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Demethoxycurcumin and its Semisynthetic Analogues as Antitubercular Agents

Dinesh Kumar Agrawal[1], Dharmendra Saikia[2], Richa Tiwari[2], Shweta Ojha[2], Karuna Shanker[1], Jonnala Kotesh Kumar[1], Anil Kumar Gupta[2], Sudeep Tandon[3], Arvind Singh Negi[1], Suman Preet Singh Khanuja[2]

[1] Analytical Chemistry Division, Central Institute of Medicinal and Aromatic Plants (CIMAP-CSIR), Lucknow, India
[2] Genetic Resources and Biotechnology Division, Central Institute of Medicinal and Aromatic Plants (CIMAP-CSIR), Lucknow, India
[3] Process Division, Central Institute of Medicinal and Aromatic Plants (CIMAP-CSIR), Lucknow, India

Abstract

Demethoxycurcumin, isolated from the rhizomes of *Curcuma longa*, was found to possess antitubercular activity against *Mycobacterium tuberculosis* H_{37}Rv strain at 200 μg/mL. Derivatisation of this active principle yielded a potent agent **6**, exhibiting considerable activity with a minimum inhibitory concentration (MIC) value of 7.8 μg/mL.

Key words

Curcuma longa · Zingiberaceae · *Mycobacterium tuberculosis* · demethoxycurcumin · phenolic derivatives · antitubercular activity

Abbreviations

H_{37}Rv: Mycobacterium tuberculosis H_{37}Rv strain
MIC: minimum inhibitory concentration

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Turmeric (*Curcuma longa* L.) has been used in the Indian system of medicine for the treatment of coughs, fever, jaundice, liver, urinary diseases, wounds, inflammatory troubles of the joints and others [1]. Various medicinal properties of turmeric like antioxidant, anticancer, antimalarial, hepatoprotective, anti-inflammatory and wound healing are scientifically well established now [2].

The present study was intended to identify the antitubercular principle from *C. longa* chloroform extracts. Of the three compounds isolated, i. e., curcumin **1**, demethoxycurcumin **2** and bisdemethoxycurcumin **3**, we could identify **2** as the compound responsible for the antitubercular activity in the extracts. This paper is the first report on demethoxycurcumin as an antitubercular agent. Furthermore, semisynthetic modifications of demethoxycurcumin yielded an analogue with an antitubercular activity twenty five times higher than that of the parent.

The chloroform extract of rhizomes showed antitubercular activity against H_{37}Rv at 1000 μg/mL while the purified curcuminoid, demethoxycurcumin **2**, was found to be active at 200 μg/mL (**Table 1** and **Fig. 1**). Demethoxycurcumin **2** was chemically modified at its phenolic hydroxy positions to get four dif-

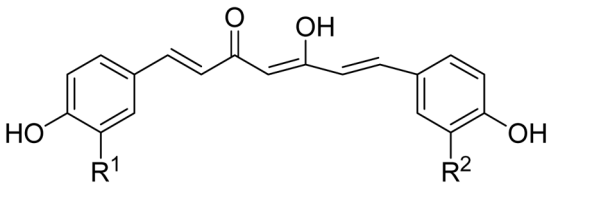
ferent derivatives **4** – **7**. Compound **6** possessed potent antitubercular activity (MIC = 7.812 μg/mL), while **7** showed moderate activity (MIC = 125 μg/mL) (**Table 1** and **Fig. 2**). Compounds **1**, **3**, **4** and **5** were inactive even at 250 μg/mL. In its ^{1}H-NMR spectrum, compound **6** showed a broad doublet at δ 4.68 ($OCH_2 \times 2$), a doublet at δ 6.05 ($=CH \times 2$), a multiplet at δ 6.35 ($=CH \times 2$), a triplet at δ 0.96 ($=CH \times 2$) and a distorted quartet at δ 4.1 ($OCH_2 \times 2$) indicating attachment of two $-OCH_2-CH=CH-COOCH_2CH_3$ units at the phenolic positions. Electrospray mass spectrometry confirmed its molecular formula as $C_{32}H_{33}O_9$. The ^{1}H-NMR spectrum of compound **7** exhibited two distinct singlets at δ 4.68 (OCH_2), δ 4.74 (OCH_2), a broad triplet at δ 1.33 ($CH_3 \times 2$) and a distorted quartet at δ 4.28 ($OCH_2 \times 2$) to indicate attachment of the $-OCH_2COOCH_2CH_3$ units at the phenolic positions. Electrospray mass spectrometry confirmed its molecular formula as $C_{28}H_{30}O_9$. The purities of the semi-synthetic derivatives **6** and **7** were 94.7% and 96.2%, respectively.

