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Original article

Depsides as non-redox inhibitors of leukotriene B_4 biosynthesis and HaCaT cell growth, 2. Novel analogues of obtusatic acid

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Abstract – A series of obtusatic acid analogues has been synthesized and evaluated as inhibitors of leukotriene B_4 (LTB_4) biosynthesis and as antiproliferative agents. The 4-*O*-benzylated and the 4-*O*-demethylated congeners were the most potent inhibitors of LTB_4 production of the depside class of compounds, with IC_{50} values in the submicromolar range. Furthermore, these compounds do not function as redox-based inhibitors because they were not reactive 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. Some obtusatic acid congeners were also potent inhibitors of keratinocyte growth. Growth inhibition was not mediated by damage to the cell membrane, as the activity of lactate dehydrogenase released from the cytoplasm was in the control range. © 2000 Éditions scientifiques et médicales Elsevier SAS

antiproliferative activity / keratinocytes / lactate dehydrogenase release / leukotriene biosynthesis / obtusatic acid

1. Introduction

Leukotrienes play an important role in the pathology of a variety of inflammatory and allergic diseases [1]. However, the therapeutic value of inhibitors and antagonists of these mediators in inflammatory diseases other than asthma is less clear [2]. A large number of compounds act as inhibitors of leukotriene B_4 (LTB_4) biosynthesis through interaction with free radicals [3], as the conversion of arachidonic acid to leukotrienes via the 5-lipoxygenase (5-LO) pathway is a radical-based oxidation [4]. However, the ability of 5-LO to catalyse drug oxidation implies that reactive radical species could be formed during the process of inhibition. Because of the potential toxicity of such redox inhibitors it is of importance to identify compounds acting by a non-redox mechanism. There are also inhibitors that bind to FLAP (5-lipoxygenase-activating-protein), and prevent 5-LO activation by preventing the translocation of the enzyme from the cytosol to the membrane and inhibit LTB_4 production [5].

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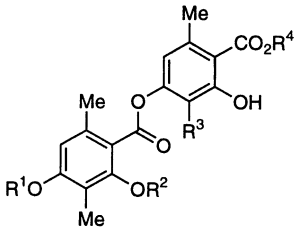
Previously we have described the characteristics of inhibition of LTB_4 biosynthesis in bovine polymorphonuclear leukocytes (PMNL) by lichen-derived metabolites isolated from *Parmelia* species, and the didepside diffractaic acid **1** was identified as a non-redox inhibitor [6]. Also, several structurally dissimilar lichen depsides have been reported to inhibit peptide leukotriene formation [7] and 5-LO from porcine leukocytes [8]. In addition, lichen depsides have also been described to inhibit prostaglandin biosynthesis [9].

In our continuing study aimed at the discovery and development of potential anti-inflammatory and antiproliferative drug candidates for the treatment of skin diseases such as psoriasis, we synthesized a series of modified analogues of **1** (*figure 1*) and its congener barbatic acid **2**, to explore the effect of increased lipophilicity and some redox properties on the biological activity of the compounds. The combined inhibitory action against LTB_4 biosynthesis and keratinocyte cell growth of these derivatives, represented by ethyl 4-*O*-demethylbarbatate **3** [10], suggested a beneficial effect against psoriasis and that synthesis of derivatives may be of interest.



1 R^1= Me, R^2 = Me, R^3 = Me, R^4 = H
2 R^1= Me, R^2 = H, R^3 = Me, R^4 = H
3 R^1= H, R^2 = H, R^3 = Me, R^4 = Et
4 R^1= Me, R^2 = H, R^3 = H, R^4 = H

