N-ω-Carbethoxypentyl-4-quinolones: A New Class of Leukotriene Biosynthesis Inhibitors

Nicoletta Desideri[a]*, Isabella Sestili[a], Maria Luisa Stein[a], Stefano Manarini[b], Giuseppe Dell'Elba[b], and Chiara Cerletti[b]

[a] Dipartimento di Studi farmaceutici, Università "La Sapienza" di Roma, P.le A. Moro 5, 00185 Roma.

[b] Istituto di Ricerche farmacologiche Mario Negri, Consorzio Mario Negri Sud, 66030 S. Maria Imbaro, Italy

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Summary

6-[(4-Quinolinyl)oxy]hexanoic acids and the corresponding esters were designed and synthesized as inhibitors of the production of arachidonic acid metabolites. The inhibitory activities were assayed *in vitro* by evaluation of serum leukotriene B_4 and thromboxane B_2 production. While all 6-[(4-quinolinyl)oxy]hexanoic acids and their esters proved to be inactive, the *N*-alkyl-4-quinolones, obtained as by-products in their synthesis, were found to be a new class of leukotriene biosynthesis inhibitors.

Introduction

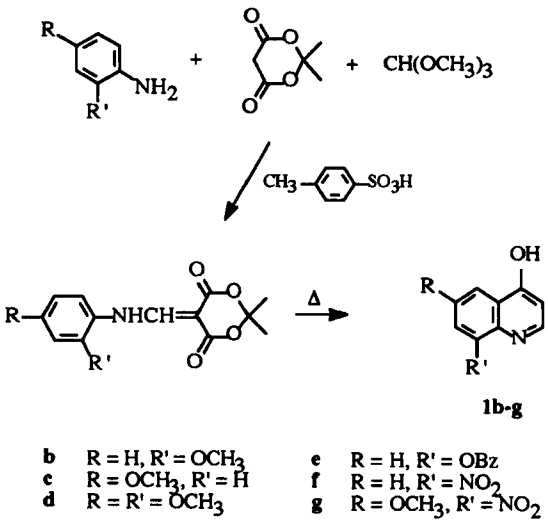
Lipoxygenases (LOs) are a class of non-heme iron dioxygenases which catalyze the oxidation by molecular oxygen of *cis,cis*-1,4-pentadiene systems. In human leukocytes 5-LO metabolizes arachidonic acid (AA) to 5-hydroperoxyeicosatetraenoic acid (5-HPETE), which can be converted to a series of biologically active compounds called leukotrienes (LTs). LTs are involved in a broad variety of functions and diseases including psoriasis, arthritis, inflammatory bowel disease, and asthma[1]. Therefore, specific 5-LO inhibitors could find applications in the treatment of such disorders.

Inhibitors of LT biosynthesis can be divided into four classes based on their mechanism of action. Three of these classes act directly on the 5-LO enzyme via a redox, a nonredox, or an iron ligand mechanism, the fourth class prevents the activation of 5-LO by binding to a membrane protein termed 5-lipoxygenase-activating protein (FLAP)[1]. From a structural point of view, these compounds fall into a large variety of categories, and quinoline derivatives are largely represented[1,2].

Following in our research on inhibitors of enzymes implicated in AA metabolism[3–6], we synthesized a series of 6-[(4-quinolinyl)oxy]hexanoic acids (**4a–l**). In order to confer the ability to bind iron at the catalytic site of the enzyme, the quinoline ring was substituted with a hydroxy (**4h**) or an amino (**4i–l**) group in the 8 position. Moreover the introduction of a methoxy (**4c**, **g**, **l**) group in the 6 position could address towards the antioxidant mechanism by affecting the redox properties of the compounds.

Chemistry

The substituted 4-hydroxyquinolines (**1b–g**) were prepared as previously described for the synthesis of **1a,b,f,g**[7]: the appropriate aniline was condensed with trimethyl orthoformate and 2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid) in the presence of catalytic amounts of *p*-toluensulfonic acid, and the intermediate was cyclized by heating in Dowtherm A (Scheme 1).



b R = H, R' = OCH_3 **e** R = H, R' = OBz
c R = OCH_3, R' = H **f** R = H, R' = NO_2
d R = R' = OCH_3 **g** R = OCH_3, R' = NO_2

