITMAV170475 was obtained from Vincent Delespaux, Institute of Tropical Medicine Antwerp, Belgium and adapted to culture in HMI9/20% FBS following the procedure of Hirumi and Hirumi [54]. All *T. b. brucei* strains and *T. evansi* were cultured at 37 °C in a $CO_2$ incubator using HMI9 media (BioSera, Ringmer, UK) supplemented with 10% fetal bovine serum (FBS, BioSera) exactly as described previously [55]. Amastigotes of *L. mexicana* (strain MNYC/BZ/62/M379) were maintained in axenic culture at 33 °C in a $CO_2$ incubator using Schneider's Drosophila medium (Gibco) supplemented with 20% FBS and 0.3% gentamycin as described [56]. Promastigotes of *L. major* (Friedlin strain) were cultured in HOMEM medium (Invitrogen) supplemented with 10% FBS at 25 °C as described [57]. Human Embryonic Kidney (HEK) cells of strain 293 T were cultured as described by Rodenko et al. [58] in a medium based on Dulbecco's modified Eagle's medium (Sigma) with 10% newborn bovine serum (Invitrogen).

### 5.4.2. Cytotoxicity and antiprotozoal activity

All compounds were screened for cytotoxicity against the human cell line HEK293 exactly as described [58]. Briefly, HEK cells were grown in 96-well plates at 37 °C under a 5% $CO_2$ atmosphere and to each well an equal volume of a doubling dilution of test compound was added, after which the plate was incubated another 16 h prior to the addition of the Alamar Blue (resazurin, Sigma) reagent. The highest concentration of test compound was 250 μM, unless restricted by solubility. Fluorescence ($\lambda_{exc}$ = 530 nm; $\lambda_{em}$ = 590 nm) was determined after an additional 24 h incubation, in a Perkin–Elmer LS55 fluorimeter. $EC_{50}$ values (defined as Effective Concentration for 50% reduction of fluorescence in the Alamar Blue assay, below) were determined by non-linear regression,

fitting data to a sigmoid curve with variable slope (Prism version 4, GraphPad).

Tests for trypanocidal (*T. b. brucei*) and leishmanicidal activity utilized similar protocols based on Alamar Blue fluorescence in 96-well plates, using the culture condition described in the previous section and protocols exactly as described [49,55–57]. An Alamar Blue procedure was validated for the culture adapted *T. evansi*, using cell counts in a hemocytometer under phase-contrast microscopy to confirm correlation between Alamar Blue and cell survival after exposure to various standard trypanocides. Test conditions were optimized after (1) variation of trypanosome cell number at start of drug test, (2) incubation of trypanosome culture with test compound and (3) incubation time with the Alamar blue reagent. These parameters were resolved as $1.5 \times 10^5$ trypanosomes/ml and 48 h and 24 h, respectively, for the incubations at 37 °C in a $CO_2$ incubator in HMI9/20% FBS.

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