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Original article

Depsides as non-redox inhibitors of leukotriene B_4 biosynthesis and HaCaT cell growth. 1. Novel analogues of barbatic and diffractaic acid

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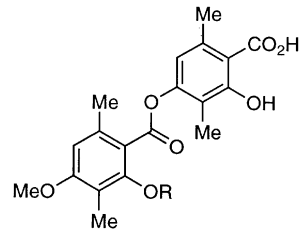
Abstract – A series of barbatic and diffractaic acid analogues has been synthesized and evaluated as inhibitors of leukotriene B_4 (LTB_4) biosynthesis and as antiproliferative agents. The 4-*O*-demethyl barbatic and diffractaic acid derivatives were among the most active compounds in both assays. In particular, ethyl 4-*O*-demethylbarbatate was the most potent LTB_4 biosynthesis inhibitor of this series, with an IC_{50} value in the submicromolar range. Because the compounds did not show appreciable reactivity against a stable free radical, 2,2-diphenyl-1-picrylhydrazyl, and did not produce appreciable amounts of deoxyribose degradation as a measure of their potency to generate hydroxyl radicals, a simple redox effect could not explain their biological activity. Also, there was no nonspecific cytotoxicity as documented by the activity of lactate dehydrogenase released from the cytoplasm of keratinocytes, which was in the control range. © 1999 Éditions scientifiques et médicales Elsevier SAS

barbatic acid / diffractaic acid / antiproliferative activity / keratinocytes / lactate dehydrogenase release / leukotriene biosynthesis

1. Introduction

Depsides are a distinct class of lichen-derived compounds which are formed by condensation of two or more hydroxybenzoic acids whereby the carboxyl group of one molecule is esterified with a phenolic hydroxyl group of a second molecule. One of the most common secondary metabolites of many lichen species [1] is the didepside diffractaic acid (**1**, *figure 1*). This compound has been shown by several groups to exhibit antiviral [2], antitumour [3], analgesic and antipyretic [4] properties. Among several structurally dissimilar lichen-derived metabolites isolated from *Parmelia* species, we have identified **1** as a non-redox inhibitor of the biosynthesis of leukotriene B_4 (LTB_4) in bovine polymorphonuclear leukocytes (PMNL) [5]. Leukotrienes are derived from the biotransformation of arachidonic acid through the action of 5-lipoxygenase (5-LO) and play an important role in a variety of pathophysiological states in man, particularly those involving inflammation [6]. Furthermore, we found that **1** is also a potent antiproliferative agent against the growth of human keratinocytes [7]. These combined inhibitory actions against 5-LO and keratinocyte cell growth suggested a beneficial effect against inflammatory and hyperproliferative skin diseases such as psoriasis, since both pathological features were targeted.



1 R = Me
2 R = H

Figure 1. Structures of diffractaic acid (**1**) and barbatic acid (**2**).

As part of our continuing search for agents suitable for the treatment of inflammatory and hyperproliferative skin

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diseases such as psoriasis, we have synthesized several novel analogues of **1** and its congener barbatic acid (**2**), modified at the carboxylic acid function and the 4-methoxy group in the benzoyloxy moieties, to explore the effect of increased lipophilicity and some redox properties on the biological activity of the compounds. The redox properties were evaluated in terms of reactivity against the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), in order to evaluate the antioxidant potential, and deoxyribose degradation was determined as a measure of hydroxyl radical formation [8]. The ability of the novel compounds to inhibit the growth of human keratinocytes was evaluated in HaCaT cells [9], and inhibition of LTB_4 biosynthesis was assayed in bovine polymorphonuclear leukocytes [8].

2. Chemistry

Unambiguous syntheses of the lichen depsides **1** and **2** have been reported [10]. The mononuclear precursors for the novel depsides were obtained from ethyl 2,4-dihydroxy-3,6-dimethylbenzoate (**3**) following the methodology of Elix [10] which proved to be particularly suitable for the large scale preparation of this starting material (*figure 2*). Esters **4a**–**4e** were directly obtained from **3** by transesterification in the presence of the corresponding sodium alkoxides and alcohols [10]. Alkylation of **3** with a one molar proportion of dimethyl sulfate or the pertinent benzyl chlorides in the presence of potassium carbonate, selectively yielded the corresponding 4-methoxy derivative **5** or 4-benzyloxy derivatives **9a** and **b**. Methylation of the second hydroxy group of these derivatives with dimethyl sulfate gave the 2-methoxy derivatives **6** and **10a** and **b**. Subsequent hydrolysis of the esters **5**, **6**, **9a** and **b**, and **10a** and **b** yielded the requisite acids **7**, **8**, **11a** and **b**, and **12a** and **b**, respectively, for the A-ring of the desired depsides. Depside formation between these acids and the phenolic esters **3** and **4a**–**e** was achieved by treatment with trifluoroacetic anhydride in anhydrous toluene and yielded the barbatic and diffractaic acid analogues **13a**–**f**, **14a**–**f**, **15a**–**f** and **17a**–**f**, **18a**–**f**, **19a**–**f**, respectively (*figure 3*). Hydrogenolytic cleavage of the benzyl ethers **14a**–**e** and **18a**–**e** over palladium/carbon produced the phenolic analogues **16a**–**e** and **20a**–**e**, respectively, whereas benzyl esters **14f** and **18f** were cleaved to the acids **16f** and **20f**, respectively. Analogously, benzyl esters **13f** and **17f** yielded the parent compounds **2** and **1**, respectively.