The objective of the synthesis of **6** and **7** was to increase the lipophilicity of **2** by attaching fatty acid ester chains at the phenolic hydroxy groups. Earlier studies, [3], [4], [5], [6] found that molecules with moderate to high lipophilicity exhibit better antitubercular activity due to the lipophilic nature of the *Mycobacterium tuberculosis* cell wall. Therefore, transport of polar compounds through the outer lipid layer of mycobacteria is retarded [7]. Fitzpatrick [8] reported antitubercular activity in aqueous extracts of *C. longa* leaves in broth dilution assays at MIC < 1 : 40 dilution. In the tube dilution test, Grange and Davey [9] found the 95% ethanolic extract of *C. longa* to be active at 1 : 80 dilution against H_{37}Rv. Schraufstatter and Bernt [10] reported curcumin's antimycobacterial activity at 1 : 10 000 dilution.

Table 1 Antimycobacterial activity of *C. longa* extract, curcuminoids and semisynthetic derivatives against *M. tuberculosis* H_{37}Rv strain with the BACTEC assay.

S. No.	Compound	MIC (μg/mL)
1.	Chloroform extract	1000
2.	Curcumin (**1**)	Inactive
3.	Demethoxycurcumin (**2**)	200
4.	Bisdemethoxycurcumin (**3**)	Inactive
5.	**4**	Inactive
6.	**5**	Inactive
7.	**6**	7.812
8.	**7**	125
9.	Rifampicin	2.0

Mean of three experiments in replicates.



1 (Curcumin): $R^1 = R^2 = OCH_3$;
2 (Demethoxycurcumin): $R^1 = H$; $R^2 = OCH_3$;
3 (Bisdemethoxycurcumin): $R^1 = R^2 = H$.

Fig. 1 Structures of curcumin **1**, demethoxycurcumin **2** and bisdemethoxycurcumin **3**.

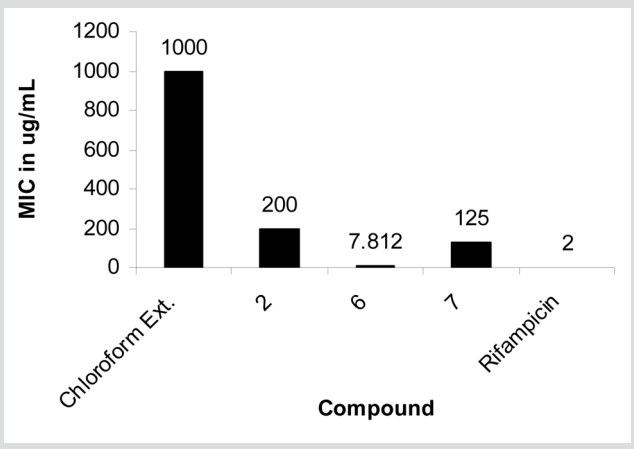


Fig. 2 Determination of MIC (μg/mL) of extract and active constituents against *M. tuberculosis* H_{37}Rv strain with the BACTEC assay.

But no further studies have been undertaken thereafter to identify the molecule responsible. Thus, the present article is the first report on antimycobacterial activity of demethoxycurcumin **2**. The curcuminoid content was determined in chloroform, acetonitrile, ethanol and ethyl acetate extracts of one and two year old rhizomes of *C. longa* using analytical HPLC. It was observed that the concentrations of **1**, **2** and **3** were higher in the two year old rhizomes (chloroform extract – 118%, acetonitrile extract – 24%, ethanol extract – 54% and ethylacetate extract – 37% higher) as compared to the corresponding one year old rhizome extracts. The purities of isolated compounds **1**, **2** and **3** were found to be 99.4%, 98.8% and 98.6%, respectively.

In conclusion, the present study provides scientific support for the traditional use of *C. longa* against various infections and fever. In some previous reports, the antimycobacterial activity of *C. longa* was reported at extract level only. Now, demethoxycurcumin has been found as a lead molecule for antitubercular activity. Two of its semisynthetic derivatives possessed better activity. Its structure-activity relationship (SAR) is under study, which may further support optimisation of a better antitubercular drug candidate in the future.

Materials and Methods

The rhizomes of *C. longa* (family: Zingiberaceae) were collected from CIMAP research farm, Lucknow (Accession No. CIMAP-1554) in October, 2006. The plant was authenticated by a CIMAP taxonomist. The rhizomes were dried at room temperature (25 – 35 °C), pulverised and stored at 10 – 15 °C until extraction. *Mycobacterium tuberculosis* H_{37}Rv (ATCC 27294) culture was obtained from National JALMA Institute for Leprosy and other Mycobacterial Diseases, Agra, India.

