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Chemical Transformation and Biological Studies of Marine Sesquiterpene (S)-(+)-Curcuphenol and Its Analogs

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Abstract

Chemical transformation studies of the marine sesquiterpene phenol (S)-(+)-curcuphenol (**1**) isolated from the Jamaican sponges, *Didiscus oxeata* and *Myrmekioderma styx*, were accomplished. In order to optimize the activity and better understand the SAR of (S)-(+)-curcuphenol, nineteen semisynthetic analogs were prepared and evaluated for activity against infectious diseases. A number of analogs showed significant activity against Mtb and *Leishmania donovani*, while showing good to moderate activities in antibacterial and antifungal assays as well as against *P. falciparum* (D6 clone) and (W2 clone). The analogs **a**, **c**, **h**, and **r** exhibited Mtb activity with MICs of 24.6, 41.2, 6.90, and 50.5 μ M, respectively. Analog **f** shows enhanced activity against *L. donovani* with an IC₅₀ of 0.6 μ M and IC₉₀ of 40 μ M respectively.

Keywords

Curcuphenol; Marine Natural Products; Leishmania; Malaria

1. Introduction

(S)-(+)-Curcuphenol (**1**) is a biologically active sesquiterpene phenol that has been isolated from the marine sponges *Didiscus oxeata*, *D. flavus*, *Myrmekioderma styx*, and *Epipolasis sp.* [1,2,3,4]. (S)-(+)-Curcuphenol has shown activity against several human cancer cell lines (A-549, HCT-8, and MDAMB) with MIC's of 0.1-10 μ g/mL [3]. A recent investigation showed **1** to have cytotoxicity against cancer cell lines in a manner independent of a p53 mechanism [18]. Additionally, (S)-(+)-curcuphenol inhibited activity of gastric H⁺, K⁺-ATPase by 50% at concentrations of 8.3 μ M and 23 μ M, respectively [4]. Both (+) and (-)-curcuphenol have been reported to have antimicrobial activity against *Staphylococcus aureus* [1,5]. Furthermore, (S)-(+)-curcuphenol has shown activity against *Candida albicans*, [1,3] *Cryptococcus neoformans* [1], and methicillin-resistant *Staphylococcus aureus* [1]. (S)-(+)-Curcuphenol and the closely related (S)-(+)-15-hydroxycurcuphenol (**2**) also display in vitro antimalarial activity against *Plasmodium falciparum* (D6 clone) and *P. falciparum* (W2 clone) [1]. The enantiomer, (R)-(-)-curcuphenol, has been isolated from the gorgonian soft coral *Pseudopterogorgia rigida* [5] and the terrestrial plant *Lasiantha podocephala* [6,7] and has

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Supporting Information: Supporting information containing mass spectral data and NMR is available upon request.

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shown activity against *S. aureus* and *Vibrio anguillarum* [5]. (R)-(-)-curcuminone (**3**) and (R)-(-)-curcuhydroquinone (**4**) have also displayed activity against *S. aureus* and *V. anguillarum*. The closely related compound xanthorrhizol (**5**) has shown protective effects against cisplatin induced cytotoxicity and eliminated any cisplatin-induced DNA-binding activity of NF- κ B [8]. Additionally, xanthorrhizol (**5**) has reported activity against *C. albicans*, toxicity to *Artemia salina*, and cytotoxicity against a human nasopharyngeal carcinoma cell line (KB) [9]. An enhancement of the antituberculosis activity of **1** was observed during administration with cyanthiwigin B (**6**) [2]. A recent study by Takamatsu *et. al.* found **1** to have strong antioxidant activity but concluded that it may not enter into cells due to poor cellular uptake or reduced medium solubility [10]. Total enantioselective synthesis of curcumin and closely related compounds has been accomplished by numerous groups [11-17].

Semisynthetic studies of natural products have been a reliable method for the generation of more active and less toxic derivatives and establishment of structure-activity relationships (SAR). In an attempt to improve the activity and understand the SAR of (S)-(+)-curcumin (**1**), we report herein the chemical modification of **1** at the C1 position (Scheme 1). The twenty analogs reported here were evaluated for biological activity against several infectious diseases. The results from these assays can be seen in Tables 2-5.

The esterification of curcumin with a variety of carboxylic acids in the presence of N,N'-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) produced twenty analogs in yields greater than 90%. In all cases, the reactions proceeded efficiently at ambient temperatures and were complete within 20-24 hours. Reactions using dichloromethane as a solvent yielded the best results. Esterification produced a number of cyclic and heterocyclic ester and the results are presented in Table 1. This method was effective for coupling of both simple as well as complex carboxylic acids, and it is noteworthy, that two of the carboxylic acids used in the coupling of curcumin are clinically used drugs. Diflunisal (used in formation of **l**) is a nonsteroidal analgesic, anti-inflammatory, and antipyretic drug. Chlorambucil (used in formation of **m**) is chemotherapeutic agent that is given as a treatment for some types of cancer.