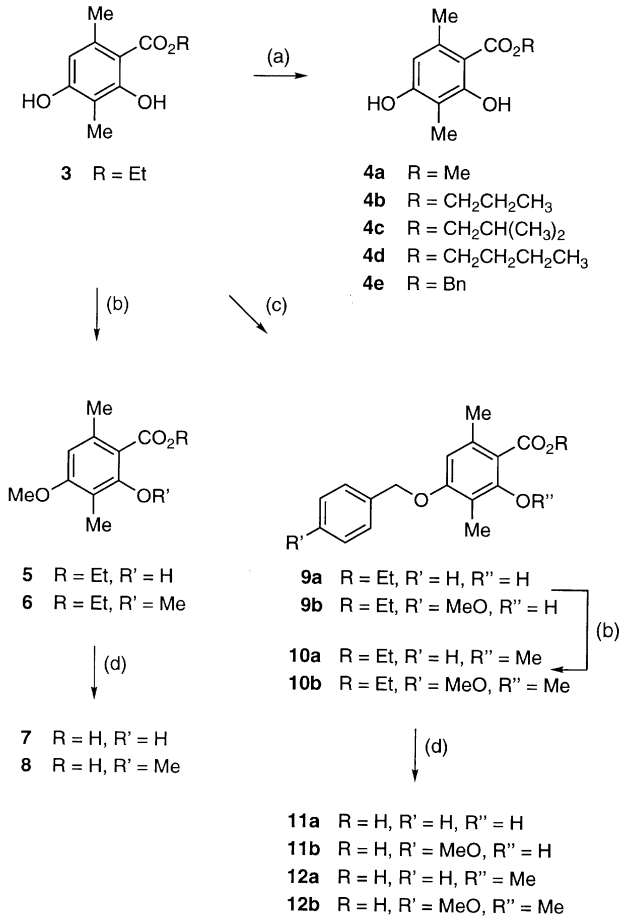


Figure 2. Reagents: (a) Na, ROH, Δ, 24 h; (b) Me_2SO_4, K_2CO_3, acetone, Δ, 24 h; (c) 4-R´$C_6H_4CH_2Cl$, K_2CO_3, acetone, Δ, 24 h; (d) KOH, H_2O, DMSO, 90 °C.

3. Biological results and discussion

Inhibition of LTB_4 biosynthesis by the barbatic and diffractaic acid analogues was determined in bovine polymorphonuclear leukocytes. As shown in *table I*, activity of barbatic acid (**2**), with an IC_{50} value of 7.8 μM, was similar to that of **1**. The biological activity was reduced when the free carboxylic acid groups were esterified. The esters **13a**–**f** of **2** were either moderately active or inactive even at concentrations up to 20 μM. Also, esterification of **1** resulted in less active or inactive compounds (**17a** and **c**–**f**), except for **17b**, where activity was retained. In general, introduction of a 4-benzyl or 4-methoxybenzyl group into **1** and **2**, as in **14a**–**f**, **15a**–**f**, **18a**–**f**, and **19a**–**f** dramatically reduced inhibitory action against LTB_4 biosynthesis. Most of these compounds were inactive at 20 μM. Exceptions were the

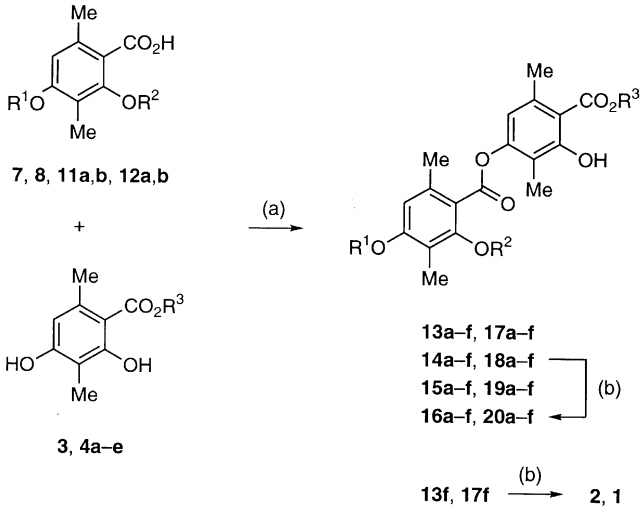


Figure 3. Reagents: (a) $(CF_3CO)_2O$, toluene, room temperature; (b) Pd/C, EtOAc, room temperature. R^1, R^2, and R^3 are defined in *table I*.