Ground rhizomes (145 g) were extracted with chloroform (1000 mL) after defatting with hexane in a Soxhlet apparatus to get 5.22 g of a residue. The residue (5.1 g) was chromatographed on silica gel (180 g, 60 – 120 mesh, 5 × 70 cm glass) and was eluted with increasing polarity mixtures of hexane-chloroform and chloroform-acetone to get curcumin **1**, demethoxycurcumin **2** and bisdemethoxycurcumin **3** in 0.69%, 0.09% and 0.17% yields. The isolated compounds **1**, **2** and **3** were characterised by spectroscopy (see Supporting Information for spectra) [11], [12].

Quantification of **1**, **2** and **3** in the rhizome extracts was done by reverse phase HPLC (Waters) using a C-18 column (Symmetry;

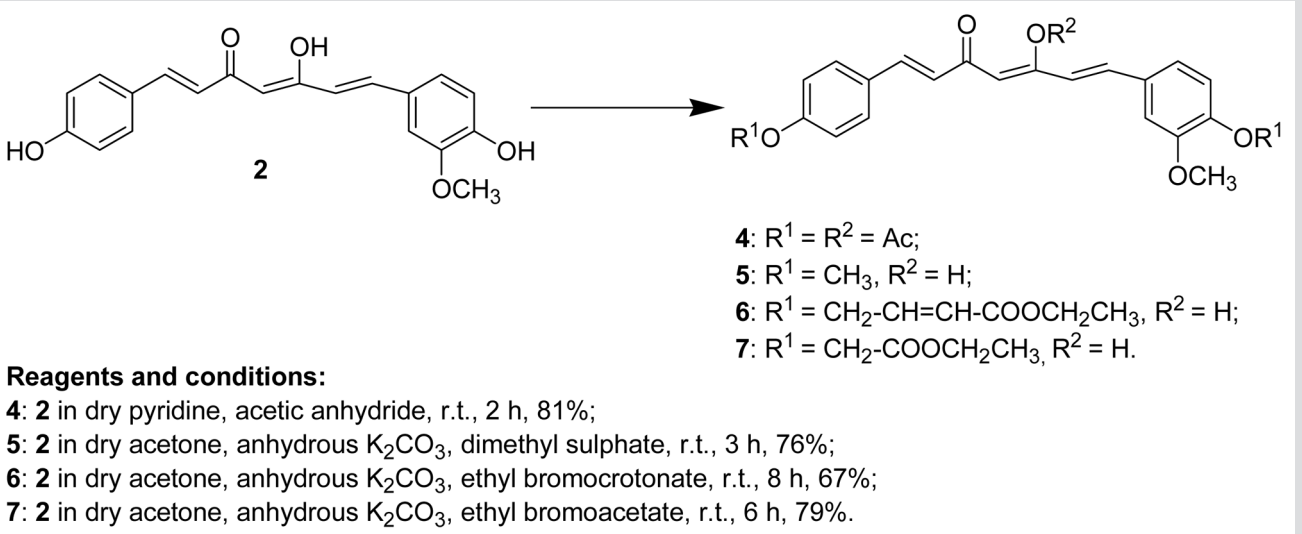


Fig. 3 Modification of **2** into **4**, **5**, **6** and **7**.

Table 2 Physical and spectral data of semisynthetic derivatives **4** – **7** of demethoxycurcumin.