2. Materials and methods

2.1 General Procedure

(S)-(+)-Curcumin (**1**) (0.75 mmol) was dissolved in dry methylene chloride (1.5 mL). To this solution, the carboxylic acid (0.75 mmol) and DMAP (catalytic amount) was added and stirred for about 5 minutes followed by the addition of DCC (0.75 mmol). The reaction was stirred at room temperature and the reaction progress was monitored using thin layer chromatography (TLC). The reaction was complete after 24 hours. The reaction mixture was filtered and evaporated. The residue was fractionated on silica gel prep TLC GF₂₅₄ 2000 μ m, using hexane:ethyl acetate (80:20). This procedure was applied to different carboxylic acids to afford the resulting products as oils or amorphous solids with 91-98% yields. The efficiency, operational simplicity, and high yields make this method useful for the coupling of this natural product phenol with commercially available carboxylic acids.

All products were characterized by ¹H NMR, ¹³C NMR, and mass spectral data. 1D and 2D NMR spectra were recorded using a Bruker Avance DRX-400 spectrometer. Chemical shifts, with δ values expressed in parts per million (ppm), are referenced to the residual solvent signals with resonances at $\delta_{\text{H}}/\delta_{\text{C}}$ 7.26/77.0 (CDCl₃). Liquid chromatography mass spectra (LCMS) was recorded on a Waters Micromass ZQ or a Waters Acquity UPLC in APCI mode. TLC was performed on aluminum sheets (Si gel 60 F₂₅₄, Merck KGaA, Germany) with a developing system of acetone-hexane (80:20).

Caution should be used in handling this class of compounds. One of the investigators became sensitized with a dermal skin allergy resembling an urushiol allergy during the course of this investigation.

2.2 Molecular Modeling

Molecular modeling studies were performed on a Silicon Graphics Octane2 workstation. The molecular structure was constructed using standard geometries and standard bond lengths with Sybyl 6.8 and was manipulated using standard Tripos force. The initial conformations of the molecule were obtained from 10 rounds of simulated annealing experiments. In each round of simulation, the molecule was heated to 500K within 500fs and then allowed to cool down to 200K within 5000fs. The ten optimal energy conformations were selected from the 1200 conformations generated and were minimized using Powell's method, MMFF94 force field and partial charges, until a root-mean-square deviation 0.001 kcal/mol/Å was achieved. A distance-dependent dielectric of 1.00 was used throughout the calculation. Subsequent minimization using conjugate gradient and BFGS algorithms were used to arrive at the final minimized structures. Finally, from these refined conformations, the conformers with the lowest total energy were selected for the final molecular conformation.

2.3 In Vitro Assays

Compounds were screened for antileishmanial [19], antimalarial [20], antituberculosis [21], and anti-HIV-1 [22] activity and assays were performed according to previously published procedures.

3. Results and Discussion

3.1 Anti-HIV-1

Isoquinoline derivatives have previously been patented for treatment of HIV-1 through inhibition of HIV-1 reverse transcriptase [23], as HIV protease inhibitors [24], and even as viral entry inhibitors against HIV [25]. Additionally, indole alkaloids have been patented as HIV-1 reverse transcriptase inhibitors [26] and indole substitution has been reported to dramatically increase anti-HIV-1 activity [27]. It is therefore not surprising that analogs **a**, **c**, **j**, and **r** showed activity (IC₅₀ 31.2, 29.2, 18.4, and 18.2 μM, respectively) against HIV-1 due to the presence of these moiety in the substituent. Alkylation of the indole nitrogen has been previously published to increase anti-HIV-1 activity [28].

3.2 Anti-tuberculosis activity

Isoquinoline has been included in a recent patent for *Mycobacterium* serine/threonine kinases as a tuberculosis drug target [29] and quinoline derivatives as mycobacterial inhibitors [30]. Indole derivative activity against *M. tuberculosis* have also previously been reported [31,32]. Analog **a**, **b**, **c**, and **h** have shown moderate activity (MIC 24.6, 33.9, 41.2, and 6.90 μM, respectively) in our *M. tuberculosis* screening assays. This reinforces previous data suggesting a positive correlation between quinoline and indole substituents and an increase in mycobacterial inhibitory activity.

3.3 Anti-malarial activity

Curcuphenol has no antimalarial activity against the chloroquine-sensitive D6 clone of *Plasmodium falciparum* but is active against the chloroquine resistant W2 clone with an IC₅₀ of 16.5 μM. No clear SAR is apparent for the increase in activity against the D6 clone found for analogs **p** and **r** (IC₅₀ 6.8, and 5.1 μM, respectively). An increase in activity was observed for the W2 clone in analogs **a**, **d**, **e**, **h**, **i**, **n**, **p**, **q** and **r** (IC₅₀ 11.5, 4.9, 12.9, 8.7, 1.6, 1.6, 3.4, 10.8, and 3.7 μM, respectively), however no SAR was clearly apparent.