4-benzyloxydiffractates **14c**, **e** and **f** of **2** which showed comparable activity.

Since a major class of leukotriene biosynthesis inhibitors often contains a hydroxylated aromatic ring [11], we have speculated that demethylation of the 4-methoxy group of **1** and **2** might improve their activity. As expected, 4-*O*-demethyl barbatic and diffractaic acid derivatives **16a**–**f** and **20a**–**f**, respectively, inhibited LTB_4 biosynthesis with IC_{50} values in the low micromolar range. With the exception of the free acids **16f** and **20f**, these analogues approached the potency of their respective parent compounds or were even more potent than these. In particular, the ethyl esters **16b** and **20b** were the most potent inhibitors of LTB_4 biosynthesis of this series. Potency of compound **16b**, with an IC_{50} of 0.8 μM, was comparable to that of the standard inhibitor nordihydroguaiaretic acid.

One chemical feature of many inhibitors of LTB_4 biosynthesis is their ability to remove free radicals, since the conversion of arachidonic acid into LTB_4 is an oxidative process. Therefore, we have evaluated the depsides for their ability to react with the stable free radical DPPH to give the reduced 2,2-diphenyl-1-picrylhydrazine. *Table I* shows that no appreciable amount of reduced hydrazine was formed by these compounds, documenting their lack of reactivity against stable free radicals. This suggests that a simple redox effect does not explain their activity in the LTB_4 assay. Rather, activity appears to be due to specific enzyme interaction.

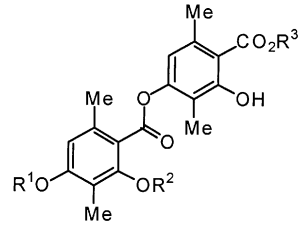
Moreover, the results obtained from the deoxyribose assay (*table I*) also suggest that hydroxyl radicals are not involved in the mechanism of enzyme inhibition by the novel depsides. The deoxyribose assay is a sensitive test for the production of hydroxyl radicals [12]. The release of 2-thiobarbituric acid reactive material is expressed as malondialdehyde (MDA) and reflects a measure for hydroxyl-radical generation. However, we did not observe any deoxyribose degradation from depsides, even for compounds **16a**–**f** with three phenolic hydroxyl groups.

In vitro antiproliferative activities were determined in 24-well culture dishes against the growth of HaCaT cells. This nontransformed human cell line can be used as a model for highly proliferative epidermis [13]. The parent compounds **1** and **2** were potent inhibitors of cell growth with IC_{50} values of 2.6 and 4.1 μM, respectively (*table I*). While the esters **13a**–**c** of **2** showed comparable or slightly improved activity, the corresponding esters **17a**–**c** of **1** were inactive. However, butyl ester **17e** displayed potent antiproliferative activity. Furthermore, among the 4-benzylated derivatives of **1** were also some antiproliferative active agents. Similar to the results obtained in the LTB_4 assay, 4-*O*-demethylation of barbatic and diffractaic acid (**16a**–**f** and **20a**–**f**, respectively) generally produced active compounds, although there were no improvements as compared to the parent depsides.

There is little or no correlation between the in vitro antiproliferative activity of the compounds and their ability to inhibit LTB_4 biosynthesis. With respect to both features, well-balanced representatives are found among the esters of diffractaic acids and the 4-*O*-demethylated analogues of **1** and **2**. Unfortunately, the potent LTB_4 biosynthesis inhibitor **20b** is inactive at 20 μM. The most potent inhibitor of keratinocyte growth, ethyl diffractate (**13b**), is also an inhibitor of LTB_4 biosynthesis. Likewise, the most potent inhibitor of LTB_4 biosynthesis, ethyl 4-*O*-demethylbarbatate (**16b**), also displays antiproliferative activity.

Keratinocytes were also tested for their susceptibility to the action of the most potent depsides on plasma membrane integrity. As a measure of cytotoxicity, release of lactate dehydrogenase into the culture medium was determined [14]. In these experiments, all potent inhibitors of keratinocyte growth showed values in the control range, documenting that their activity was due to cytostatic rather than cytotoxic effects. This may be advantageous as compared with the topical antipsoriatic agent anthralin, which is known to induce inflammation of the healthy skin surrounding a psoriatic lesion. As a result of the strong hydroxyl radical generating activity of this

Table I. Redox properties, inhibition of LTB_4 biosynthesis, antiproliferative activity and cytotoxicity against HaCaT cells of barbatic and diffractaic acid derivatives.