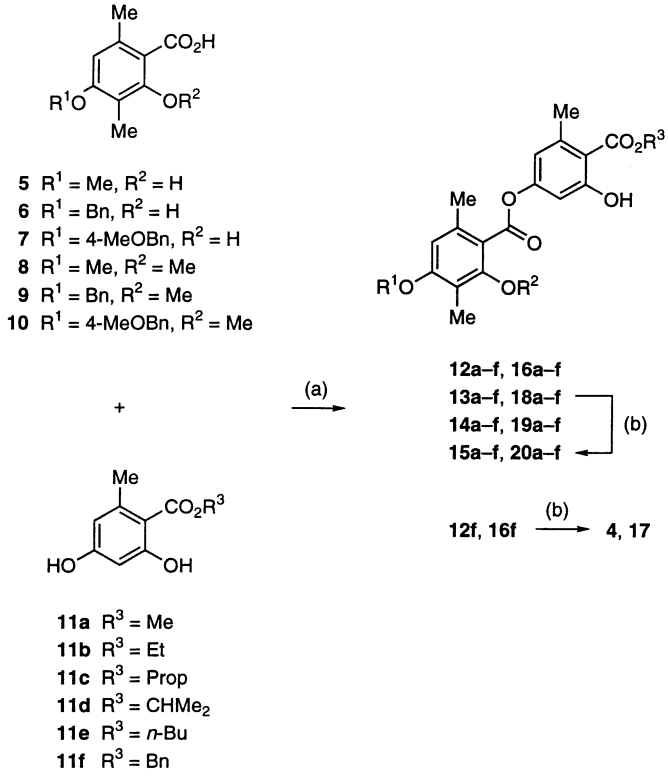
Figure 1. Structures of diffractaic acid (**1**), barbatic acid (**2**), ethyl 4-*O*-demethylbarbatate (**3**) and obtusatic acid (**4**).

In the present paper we report the preparation and evaluation of a further series of lichen depsides which are analogues of another common lichen metabolite, obtusatic acid **4** [11]. Even though it is widely distributed in various lichen species, the biological activity of **4** is rarely reported in the literature. Accordingly, **4** and its novel analogues were assayed for inhibition of LTB_4 biosynthesis in bovine polymorphonuclear leukocytes [12], and their ability to inhibit the growth of human keratinocytes was evaluated in HaCaT cells [13]. Furthermore, their 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, deoxyribose degradation was determined as a measure of hydroxyl radical formation [12].

2. Chemistry

The appropriate precursor acids and esters required for the synthesis of the tested compounds of *table I* have been reported. Following the methodology of Elix [14], the 4-substituted 2-hydroxy-3,6-dimethylbenzoic acids **5**–**7** and 2-methoxy-3,6-dimethylbenzoic acids **8**–**10** for the A-ring of the depsides were prepared exactly as we reported previously [10]. The B-ring precursors, the 2,4-dihydroxy-6-methylbenzoates **11a** and **11c**–**f**, were obtained directly from the corresponding ethyl ester **11b** [15] by transesterification.

Condensation of the benzoic acids **5**–**10** with the esters **11a**–**f** in the presence of trifluoroacetic anhydride and anhydrous toluene gave the obtusatic and 2-*O*-methylobtusatic acid analogues **12a**–**f**, **13a**–**f**, **14a**–**f** and **16a**–**f**, **18a**–**f**, **19a**–**f**, respectively (*figure 2*). Hydrogenolytic cleavage of the benzyl ethers **13a**–**e** and **18a**–**e**



5 R^1 = Me, R^2 = H
6 R^1 = Bn, R^2 = H
7 R^1 = 4-MeOBn, R^2 = H
8 R^1 = Me, R^2 = Me
9 R^1 = Bn, R^2 = Me
10 R^1 = 4-MeOBn, R^2 = Me

11a R^3 = Me
11b R^3 = Et
11c R^3 = Prop
11d R^3 = $CHMe_2$
11e R^3 = *n*-Bu
11f R^3 = Bn

12a–f, 16a–f
13a–f, 18a–f
14a–f, 19a–f
15a–f, 20a–f

12f, 16f → **4, 17**

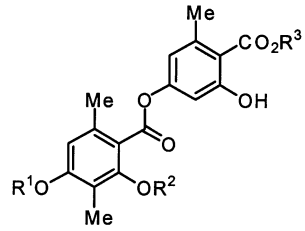
Figure 2. Reagents: (a) $(CF_3CO)_2O$, toluene, room temperature; (b) Pd/C, EtOAc, room temperature.

over palladium/carbon produced the phenolic analogues **15a**–**e** and **20a**–**e**, respectively, whereas benzyl esters **13f** and **18f** were cleaved to the acids **15f** and **20f**, respectively. Finally, hydrogenolytic cleavage of the benzyl esters **12f** and **16f** yielded obtusatic acid **4** and 2-*O*-methylobtusatic **17**, respectively.