Scheme 1

The *O*-alkylquinolines **2a–g** were synthesized starting from the sodium salt of the appropriate 4-hydroxyquinoline, which was prepared with sodium ethoxide in ethyl alcohol; this salt was solubilized in dimethyl sulfoxide and reacted with ethyl ω-bromohexanoate at room temperature. Mixtures of *O*-alkyl (**2a**, **2e**) and *N*-alkyl (**3a**, **3e**) derivatives were obtained from **1a** or **1e** (Scheme 2, method A); the two esters were separated by column chromatography on silica gel eluting with ethyl acetate. On the bases of NMR and IR data, the *O*-alkyl and *N*-alkyl structure was assigned at the first and at the second eluted compound, respectively. The C-5 proton in NMR spectra of *N*-alkyl quinolones is shifted downfield in respect to that of *O*-alkyl quinolines, due to the anisotropic effect of the 4-oxo group. In contrast, the C-2 signal is shifted upfield. Therefore, signals for C-5 and C-2 usually reverse their positions. The coupling costants of C-2 ($J_{2\text{-}3}$) and of C-5 ($J_{5\text{-}6}$ and/or $J_{5\text{-}7}$) allow unequivocal assignment of the signals. The presence in the IR spectra of the *N*-alkyl derivatives of a band around 1610 cm^{-1} (due to the 4-carbonyl group) confirms the structure.

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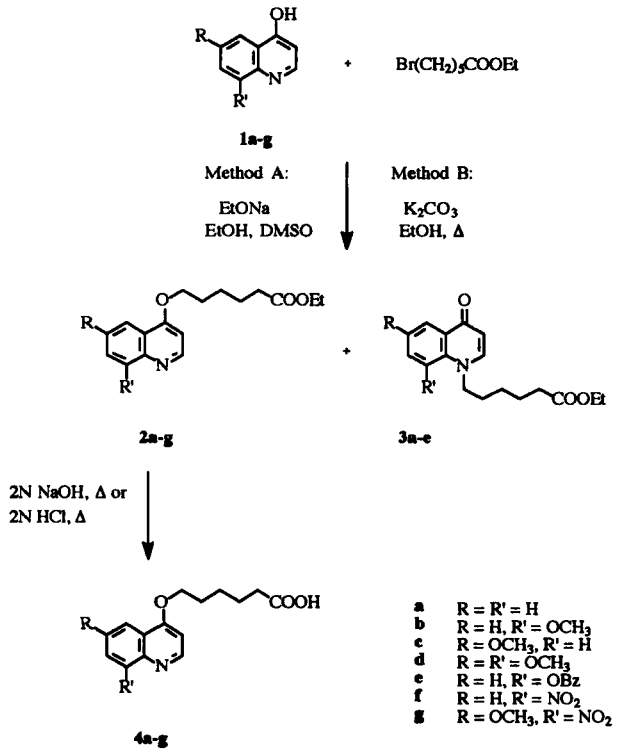
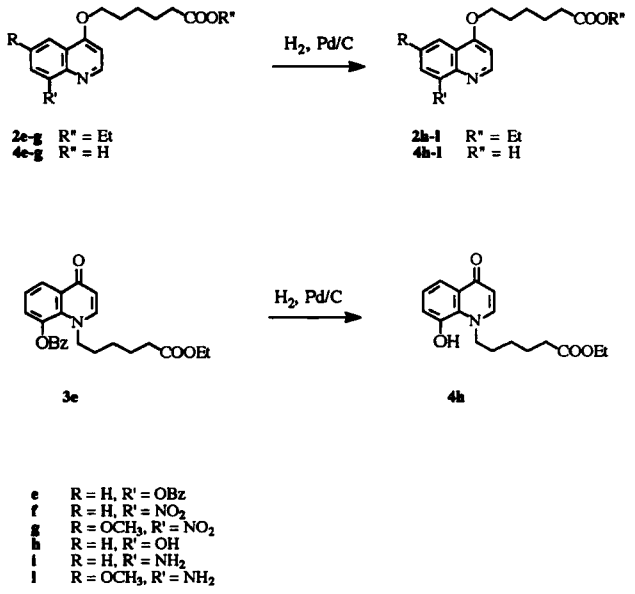


Scheme 2



Scheme 3

In order to obtain also the *N*-alkylquinolones **3b-d**, the alkylation was performed by heating an ethanolic solution of 4-hydroxyquinoline (**1b–d**) and ethyl ω-bromohexanoate in the presence of potassium carbonate. Also this method led to a mixture of *O*-alkyl and *N*-alkyl derivatives with prevalence of the first one (Scheme 2, method B).