Compound	R^1	R^2	R^3	k_{DPPH}[a] (M^{-1} s^{-1})	DD ($^{\cdot}OH$)[b]	LTB_4[c] IC_{50} (μM)	AA[d] IC_{50} (μM)	LDH[e] (mU)
1	Me	Me	H	0.63 ± 0.01	0.12 ± 0.07	7.6	2.6	136
2	Me	H	H	0.14 ± 0.07	0.09 ± 0.01	7.8	4.1	148
13a	Me	H	Me	0.93 ± 0.01	0.16 ± 0.04	18.6	4.8	148
13b	Me	H	Et	0.31 ± 0.05	0.18 ± 0.06	11.3	1.9	143
13c	Me	H	Prop	0.29 ± 0.01	0.21 ± 0.03	10.5	3.2	143
13d	Me	H	$CHMe_2$	ND	ND	> 20	> 20	ND
13e	Me	H	Bu	ND	ND	> 20	> 20	ND
13f	Me	H	CH_2Ph	0.45 ± 0.13	0.08 ± 0.01	19.2	> 20	ND
14a	$PhCH_2$	H	Me	ND	ND	> 20	19.0	150
14b	$PhCH_2$	H	Et	ND	ND	> 20	16.7	142
14c	$PhCH_2$	H	Prop	0.55 ± 0.04	0.19 ± 0.03	9.0	> 20	ND
14d	$PhCH_2$	H	$CHMe_2$	0.78 ± 0.01	0.23 ± 0.01	16.2	> 20	ND
14e	$PhCH_2$	H	Bu	0.51 ± 0.01	0.11 ± 0.03	9.0	9.0	150
14f	$PhCH_2$	H	CH_2Ph	0.96 ± 0.01	0.26 ± 0.01	7.9	8.4	140
15a	4-$MeOPhCH_2$	H	Me	ND	ND	> 20	> 20	ND
15b	4-$MeOPhCH_2$	H	Et	ND	ND	> 20	> 20	ND
15c	4-$MeOPhCH_2$	H	Prop	ND	ND	> 20	> 20	ND
15d	4-$MeOPhCH_2$	H	$CHMe_2$	0.72 ± 0.01	0.19 ± 0.01	15.0	> 20	ND
15e	4-$MeOPhCH_2$	H	Bu	ND	ND	> 20	> 20	ND
15f	4-$MeOPhCH_2$	H	CH_2Ph	ND	ND	> 20	> 20	ND
16a	H	H	Me	0.34 ± 0.01	0.21 ± 0.02	2.1	8.2	143
16b	H	H	Et	0.52 ± 0.07	0.02 ± 0.01	0.8	8.4	149
16c	H	H	Prop	0.91 ± 0.60	0.12 ± 0.01	5.7	3.6	149
16d	H	H	$CHMe_2$	0.43 ± 0.14	0.16 ± 0.01	2.6	8.0	146
16e	H	H	Bu	0.67 ± 0.09	0.72 ± 0.01[f]	5.0	3.8	140
16f	H	H	H	0.81 ± 0.03	0.01 ± 0.01	14.0	8.2	140
17a	Me	Me	Me	0.85 ± 0.03	0.19 ± 0.01	13.2	> 20	ND
17b	Me	Me	Et	0.57 ± 0.01	0.21 ± 0.01	5.3	> 20	ND
17c	Me	Me	Prop	0.93 ± 0.01	0.06 ± 0.02	19	> 20	ND
17d	Me	Me	$CHMe_2$	ND	ND	> 20	14.0	170[f]
17e	Me	Me	Bu	ND	ND	> 20	4.1	167[f]
17f	Me	Me	CH_2Ph	ND	ND	> 20	> 20	ND
18a	$PhCH_2$	Me	Me	ND	ND	> 20	> 20	ND
18b	$PhCH_2$	Me	Et	0.97 ± 0.02	0.44 ± 0.02[f]	18.3	> 20	ND
18c	$PhCH_2$	Me	Prop	ND	ND	> 20	ND	ND
18d	$PhCH_2$	Me	$CHMe_2$	ND	ND	> 20	ND	ND
18e	$PhCH_2$	Me	Bu	ND	ND	> 20	ND	ND
18f	$PhCH_2$	Me	CH_2Ph	ND	ND	> 20	ND	ND

[a] Reducing activity against 2,2-diphenyl-1-picrylhydrazyl with equimolar amounts of test compound. [b]Deoxyribose degradation as a measure of hydroxyl-radical formation. Indicated values are μmol of malondialdehyde/mmol of deoxyribose released by 75 μM of test compound (controls < 0.1). [c]Inhibition of LTB_4 biosynthesis in bovine PMNL. Inhibition was significantly different with respect to that of the control; $n = 3$ or more, $P < 0.01$. Nordihydroguaiaretic acid was used as the standard inhibitor (IC_{50} = 0.4 μM) [8]. [d]Antiproliferative activity against HaCaT cells. Inhibition of cell growth was significantly different with respect to that of the control, $n = 3$, $P < 0.01$. [e]Activity of LDH (mU) release in HaCaT cells after treatment with 2 μM test compound ($n = 3$, SD < 10%). [f]Values are significantly different with respect to vehicle control ($P < 0.05$). ND = not determined. [g]Positive control [8].