Compd. No.	State	Yield (%)	^{1}H NMR (300 MHz, $CDCl_3$)	ESI Mass (MeOH)
4	Oil	81	δ 2.31 (s, 6H, 2×OAc), 2.45 (s, 3H, OAc), 3.87 (s, 3H, OCH_3), 6.23 (s, 1H, CO-CH = C-OH), 6.68 – 6.73 (d, 2H, = CH-CO-, J = 15.3 Hz), 6.76 – 6.82 (m, 4H, aromatic protons), 7.03 – 7.07 (m, 1H, aromatic proton), 7.52 – 7.59 (m, 2H, aromatic protons), 8.08 – 8.13 (d, 2H, CH = C-CO-, J = 15.9 Hz).	Positive mode: 487.0 $[M + Na]^+$, 502.9 $[M + K]^+$; Negative mode: 462.9 $[M - 1]^-$.
5	Oil	76	δ 3.76 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 5.80 (s, 1H, CO-CH = C-OH), 6.47 – 6.52 (d, 2H, = CH-CO-, J = 15.9 Hz), 6.86 – 6.89 (d, 2H, aromatic protons, J = 8.4 Hz), 6.90 – 6.93 (d, 2H, aromatic protons, J = 8.7 Hz), 7.07 (d, 1H, aromatic proton, J = 1.5 Hz), 7.13 – 7.15 (d, 1H, aromatic proton, J = 8.4 Hz), 7.49 – 7.54 (d, 2H, CH = C-CO, J = 15.9 Hz), 7.58 – 7.60 (d, 1H, aromatic proton, J = 6.0 Hz), 7.63 – 7.65 (d, 1H, aromatic proton, J = 6.0 Hz).	367.1 $[M + Na]^+$.
6	Oil	67	δ 0.95 – 0.97 (t, 6H, 2×CH_3), 3.81 (s, 3H, OCH_3), 4.1 (distorted quartet, 4H, OCH_2-C), 4.68 (bd, 2H, OCH_2-C =), 5.68 (s, 1H, CO-CH = C-OH), 6.03 – 6.08 (d, 2H, 2× = CH-CO, J = 12.9 Hz), 6.35 – 6.41 (d, 2H, 2× = CH-CO, J = 15.9 Hz), 6.65 – 6.67 (d, 1H, aromatic proton, J = 6.0 Hz), 6.97 (bs, 1H, aromatic proton), 7.05 – 7.08 (bs, 2H, 2×CH =), 7.37 – 7.40 (d, 2H, aromatic protons, J = 8.4 Hz), 7.49 – 7.52 (d, 2H, aromatic protons, J = 7.5 Hz), 7.4 – 7.7 (d, 1H, aromatic proton)), 7.69 – 7.72 (d, 2H, CH = C-CO-, J = 15.9 Hz).	563.0 $[M + H]^+$, 585.1 $[M + Na]^+$; Negative ESI: 560.8 $[M - 1]^-$.
7	Oil	79	δ 1.31 – 1.34 (bt, 6H, 2×CH_3), 3.96 (s, 3H, OCH_3), 4.25 – 4.31 (bq, 4H, 2×OCH_2-C), 4.68 (s, 2H, OCH_2), 4.74 (s, 2H, OCH_2), 5.82 (s, 1H, CO-CH = C-OH), 6.50 – 6.55 (d, 2H, 2× = CH-CO-, J = 15.6 Hz), 6.80 – 6.83 (d, 1H, aromatic proton, J = 8.7 Hz), 6.93 – 6.96 (d, 2H, aromatic proton, J = 8.7 Hz), 7.12 – 7.13 (m, 3H, aromatic protons), 7.51 – 7.54 (d, 1H, aromatic protons, J = 8.7 Hz), 7.58 – 7.63 (d, 2H, CH = C-CO-, J = 15.9 Hz).	511.1 $[M + H]^+$, 533.1 $[M + Na]^+$.

250×4.6 mm) and acetonitrile (5% THF): 0.1 M $NaHPO_4$ (pH 3.8) = 57 : 43 as mobile phase (buffer) with a flow rate of 1.0 mL/min. Data acquisition was done at 425 nm. Retention times (t_R) of **1**, **2** and **3** were 13.65, 12.25 and 10.98 min, respectively.

Demethoxycurcumin **2** was modified into triacetate **4**, dimethoxy ether **5** and also to two novel lipophilic analogues, i.e., 4-{4-[7-(3-methoxy-4-methylphenyl)-3,5-dioxohepta-1,6-dienyl]-phenoxy}-but-2-enoic acid ethyl ester **6** and {4-[7-(4-ethoxycarbonylmethoxy-3-methoxyphenyl)-3,5-dioxohepta-1,6-dienyl]-phenoxy}-acetic acid ethyl ester **7** at both its phenolic positions (**Fig. 3**) [13]. The structures of **4** – **7** were confirmed by spectroscopy (**Table 2**). All compounds **1** – **7** were evaluated for *in vitro* antitubercular activity with the BACTEC 460 Radiometric Susceptibility Assay (a detailed protocol is provided in the Supporting Information) against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC 27294). Rifampicin (Sigma Biochemicals; 95% pure) was used as positive control.

Supporting information

^{1}H-, ^{13}C-NMR and mass spectra of compound **1** – **3** and a detailed bioassay description are available as Supporting Information.

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Correspondence
Dr. Arvind Singh Negi
Analytical Chemistry Division
Central Institute of Medicinal and Aromatic Plants (CIMAP-CSIR)
Kukrail Picnic Spot Road
P. O. CIMAP
Lucknow – 226015
India
Phone: +91-522-2342676, Ext. 327
Fax: +91-522-2342666
arvindcimap@rediffmail.com