3.4 Anti-leishmania activity

Curcuphenol shows anti-leishmania activity (IC_{50} 11 μ M) close to that of pentamidine and amphotericin B (IC_{50} 4.7 and 1.2 μ M, respectively). Analogs **d**, **f** and **h** have shown increased anti-leishmania activity with IC_{50} values of 4.7, 0.6 and 9.2 μ M, respectively. The activity of **f** is of particular interest because of in vitro activity better than the previously mentioned drugs approved for treatment of leishmaniasis.

3.5 Anti-microbial activity

Curcuphenol has reported activity against a number of microbial targets. The generated derivatives were tested against a panel of microbes with the results being reported in Table 3. Slight increases in activity were seen against *Pseudomonas aeruginosa* (**e**) and for several derivatives against *Mycobacterium intracellulare* (**a**, **d**, **e**, **f**, **h**, **i**, **l**, **o**, **p**). However, dramatic increases against microbial targets were not observed.

3.6 Anti-tumor

The derivatives were screened against Leiomyosarcoma tumor cells (ST-12X). Curcuphenol shows only slight in vitro tumor inhibition (2.6%) at a concentration of 1 μ g/mL. Analogs **a-n** were also evaluated for their cytotoxic activity at concentrations of 1 μ g/mL and 10 μ g/mL. Analogs **m** and **n** were the most promising with an increase to 28.7% and 20.0% in vitro tumor inhibition at 1 μ g/mL, respectively. Analogs **b**, **d**, **h**, and **i** also showed mild increases in anti-tumor activity at 1 μ g/mL (11.2%, 4.4%, 7.6%, and 3.8%, respectively). Many of the screened derivatives showed dramatic increases at concentrations of 10 μ g/mL with analogs **a-j** and **n** showing greater than 95% tumor inhibition.

3.7 Contact Dermatitis

As previously mentioned, one of the researchers became acutely sensitized during the course of investigation of this class of compounds. A recent study indicates a novel mechanism of contact allergy to urushiol analogs through hydroperoxidation of side chain unsaturations by lipoxygenase [33] to cause polymerization. However, internal cyclization to form an eight membered ring may also have validity because of the biological alleopathic activity of the structurally related (+)-heliannuol A (**7**) [34,35] in terrestrial plant. This would give biological significance to related naturally occurring urushiols and sesquiterpenes previously found to cause contact dermatitis

3.8 Molecular Modeling

We report here the minimized energy conformer of curcuphenol with the active curcuphenol derivative compound **f**. Comparison of the conformers shows a change in the lowest energy conformation due to steric hindrance within compound **f**. The change in the overall lowest energy conformation and the relative three dimensional space occupied may have contributed to the observed increase in activity.

4. Conclusions

Recent investigations into curcuphenol and related compounds have yielded a variety of activity profiles and stereoselective strategies for its synthesis. Our study has reported derivatives of curcuphenol with increased activity against tuberculosis, anti-HIV-1, leishmania, and cancer. The most impressive of these activities is that of compound **f** with activity against leishmania almost a log order better than pentamidine. In addition, we report the lowest energy conformation for curcuphenol and the subsequently highly active anti-leishmania derivative, compound **f**.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Figure 2.
Minimized Lowest Energy Conformation of Curcuphenol

Figure 3.
Minimized Lowest Energy Conformation of Compound **f**.

Scheme 1.

Table 1
Coupling of curcuphenol with various carboxylic acids

Entry	Product	Yield (%)	Entry	Product	Yield (%)
a		95	m		98
b		91	n		95
c		96	o		97
d		95	p		98
e		96	q		91
f		96	r		95
h		95	s		98
i		92	t		97
j		96	u		92