Table I. (continued).

Compound	R^1	R^2	R^3	k_{DPPH}[a] (M^{-1} s^{-1})	DD ($^{\cdot}OH$)[b]	LTB_4[c] IC_{50} (μM)	AA[d] IC_{50} (μM)	LDH[e] (mU)
19a	4-$MeOPhCH_2$	Me	Me	ND	ND	> 20	> 20	ND
19b	4-$MeOPhCH_2$	Me	Et	ND	ND	> 20	> 20	ND
19c	4-$MeOPhCH_2$	Me	Prop	ND	ND	> 20	ND	ND
19d	4-$MeOPhCH_2$	Me	$CHMe_2$	ND	ND	> 20	ND	ND
19e	4-$MeOPhCH_2$	Me	Bu	ND	ND	> 20	ND	ND
19f	4-$MeOPhCH_2$	Me	CH_2Ph	ND	ND	> 20	ND	ND
20a	H	Me	Me	0.93 ± 0.01	0.18 ± 0.01	7.8	9.0	138
20b	H	Me	Et	0.64 ± 0.01	0.10 ± 0.02	1.4	> 20	ND
20c	H	Me	Prop	0.89 ± 0.05	0.01 ± 0.01	8.5	> 20	ND
20d	H	Me	$CHMe_2$	0.97 ± 0.01	0.09 ± 0.01	5.8	7.2	168
20e	H	Me	Bu	0.51 ± 0.01	0.16 ± 0.01	7.8	> 20	ND
20f	H	Me	H	0.24 ± 0.03	0.15 ± 0.01	11.0	9.8	114
anthralin[g]				24.2 ± 4.2[f]	2.89 ± 0.14[f]	37.0	0.7	294[f]

agent [15], LDH release by anthralin significantly exceeded that of the vehicle control.

In conclusion, barbatic acid analogues were consistently more active against the biosynthesis of LTB_4 and the growth of HaCaT keratinocytes than the corresponding diffractaic acid analogues. Though this may be related to their additional phenolic hydroxyl group, determination of the antioxidant and pro-oxidant potential of the compounds did not reveal any appreciable redox activity. Barbatic acid analogue **16b** has been identified as a potent non-redox inhibitor of LTB_4 biosynthesis which also displays antiproliferative activity against keratinocyte growth.

4. Experimental protocols

4.1. Chemistry

4.1.1. General

For analytical instruments and methods see reference [16].

Compounds **1**–**3** and **5**–**8** were prepared as described [10].

4.1.2. Propyl 2,4-dihydroxy-3,6-dimethylbenzoate **4b**

Sodium (0.5 g, 21.73 mmol) was dissolved in absolute propanol (50 mL) and stirred at room temperature. Then **3** (3 g, 14.28 mmol) was added to the solution and refluxed under nitrogen for 24 h. The solution was cooled, acidified with cold 10% HCl and extracted with ether (3 × 100 mL). The combined organic phase was dried over $MgSO_4$ and evaporated. The crude product was purified by flash chromatography (SiO_2, CH_2Cl_2) to give colourless crystals; FTIR 3 396, 2 980, 2 957, 1 630 cm^{-1}; ^{1}H-NMR (250 MHz, $CDCl_3$) δ 12.14 (s, 1H), 6.20 (s, 1H), 4.97 (s, 1H), 4.30 (t, *J* = 6.5 Hz, 2H), 2.48 (s, 3H), 2.10 (s, 3H), 1.80 (m, 2H), 1.03 (t, *J* = 7.4 Hz, 3H); Anal. ($C_{12}H_{16}O_4$) C, H.

Analogously, compounds **4a** and **c**–**e** were prepared from **3** (*table II*).

4.1.3. Ethyl 2-hydroxy-4-(4-methoxybenzyloxy)-3,6-dimethylbenzoate **9b**

A suspension of **3** (6 g, 28.57 mmol), anhydrous potassium carbonate (11.25 g, 81.39 mmol) and 4-methoxybenzylchloride (4.47 g, 28.57 mmol) in dry acetone (75 mL) was refluxed for 24 h, then cooled, acidified with cold 10% HCl and extracted with ether (3 × 200 mL). The combined organic phase was washed with water, dried over $MgSO_4$ and evaporated. The crude product was purified by column chromatography (SiO_2) using hexane/ethyl acetate (9:1) to afford colourless crystals; FTIR 3 438, 1 720, 1 636 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 11.91 (s, 1H), 7.35 (d, *J* = 9.5 Hz, 2H), 6.93 (d, *J* = 9.5 Hz, 2H), 6.34 (s, 1H), 5.03 (s, 2H), 4.38 (q, *J* = 7.1 Hz, 2H), 3.82 (s, 3H), 2.52 (s, 3H), 2.11 (s, 3H), 1.41 (t, *J* = 7.1 Hz, 3H). Anal. ($C_{19}H_{22}O_5$) C, H.