3. Biological results and discussion

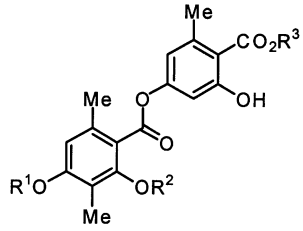
The compounds were evaluated for their potency to inhibit production of LTB_4 in Ca^{2+}-ionophore-activated bovine polymorphonuclear leukocytes [12]. The data are reported in *table I*. No significant inhibitory activity was observed for **4** and its 4-*O*-methyl analogue **17** at 20 μM concentrations. By contrast, modification of **4** and **17** to exchange the free carboxylate for an ester function provided congeners **12a**–**f** and **16a**–**f**, respectively, with inhibitory activity against LTB_4 biosynthesis. The lack of activity of **4** and **17** is an unexpected observation in light of the potency of their corresponding esters. As the precursor of LTB_4, arachidonic acid, which is the natural substrate of the 5-LO, is metabolized as free carboxylate,

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



Compound	R^1	R^2	R^3	k_{DPPH}[a] ($M^{-1}s^{-1}$)	DD ($^{\cdot}OH$)[b]	LTB_4[c] (μM)	IC_{50} AA[d] (μM)	IC_{50} LDH[e] (mU)
4	Me	H	H	ND	ND	> 20	> 20	ND
12a	Me	H	Me	0.67 ± 0.03	0.12 ± 0.01	7.2	> 20	ND
12b	Me	H	Et	0.77 ± 0.02	0.19 ± 0.01	6.8	> 20	ND
12c	Me	H	Prop	0.36 ± 0.02	0.05 ± 0.01	6.8	ND	ND
12d	Me	H	$CHMe_2$	0.17 ± 0.02	0.06 ± 0.01	5.3	ND	ND
12e	Me	H	Bu	0.59 ± 0.09	0.14 ± 0.01	10	ND	ND
12f	Me	H	CH_2Ph	ND	ND	> 20	ND	ND
13a	$PhCH_2$	H	Me	0.28 ± 0.02	0.22 ± 0.01	10	> 20	ND
13b	$PhCH_2$	H	Et	0.31 ± 0.02	0.18 ± 0.01	3.8	> 20	ND
13c	$PhCH_2$	H	Prop	0.43 ± 0.04	0.13 ± 0.01	0.7	> 20	ND
13d	$PhCH_2$	H	$CHMe_2$	0.33 ± 0.02	0.03 ± 0.01	2.5	ND	ND
13e	$PhCH_2$	H	Bu	0.98 ± 0.04	0.17 ± 0.01	9	ND	ND
13f	$PhCH_2$	H	CH_2Ph	ND	ND	> 20	ND	ND
14a	4-$MeOPhCH_2$	H	Me	ND	ND	> 20	> 20	ND
14b	4-$MeOPhCH_2$	H	Et	0.78 ± 0.07	0.15 ± 0.01	9.3	> 20	ND
14c	4-$MeOPhCH_2$	H	Prop	0.92 ± 0.05	0.11 ± 0.02	5.6	ND	ND
14d	4-$MeOPhCH_2$	H	$CHMe_2$	0.33 ± 0.06	0.19 ± 0.01	7.4	ND	ND
14e	4-$MeOPhCH_2$	H	Bu	0.26 ± 0.03	0.21 ± 0.03	11	ND	ND
14f	4-$MeOPhCH_2$	H	CH_2Ph	ND	ND	> 20	ND	ND
15a	H	H	Me	0.75 ± 0.04	0.05 ± 0.01	5.2	> 20	ND
15b	H	H	Et	0.67 ± 0.04	0.13 ± 0.01	2.4	> 20	ND
15c	H	H	Prop	0.92 ± 0.08	0.09 ± 0.01	0.3	> 20	ND
15d	H	H	$CHMe_2$	0.14 ± 0.01	0.09 ± 0.01	16	> 20	ND
15e	H	H	Bu	0.31 ± 0.01	0.18 ± 0.02	6.6	> 20	ND
15f	H	H	H	0.33 ± 0.01	0.39 ± 0.05	13	8.0	152
16a	Me	Me	Me	0.69 ± 0.08	0.04 ± 0.01	3.6	> 20	ND
16b	Me	Me	Et	0.77 ± 0.09	0.18 ± 0.01	1.3	> 20	ND
16c	Me	Me	Prop	0.20 ± 0.01	0.05 ± 0.01	7.6	ND	ND
16d	Me	Me	$CHMe_2$	0.75 ± 0.04	0.06 ± 0.01	4.2	ND	ND
16e	Me	Me	Bu	0.82 ± 0.03	0.17 ± 0.01	13	4.1	146
16f	Me	Me	CH_2Ph	ND	ND	> 20	ND	ND
17	Me	Me	H	ND	ND	> 20	3.9	156
18a	$PhCH_2$	Me	Me	0.56 ± 0.04	0.14 ± 0.03	18	> 20	ND
18b	$PhCH_2$	Me	Et	0.21 ± 0.02	0.21 ± 0.02	5.6	> 20	ND
18c	$PhCH_2$	Me	Prop	0.34 ± 0.06	0.11 ± 0.01	16	ND	ND
18d	$PhCH_2$	Me	$CHMe_2$	0.79 ± 0.06	0.19 ± 0.01	7.2	ND	ND
18e	$PhCH_2$	Me	Bu	ND	ND	> 20	ND	ND
18f	$PhCH_2$	Me	CH_2Ph	ND	ND	> 20	ND	ND
19a	4-$MeOPhCH_2$	Me	Me	ND	ND	> 20	> 20	ND
19b	4-$MeOPhCH_2$	Me	Et	0.69 ± 0.24	0.18 ± 0.03	11	> 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	0.68 ± 0.08	0.12 ± 0.01	14	4.8	184[f]