Entry	Product	Yield (%)	Entry	Product	Yield (%)
1		97			

Table 2

Anti-TB, anti-malarial, anti-HIV-1 and anti-leishmania data

Entry	<i>Mycobacterium tuberculosis</i> (H37Rv) μ M	Activity in vitro					Cyto-toxicity (Vero) TC ₅₀ (ng/ml)	
		<i>P. falciparum</i> (chloroquine-resistant W2 clone) IC ₅₀ μ M	<i>P. falciparum</i> (D6 clone) IC ₅₀ μ M	Anti-HIV-1		<i>Leishmania donovani</i>		
				EC ₅₀ /EC ₉₀ μ M	IC ₅₀ μ M	IC ₅₀ μ M		IC ₉₀ μ M
l	587	16.5	NA	NA	11	60	NC	
a	24.6	11.5	NA	31.2/54.0	16	NA	NC	
b	33.9	NA	NA	42.5/84.4	18	NA	NC	
c	41.2	NA	NA	29.2/48.6	12	43	NC	
d	81.5	4.9	NA	35.5/65.4	4.7	12	NC	
e	91.7	12.9	NA	40.4/78.6	11	130	NC	
f	171	NA	NA	43.1/87.6	0.6	40	NC	
h	6.90	8.7	NA	36.2/66.6	9.2	33	NC	
i	388	1.6	NA	41.5/82.5	14	30	NC	
j	70.7	NA	NA	18.4/80.9	14	NA	NC	
l	NA	NA	NA	NT	69	NA	NC	
m	NA	NA	NA	NT	46	NA	NC	
n	191	1.6	NA	NT	15	37	NC	
o	NA	NA	NA	NA/NA	12	55	NC	
p	NA	3.4	6.8	NA/NA	12	39	NC	
q	NA	10.8	NA	NA/NA	17	54	NC	
r	50.5	3.7	5.1	18.2/61.7	17	51	NC	
s	NA	NA	NA	5.50/NA	NA	NA	NC	
t	NA	NA	NA	50.6/NA	13	NA	NC	
u	NA	NA	NA	NA/NA	13	NA	NC	
Rifampin	0.6	NT	NT	NT	NT	NT	NT	
Pentamidine	NT	NT	NT	NT	4.7	10	NT	
Amphotericin B	NT	NT	NT	NT	1.2	2.5	NT	
Chloroquine	NT	0.4	0.05	NT	NT	NT	NT	
AZI³²	NT	NT	NT	0.004/NT	NT	NT	NT	

Minimum inhibitor concentration (MIC) were considered not active (NA) at sample concentrations above 128 μ g/mL. IC₅₀ and IC₉₀ are the sample concentrations that kill 50% and 90% cells compared to the solvent controls and were considered not active (NA) if activity was not observed at the concentrations tested. For malarial assays, screens were run at 4760, 1587, and 528.8 ng/mL. For Leishmania assays, screens were run at 50, 12.5 and 3.125 μ g/mL. For Anti-HIV-1, samples were considered not active (NA) above 100 μ M. NT: Not Tested; NC: Not Cytotoxic (4.7 μ g/mL);

Table 3

Anti-microbial activity data

IC 50							
Entry	<i>Candida Albicans</i> μ M	<i>Cryptococcus neoformans</i> μ M	<i>Staphylococcus aureus</i> μ M	Methicillin resistant <i>Staphylococcus</i> μ M	<i>Pseudomona aeruginosa</i> μ M	<i>Mycobacteriu intracellular</i> μ M	<i>Aspergillu fumigatus</i> μ M
1	36	44	36	34	NA	NA	130
a	NA	NA	NA	NA	NA	130	NA
b	NA	NA	NA	NA	NA	NA	NA
c	NA	NA	NA	NA	NA	NA	NA
d	NA	NA	96	83	NA	96	NA
e	NA	NA	74	88	59	59	NA
f	154	NA	62	140	NA	62	NA
h	NA	NA	NA	NA	NA	26	NA
i	NA	NA	61	61	NA	76	150
j	NA	NA	NA	NA	NA	NA	NA
l	55	55	55	44	NA	89	NA
m	NA	NA	NA	NA	NA	NA	NA
n	NA	NA	NA	NA	NA	NA	NA
o	NA	NA	NA	NA	NA	130	NA
p	NA	NA	NA	NA	NA	71	NA
q	NA	NA	NA	NA	NA	NA	NA
r	NA	NA	NA	NA	NA	NA	NA
s	NA	NA	NA	NA	NA	NA	NA
t	NA	NA	NA	NA	NA	NA	NA
u	NA	NA	NA	NA	NA	NA	NA
Amphotericin B	0.3	0.6	NT	NT	NT	NT	NT
Ciprofloxacin	NT	NT	0.3	0.3	0.3	0.9	NT

IC50 is the concentration (μ M) that affords 50% inhibition of growth. Samples which were considered not active (NA) did not show activity at the concentrations tested. The screens were run at concentrations of 50, 10, and 2 μ g/mL. NA: Not Active.

Table 4

Anti-tumor activity against primary human tumor cells.

Entry	Mean % Inhibition	
	1 µg/mL	10 µg/mL
l	2.6	-
a	-11.0	99.2
b	11.2	97.8
c	-3.8	101.4
d	4.4	100.7
e	-16.0	100.6
f	1.8	100.6
h	7.6	100.3
i	3.8	100.6
j	-8.4	99.5
l	2.4	7.2
m	28.7	31.1
n	20.0	103.7