4.1.4. Ethyl 2-methoxy-4-(4-methoxybenzyloxy)-3,6-dimethylbenzoate **10b**

A suspension of **9b** (2.20 g, 6.67 mmol), anhydrous potassium carbonate (2.76 g, 20 mmol) and dimethyl sulfate (0.6 mL, 6.67 mmol) in dry acetone (50 mL) was refluxed for 24 h, then cooled, acidified with cold 10% HCl and extracted with ether (3 × 200 mL). The combined organic phase was washed with water, dried over $MgSO_4$ and evaporated. The crude product was purified by column chromatography (SiO_2) to afford a colourless

Table II. Chemical data of starting materials, barbatic and diffractaic acid derivatives.

Compound[a]	Formula[b]	M.p. (°C)	Yield (%)	Solvent[c,d] (vol%)	Anal.[e]
4a	$C_{10}H_{12}O_4$	144; ref. [18] 145	84	MC[c]	C, H
4b	$C_{12}H_{16}O_4$	135; ref. [19] 139	93	MC[c]	C, H
4c	$C_{12}H_{16}O_4$	88; ref. [19, 20] 92	62	MC[c]	C, H
4d	$C_{13}H_{18}O_4$	120; ref. [19, 20] 123	88	MC[c]	C, H
4e	$C_{16}H_{16}O_4$	118; ref. [10] 113	55	MC[c]	C, H
9a	$C_{18}H_{20}O_4$	67; ref. [10] 67–68	65	H/EA[c] (9 + 1); M[d]	C, H
9b	$C_{19}H_{22}O_5$	80	85	H/EA[c] (9 + 1); M[d]	C, H
10a	$C_{19}H_{22}O_4$	oil; ref. [17] oil	91	H/EA[c] (9 + 1)	C, H
10b	$C_{20}H_{24}O_5$	oil	86	H/EA[c] (9 + 1)	C, H
11a	$C_{16}H_{16}O_5$	167; ref. [10] 165–167	84	H/EA[c] (1 + 1)	C, H
11b	$C_{17}H_{18}O_5$	198	92	H/EA[c] (1 + 1)	C, H
12a	$C_{17}H_{18}O_4$	120; ref. [17] 122	71	H/EA[c] (1 + 1)	C, H
12b	$C_{18}H_{20}O_5$	136	76	H/EA[c] (1 + 1)	C, H
13a	$C_{20}H_{22}O_7$	166; ref. [17] 170	77	H/EA[c] (9 + 1); M/C[d]	C, H
13b	$C_{21}H_{24}O_7$	186; ref. [21] 189	71	H/EA[c] (9 + 1); M/C[d]	C, H
13c	$C_{22}H_{26}O_7$	137; ref. [20] 138–139	89	H/EA[c] (9 + 1); M/C[d]	C, H
13d	$C_{22}H_{26}O_7$	125; ref. [20] 128–129	64	H/EA[c] (9 + 1); M/C[d]	C, H
13e	$C_{23}H_{28}O_7$	134; ref. [20] 133	90	H/EA[c] (9 + 1); M/C[d]	C, H
13f	$C_{26}H_{26}O_7$	132; ref. [10] 136–138	69	H/EA[c] (9 + 1); M/C[d]	C, H
14a	$C_{26}H_{26}O_7$	137; ref. [22] 133–134	63	H/EA[c] (9 + 1); M/C[d]	C, H
14b	$C_{27}H_{28}O_7$	148	73	H/EA[c] (9 + 1); M/C[d]	C, H
14c	$C_{28}H_{30}O_7$	108	73	H/EA[c] (9 + 1); M/C[d]	C, H
14d	$C_{28}H_{30}O_7$	124	65	H/EA[c] (9 + 1); M/C[d]	C, H
14e	$C_{29}H_{32}O_7$	114	61	H/EA[c] (9 + 1); M/C[d]	C, H
14f	$C_{32}H_{30}O_7$	128; ref. [10] 131–132	78	H/EA[c] (9 + 1); M/C[d]	C, H
15a	$C_{27}H_{28}O_8$	98	69	H/EA[c] (9 + 1); M/C[d]	C, H
15b	$C_{28}H_{30}O_8$	107	73	H/EA[c] (9 + 1); M/C[d]	C, H
15c	$C_{29}H_{32}O_8$	88	65	H/EA[c] (9 + 1); M/C[d]	C, H
15d	$C_{29}H_{32}O_8$	97	68	H/EA[c] (9 + 1); M/C[d]	C, H
15e	$C_{30}H_{30}O_8$	119	63	H/EA[c] (9 + 1); M/C[d]	C, H
15f	$C_{33}H_{32}O_8$	103	71	H/EA[c] (9 + 1); M/C[d]	C, H
16a	$C_{19}H_{20}O_7$	108; ref. [23] 108–112	87	H/EA[d]	C, H
16b	$C_{20}H_{22}O_7$	142	95	H/EA[d]	C, H
16c	$C_{21}H_{24}O_7$	146	90	H/EA[d]	C, H
16d	$C_{21}H_{24}O_7$	123	90	H/EA[d]	C, H
16e	$C_{22}H_{26}O_7$	153	89	H/EA[d]	C, H
16f	$C_{18}H_{18}O_7$	172; ref. [10] 136–138	91	H/EA[d]	C, H
17a	$C_{21}H_{24}O_7$	133; ref. [24] 127–128	69	H/EA[c] (9 + 1); M/C[d]	C, H
17b	$C_{22}H_{26}O_7$	144; ref. [25] 141–144	73	H/EA[c] (9 + 1); M/C[d]	C, H
17c	$C_{23}H_{28}O_7$	126; ref. [19] 127	82	H/EA[c] (9 + 1); M/C[d]	C, H
17d	$C_{23}H_{28}O_7$	124; ref. [19] 127	66	H/EA[c] (9 + 1); M/C[d]	C, H
17e	$C_{24}H_{30}O_7$	115; ref. [19] 115	88	H/EA[c] (9 + 1); M/C[d]	C, H
17f	$C_{27}H_{28}O_7$	123; ref. [10] 119	83	H/EA[c] (9 + 1); M/C[d]	C, H
18a	$C_{27}H_{28}O_7$	124	74	H/EA[c] (9 + 1); M/C[d]	C, H
18b	$C_{28}H_{30}O_7$	124	67	H/EA[c] (9 + 1); M/C[d]	C, H
18c	$C_{29}H_{32}O_7$	141	63	H/EA[c] (9 + 1); M/C[d]	C, H
18d	$C_{29}H_{32}O_7$	113	74	H/EA[c] (9 + 1); M/C[d]	C, H
18e	$C_{30}H_{34}O_7$	142	63	H/EA[c] (9 + 1); M/C[d]	C, H
18f	$C_{33}H_{32}O_7$	118; ref. [17] 118–120	69	H/EA[c] (9 + 1); M/C[d]	C, H
19a	$C_{28}H_{30}O_8$	130	82	H/EA[c] (9 + 1); M/C[d]	C, H
19b	$C_{29}H_{32}O_8$	98	73	H/EA[c] (9 + 1); M/C[d]	C, H
19c	$C_{30}H_{34}O_8$	122	62	H/EA[c] (9 + 1); M/C[d]	C, H
19d	$C_{30}H_{34}O_8$	117	80	H/EA[c] (9 + 1); M/C[d]	C, H
19e	$C_{31}H_{32}O_8$	111	77	H/EA[c] (9 + 1); M/C[d]	C, H
19f	$C_{34}H_{34}O_8$	89	65	H/EA[c] (9 + 1); M/C[d]	C, H