Table I. Contd.



Compound	R^1	R^2	R^3	k_{DPPH}[a] (M^{-1} s^{-1})	DD ($^{\cdot}OH$)[b]	LTB_4[c] (μM)	IC_{50} AA[d] (μM)	IC_{50} LDH[e] (mU)
20b	H	Me	Et	0.73 ± 0.02	0.15 ± 0.02	3.8	9.0	156
20c	H	Me	Prop	0.69 ± 0.05	0.07 ± 0.01	10	> 20	ND
20d	H	Me	$CHMe_2$	0.86 ± 0.03	0.11 ± 0.02	6.8	> 20	ND
20e	H	Me	Bu	0.54 ± 0.03	0.16 ± 0.01	5.6	> 20	ND
20f	H	Me	H	0.49 ± 0.03	0.12 ± 0.01	19	6.0	114
anthralin[g]				24.2 ± 4.2[f]	2.89 ± 0.14[f]	37	0.7	294[f]

[a]Reducing activity against 2,2-diphenyl-1-picrylhydrazyl with an equimolar amount 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 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) [12]. [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 [12].

carboxylic acids **4** and **17** might be expected to display greater activity than their esters. However, others have also reported that ester groups confer more potency than charged carboxylic acids [16]. These findings suggest a requirement for overall high lipophilicity in potent inhibitors of LTB_4 biosynthesis.

Replacing the 4-methoxy group with a benzyloxy substituent (**13a**–**f**) further improved the potency, at least for that of the obtusatic acid congeners **12a**–**f**. Of these compounds, the propyl ester **13c** with an IC_{50} value in the submicromolar range was 10-fold more potent than its 4-methyl congener **12c**. Introduction of a methoxy substituent into the benzyl group (**14a**–**f**) slightly decreased potency.

As revealed by our recent study the replacement of the 4-methoxy group by a hydroxyl group strongly increased the potency of the depsides against LTB_4 biosynthesis [10]. This structural change was also beneficial to the potency of the depsides of the obtusatic acid series. In particular, propyl 4-*O*-demethylobtusate **15c** was the most potent inhibitor of LTB_4 production in polymorphonuclear leukocytes of the depside class of compounds. With an IC_{50} of 0.3 μM, **15c** was as potent as the standard inhibitor nordihydroguaiaretic acid.

Inhibition of leukotriene biosynthesis by phenolic antioxidants is vastly described in the literature [3]. This is not surprising, since 5-LO is an oxidase. Accordingly, phenols can inhibit the enzyme as reducing agents and are preferentially oxidized themselves. In this fashion they can act as alternative substrates for 5-LO by 5-LO-induced oxidation of the inhibitor. However, lack of specificity can be a problem in this class due to the ubiquitous nature of oxidative processes in biological systems. Therefore, we have performed additional experiments to further characterize the nature of the inhibitory action of the depsides.

Their ability to react with the stable free radical DPPH to give the reduced 2,2-diphenyl-1-picrylhydrazine is shown in *table I*. Even though the most potent inhibitors of the depside bear phenolic functions, no appreciable amount of reduced hydrazine was formed by these compounds. This documents their lack of reactivity against stable free radicals and 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.

