Demethoxycurcumin and its Semisynthetic Analogues as Antitubercular Agents

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Abstract

Demethoxycurcumin, isolated from the rhizomes of *Curcuma longa*, was found to possess antitubercular activity against *Mycobacterium tuberculosis* H37Rv 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

H37Rv: Mycobacterium tuberculosis H37Rv strain
MIC: minimum inhibitory concentration

Supporting information available online at http://www.thieme-connect.de/ejournals/toc/plantamedica

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 H37Rv 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-
different 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 1H-NMR spectrum, compound 6 showed a broad doublet at δ 4.68 (OCH2×2), a doublet at δ 6.05 (= CH×2), a multiplet at δ 6.35 (= CH×2), a triplet at δ 0.96 (= CH×2) and a distorted quartet at δ 4.1 (OCH2×2) indicating attachment of two –OCH2–CH= CH2 δ doublets at M. tuberculosis ter antitubercular activity due to the lipophilic nature of the phenolic hydroxy groups. Earlier studies, [3], [4], [5], [6] found 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 H37Rv. Schraufstatter and Bernt [10] reported curcumin’s antimycobacterial activity at 1:10000 dilution.

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: Zingiberaeae) 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 H37Rv (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 x 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;
250×4.6 mm) and acetonitrile (5% THF): 0.1 M NaHPO₄ (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 (tR) 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-dioxohepta-1,6-di-ethyl]-phenoxy]-but-2-enic acid ethyl ester 6 and 4-[7-(4-ethoxy-carbonylmethoxy-3-methoxyphenyl)-3,5-dioxohepta-1,6-di-phenyl]-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 Radio- metric Susceptibility Assay (a detailed protocol is provided in the Supporting Information) against Mycobacterium tuberculosis H₃₇Rv (ATCC 27294). Rifampicin (Sigma Biochemicals; 95% pure) was used as positive control.

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

<table>
<thead>
<tr>
<th>Compd. No.</th>
<th>State</th>
<th>Yield (%)</th>
<th>¹H NMR (300 MHz, CDCl₃)</th>
<th>ESI Mass (MeOH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Oil</td>
<td>81</td>
<td>δ 2.31 (s, 6H, 2×OAc), 2.45 (s, 3H, OAc), 3.87 (s, 3H, OCH₃), 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 = CCO–, J = 15.9 Hz).</td>
<td>Positive mode: 487.0 [M + Na]⁺, 502.9 [M + K]⁺; Negative mode: 462.9 [M – 1]⁻.</td>
</tr>
<tr>
<td>5</td>
<td>Oil</td>
<td>76</td>
<td>δ 3.76 (s, 3H, OCH₃), 3.85 (s, 3H, OCH₃), 3.96 (s, 3H, OCH₃), 5.80 (s, 1H, CO-CH = CO–, 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 proton, 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 = CCO–, 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).</td>
<td>367.1 [M + Na]⁺.</td>
</tr>
<tr>
<td>6</td>
<td>Oil</td>
<td>67</td>
<td>δ 0.95 – 0.97 (t, 6H, 2×CH₃), 3.81 (s, 3H, OCH₃), 4.1 (distorted quartet, 4H, OCH₂-C), 4.68 (bd, 2H, OCH₂-C =), 5.68 (s, 1H, CO-CH = CO–, 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 = CCO–, J = 15.9 Hz).</td>
<td>563.0 [M + H]⁺, 585.1 [M + Na]⁺; Negative ESI: 560.8 [M – 1]⁻.</td>
</tr>
<tr>
<td>7</td>
<td>Oil</td>
<td>79</td>
<td>δ 1.31 – 1.34 (bt, 6H, 2×CH₃), 3.96 (s, 3H, OCH₃), 4.25 – 4.31 (bq, 4H, 2×OCH₂-C), 4.68 (bs, 2H, OCH₂-C =), 4.74 (s, 2H, OCH₂), 5.82 (s, 1H, CO-CH = CO–, 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 = CCO–, J = 15.9 Hz).</td>
<td>511.1 [M + H]⁺, 533.1 [M + Na]⁺.</td>
</tr>
</tbody>
</table>
Supporting information

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

Acknowledgements

The authors are thankful to the Director, National JALMA Institute for Leprosy and other Mycobacterial Diseases (ICMR), Agra, India for providing the mycobacterial strain. DKA is grateful to the Director, Dr. K. N. M. I. P. E. R. Modinagar for her support and guidance. The authors are also thankful to Dr. Tanveer Khadija, Principal, CCG Degree College, Lucknow for improving the language of this paper. The financial support from the Council of Scientific and Industrial Research (CSIR), India is duly acknowledged.

References


received April 24, 2008
revised August 28, 2008
accepted August 31, 2008

Bibliography

© Georg Thieme Verlag KG Stuttgart · New York
Published online November 7, 2008
ISSN 0032-0943

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