Table II. (continued).

Compound[a]	Formula[b]	M.p. (°C)	Yield (%)	Solvent[c,d] (vol%)	Anal.[e]
20a	$C_{20}H_{22}O_7$	161	98	H/EA[d]	C, H
20b	$C_{21}H_{24}O_7$	170	93	H/EA[d]	C, H
20c	$C_{22}H_{26}O_7$	143	97	H/EA[d]	C, H
20d	$C_{22}H_{26}O_7$	124	94	H/EA[d]	C, H
20e	$C_{23}H_{28}O_7$	112	91	H/EA[d]	C, H
20f	$C_{19}H_{20}O_7$	203; ref. [17] 207–209	95	H/EA[d]	C, H

[a]All compounds were obtained as colourless crystals except where stated otherwise. [b]All new compounds displayed ^{1}H-NMR and FTIR consistent with the assigned structure. [c]Eluant used for column chromatography. [d]Solvent for recrystallization; C = chloroform; EA = ethyl acetate; H = hexane; M = methanol; MC = methylene chloride. [e]Elemental analyses were within ± 0.4% of calculated values except where stated otherwise.

oil; FTIR 3 423, 1 700, 1 638 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.32 (d, *J* = 9.5 Hz, 2H), 6.93 (d, *J* = 9.5 Hz, 2H), 6.53 (s, 1H), 4.98 (s, 2H), 4.39 (q, *J* = 7.1 Hz, 2H), 3.82 (s, 3H), 3.76 (s, 3H), 2.29 (s, 3H), 2.13 (s, 3H), 1.38 (t, *J* = 7.1 Hz, 3H). Anal. ($C_{20}H_{24}O_5$) C, H.