A further aspect associated with inhibition of LTB_4 biosynthesis results from observations that several inhibitors can give rise to one-electron oxidation species which play a key role in enzyme inactivation [17, 18]. Therefore, we defined pro-oxidant properties of the compounds by the deoxyribose assay, which is a sensitive test for the production of hydroxyl radicals [19]. 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 the novel depsides (*table I*) that would approximately reach the amount of hydroxyl radicals generated by the standard agent anthralin [20].

Mounting evidence suggests that leukotrienes have a profound influence on the development and progression of human cancers, and some pharmacological agents that inhibit the LO-mediated signalling pathways are available to treat inflammatory and hyperproliferative diseases such as psoriasis [21]. Furthermore, lichen depsides are inhibitors of keratinocyte growth [22]. Therefore, selected compounds were evaluated in vitro for their ability to inhibit the growth of HaCaT cells, a non-transformed human cell line that can be used as a model for highly proliferative epidermis [23]. Unfortunately, the most potent inhibitors of LTB_4 biosynthesis, depsides **13c** and **15c**, were inactive at 20 μM concentrations. There was no correlation found between the potency of the depsides to inhibit LTB_4 biosynthesis and their in vitro antiproliferative activity. 2-*O*-Methylobtusatic acid **17** and its butyl ester **16e** were the most potent inhibitors of cell growth, and also the 4-O-demethylated compounds **15f** and **20a** displayed antiproliferative activity.

To determine cytotoxic effects of the potent antiproliferative agents, release of lactate dehydrogenase into the culture medium of HaCaT cells was studied [24]. This is commonly used as an indicator of plasma membrane damage. In this assay, LDH release by the standard anthralin significantly exceeded that of the vehicle control. On the other hand, LDH release of the test compounds (*table I*) was unchanged as compared to controls at 2 μM concentration, documenting that their antiproliferative activity was due to cytostatic rather than cytotoxic effects.

In conclusion, we have identified **13c** and **15c** as potent inhibitors of LTB_4 biosynthesis which, in contrast to many known compounds, do not function primarily as redox-based inhibitors. As a consequence, toxicity which may be attributed to non-specific redox enzyme inhibition can be ruled out.

4. Experimental protocols

4.1. Chemistry

4.1.1. General

For analytical instruments and methods see reference [25].

Starting compounds **4**, **5** [14], **6**, **7** [10], **8** [14], **9** and **10** [10] were prepared as described.

4.1.1.1. Propyl 2,4-dihydroxy-6-methylbenzoate **11c**

Sodium (0.5 g, 21.73 mmol) was dissolved in absolute propanol (50 mL) and stirred at room temperature. Then **11b** [15] (2.8 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; 92% yield; m.p. 123 °C; FTIR 3 428, 2 968, 1 615 cm^{-1}; ^{1}H-NMR (250 MHz, $CDCl_3$) δ 11.86 (s, 1H), 6.28 (d, *J* = 2.6 Hz, 1H), 6.21 (d, *J* = 2.6 Hz, 1H), 5.37 (s, 1H), 3.92 (s, 3H), 4.38 (t, 3H), 2.50 (s, 3H), 1.79 (m, 2H), 1.04 (t, 3H). Anal. ($C_{11}H_{14}O_4$) C, H.

Analogously, compounds **11a** and **11c–f** [14, 15, 26] were prepared from **11b**.

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

4.1.2.1. Ethyl 2-hydroxy-4-(2-hydroxy-4-methoxy-3,6-dimethylbenzoyloxy)-6-methylbenzoate **12b**

A solution of **5** (100 mg, 0.6 mmol) and **11b** [14] (126 mg, 0.6 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 3 380, 2 944, 1 750, 1 661 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 11.66 (s, 1H), 11.39 (s, 1H), 6.71 (d, 1H), 6.59 (d, 1H), 6.35 (s, 1H), 4.46 (q, 2H), 3.89 (s, 3H), 2.65 (s, 3H) 2.58 (s, 3H), 2.09 (s, 3H), 1.44 (s, 3H); Anal. ($C_{20}H_{22}O_7$) C, H.

Analogously, **12a** and **12c–f** were prepared from **5** and **11a**, **11c–f**; **13a–f** were prepared from **6** and **11a–f**; **14a–f** were prepared from **7** and **11a–f**; **16a–f** were prepared from **8** and **11a–f**; **18a–f** were prepared from **9** and **11a–f**; **19a–f** were prepared from **10** and **11a–f** (*table II*).