4.1.5. 2-Hydroxy-4-(4-methoxybenzyloxy)-3,6-dimethylbenzoic acid **11b**

A solution of aqueous potassium hydroxide (2.86 g in 7 mL H_2O, 51.0 mmol) was added to a solution of **9b** (3.00 g, 8.82 mmol) in DMSO (40 mL) and heated on a water bath for 2.5 h (TLC control). The solution was cooled to room temperature, diluted with excess water (100 mL), acidified with cold 10% HCl, and extracted with ether (3 × 100 mL). The combined organic phase was washed with water (3 × 200 mL), dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (SiO_2) using hexane/ethyl acetate (1:1) to afford colourless crystals; FTIR 3 053, 1 700, cm^{-1}; ^{1}H-NMR ($CDCl_3$, DMSO-d_6) δ 12.49 (s, 1H), 7.30 (d, *J* = 9.5 Hz, 2H), 6.99 (d, *J* = 9.5 Hz, 2H), 6.32 (s, 1H), 5.09 (s, 2H), 3.82 (s, 3H), 2.55 (s, 3H), 2.13 (s, 3H), Anal. ($C_{17}H_{18}O_5$) C, H.

Analogously, **12b** was prepared from **10b** (*table II*).

4.1.6. General procedure for the condensation of benzoic acids with phenolic esters

4.1.6.1. Ethyl 4-(4-benzyloxy-2-hydroxy-3,6-dimethylbenzoyloxy)-2-hydroxy-3,6-dimethylbenzoate **14b**

A solution of **11a** (136 mg, 0.5 mmol) and **3** [10] (107 mg, 0.5 mmol) in anhydrous toluene (2 mL) and trifluoroacetic anhydride (0.5 mL) was stirred at room temperature for 2.5 h (TLC control). The solvent was removed under reduced pressure and the residue was purified by column chromatography (SiO_2) using hexane/ethyl acetate (9:1). The product was recrystallized from MeOH/$CHCl_3$ to give colourless crystals; FTIR 3 430, 2 965, 2 900, 1 665, 1 618 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 11.99 (s, 1H), 11.53 (s, 1H), 7.32–7.47 (m, 5H), 6.51 (s, 1H), 6.44 (s, 1H), 5.17 (s, 2H), 4.44 (q, *J* = 7.1 Hz, 2H), 2.66 (s, 3H), 2.55 (s, 3H), 2.17 (s, 3H), 2.08 (s, 3H), 1.43 (t, *J* = 7.13 Hz, 3H); Anal. ($C_{27}H_{28}O_7$) C, H.

Analogously, **13a–f** were prepared from **7** [10] and **3** and **4a–c**; **14a** and **c–f** were prepared from **11a** [10] and **4a–c**; **15a–f** were prepared from **11b** and **3** and **4a–c**; **17a–f** were prepared from **8** [10] and **3** and **4a–c**; **18a–f** were prepared from **12a** [17] and **3** and **4a–c**; **19a–f** were prepared from **12b** and **3** and **4a–c** (*table II*).

4.1.7. General procedure for hydrogenolysis

4.1.7.1. Ethyl 4-(2,4-dihydroxy-3,6-dimethylbenzoyloxy)-2-hydroxy-3,6-dimethylbenzoate **16b**

A suspension of **14b** (112 mg, 0.25 mmol) and 10% palladium/carbon (25 mg) in dry ethyl acetate (2 mL) was stirred in H_2 for 2 h (TLC control). The suspension was then filtered through celite, and the filtrate was evaporated. The residue was purified by column chromatography (SiO_2) using hexane/ethyl acetate (9:1) to give colourless crystals; FTIR 3 456, 2 943, 1 665, cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 12.00 (s, 1H), 11.71 (s, 1H), 6.50 (s, 1H), 6.30 (s, 1H), 5.29 (s, 1H), 4.43 (q, *J* = 7.1 Hz, 2H), 2.61 (s, 3H), 2.54 (s, 3H), 2.12 (s, 3H), 2.08 (s, 3H), 1.43 (t, *J* = 7.1 Hz, 3H); Anal. ($C_{20}H_{22}O_7$) C, H.

Analogously, **16a** and **c–f** were prepared from **14a** and **c–f**; **20a–f** were prepared from **18a–f**; **1** and **2** were prepared from **17f** and **13f**, respectively (*table II*).

4.2. Biological assay methods

The procedures for the biological assays presented in *table I* were described previously in full detail: determination of the reducing activity against 2,2-diphenyl-1-picrylhydrazyl [8], deoxyribose degradation [8], inhibi-

tion of LTB_4 biosynthesis [8], inhibition of HaCaT cell proliferation [9], and release of LDH into culture medium [14].

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