4.1.3. General procedure for hydrogenolysis

4.1.3.1. 2-Hydroxy-4-(2,4-dimethoxy-3,6-dimethylbenzoyloxy)-6-methylbenzoic acid **17**

A suspension of **16f** (100 mg, 0.22 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 3 446, 2 953, 1 675, cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 11.42 (s, 1H), 6.67 (d, 1H), 6.61 (d,

Table II. Chemical data of obtusatic acid derivatives.

Compound[a]	Formula[b]	M.p. (°C)	Yield (%)	Anal.[c]
4	$C_{18}H_{18}O_7$	203; ref [14] 194–195	87	C, H
12a	$C_{19}H_{20}O_7$	173	46	C, H
12b	$C_{20}H_{22}O_7$	186	48	C, H
12c	$C_{21}H_{24}O_7$	169	57	C, H
12d	$C_{21}H_{24}O_7$	157	42	C, H
12e	$C_{22}H_{26}O_7$	114	56	C, H
12f	$C_{25}H_{24}O_7$	127; ref [14] 126–128	56	C, H
13a	$C_{25}H_{24}O_7$	172	49	C, H
13b	$C_{26}H_{26}O_7$	133	55	C, H
13c	$C_{27}H_{28}O_7$	116	41	C, H
13d	$C_{27}H_{28}O_7$	116	48	C, H
13e	$C_{28}H_{30}O_7$	108	50	C, H
13f	$C_{31}H_{28}O_7$	110; ref [14] 110–111	67	C, H
14a	$C_{26}H_{26}O_8$	139	43	C, H
14b	$C_{27}H_{28}O_8$	156	56	C, H
14c	$C_{28}H_{30}O_8$	107	48	C, H
14d	$C_{28}H_{30}O_8$	164	36	C, H
14e	$C_{29}H_{32}O_8$	122	59	C, H
14f	$C_{32}H_{30}O_8$	148	63	C, H
15a	$C_{18}H_{18}O_7$	142	87	C, H
15b	$C_{19}H_{20}O_7$	147	91	C, H
15c	$C_{20}H_{22}O_7$	140	90	C, H
15d	$C_{20}H_{22}O_7$	136	79	C, H
15e	$C_{21}H_{24}O_7$	174	93	C, H
15f	$C_{17}H_{16}O_7$	182; ref [14] 182	82	C, H
16a	$C_{20}H_{22}O_7$	168	62	C, H
16b	$C_{21}H_{24}O_7$	108	57	C, H
16c	$C_{22}H_{26}O_7$	113	49	C, H
16d	$C_{22}H_{26}O_7$	83	53	C, H
16e	$C_{23}H_{28}O_7$	66	55	C, H
16f	$C_{26}H_{26}O_7$	83	43	C, H
17	$C_{19}H_{20}O_7$	93	87	C, H
18a	$C_{26}H_{26}O_7$	103	47	C, H
18b	$C_{27}H_{28}O_7$	147	49	C, H
18c	$C_{28}H_{30}O_7$	162	54	C, H
18d	$C_{28}H_{30}O_7$	96	59	C, H
18e	$C_{29}H_{32}O_7$	142	62	C, H
18f	$C_{32}H_{30}O_7$	109	69	C, H
19a	$C_{27}H_{28}O_8$	143	60	C, H
19b	$C_{28}H_{30}O_8$	136	51	C, H
19c	$C_{29}H_{32}O_8$	140	47	C, H
19d	$C_{29}H_{32}O_8$	98	59	C, H
19e	$C_{30}H_{30}O_8$	107	64	C, H
19f	$C_{33}H_{32}O_8$	116	47	C, H
20a	$C_{19}H_{20}O_7$	143	89	C, H
20b	$C_{20}H_{22}O_7$	174	96	C, H
20c	$C_{21}H_{24}O_7$	183	91	C, H
20d	$C_{21}H_{24}O_7$	156	78	C, H
20e	$C_{22}H_{26}O_7$	192	83	C, H
20f	$C_{18}H_{18}O_7$	210	92	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]Elemental analyses were within ± 0.4% of calculated values.

1H), 6.54 (s, 1H), 3.89 (s, 3H), 3.82 (s, 3H), 2.58 (s, 3H), 2.43 (s, 3H), 2.14 (s, 3H); Anal. ($C_{19}H_{20}O_7$) C, H.

Analogously, **15a–f** and **20a–f** were prepared from **13a–f** and **18a–f**, 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 [12], deoxyribose degradation [12], inhibition of LTB_4 biosynthesis [12], inhibition of HaCaT cell proliferation [13], and release of LDH into culture medium [24].

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