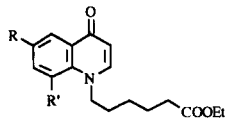
The acids **4a, 4c–g** were prepared by alkaline hydrolysis of the corresponding esters **2a, 2c–g** and were isolated as inner salts, as shown by IR spectra. The acid **4b** was obtained by acid hydrolysis of **2b** and isolated as hydrochloride (Scheme 2).

Reduction of nitro quinolines **2f, 2g, 4f** and **4g** to amino-quinolines **2i, 2l, 4i** and **4l** was achieved by catalytic hydrogenation over 5% Pd/C, at 45 psi and room temperature. The 8-hydroxy compounds **2h, 3h** and **4h** were prepared by hydrogenolysis of the corresponding benzyl ethers **2e, 3e** and **4e** in analogous conditions (Scheme 3).

Results and Discussion

The biological activity of ethyl 6-[(4-quinolinyl)oxy]hexanoates (**2a–d, 2h–l**), ethyl 6-(4-oxo-1*H*-quinolin-1-yl)hexanoates (**3a–d, 3h**), and 6-[(4-quinolinyl)oxy]hexanoic acids (**4a–d, 4h–l**) was evaluated *in vitro* by monitoring the inhibition of LTB_4 and thromboxane (Tx) B_2 production by human whole blood stimulated with calcium ionophore A23187. TxB_2 was determined by specific radioimmunoassays (RIA) on unextracted plasma samples as previously described[8]. LTB_4 was dosed by HPLC, after solvent extraction of the samples[9].

Table 1. Effect of ethyl 6-(4-oxo-1*H*-quinolin-1-yl)hexanoates **3a–d** and **3h** on human serum LTB4 generation.



Comp	R	R'	LTB_4 (%)* 50 μM	100 μM	200 μM	IC_{50} (μM)
3a	H	H	0	37±31	58±13	161
3b	H	OCH_3	20±22	40±28	69±14	134
3c	OCH_3	H	0	21±28	53±20	190
3d	OCH_3	OCH_3	32±20	46±28	75±3	114
3h	H	OH	9	19	43±3	>200

*Percent of inhibition with respect to control values. The reported values are means ± SD of 2–3 different experiments, each performed in duplicate.

No significant reduction of prostanoid generation occurred in blood incubated in the presence of all ethyl 6-[(4-quinolinyl)oxy]hexanoates (**2a–d, 2h–l**) and 6-[(4-quinolinyl)oxy]hexanoic acids (**4a–d, 4h–l**) up to 200 μM (data not shown). Only ethyl 6-(4-oxo-1*H*-quinolin-1-yl)hexanoates (**3a–d, 3h**) inhibited LTB_4 biosynthesis. As shown in Table 1, the IC_{50} values, calculated from the dose-response plots, for the inhibition of LTB_4 production ranged between 114 and 190 μM for quinolones **3a–d**, while this value was higher than the maximum tested concentration for 8-hydroxyquinolone **3h**; the inhibition at this dose resulted 43%. In the same experimental conditions, the potent reference compound, L-663,536, reportedly inhibited LTB_4 synthesis with a mean IC_{50} of 1.1 ± 0.2 μM [10]. Reduction of LTB_4 synthesis was not accompanied by variations of TxB_2 production; only the 6,8-dimethoxyquinolone **3d** showed an inhibitory effect on

Table 2: Chemical and physical data of ethyl 6-[(4-quinolinyl)oxy]hexanoates (**2a–l**).

Cpd	R	R′	Formula[a]	Mp (°C) (solvent)	Yield (%)	IR: cm^{-1}[b]	^1H NMR: δ[c]
2a	H	H	$C_{17}H_{21}NO_3$[d]	97–99[e] ($EtOH/Et_2O$)[e]	30[f]	1720 (COO)	8.60 (d, $J_{2,3}$ = 5 Hz, 1H, 2-H), 8.25–7.90 (m, 2H, 5-H, 8-H), 7.80–7.25 (m, 2H, 6-H, 7-H), 6.50 (d, $J_{2,3}$ =5 Hz, 1H, 3-H), 4.20–3.85 (m, 4H, OCH_2, $COOCH_2$), 2.20 (t, 2H, CH_2COO), 2.00–1.25 (m, 6H, $3CH_2$), 1.20 (t, 3H, CH_3).
						2800–2400 (NH), 1710 (COO)[e]	9.25 (d, $J_{2,3}$ = 6 Hz, 1H, 2-H), 8.60–7.80 (m, 4H, 5-H-8-H), 7.60 (d, $J_{2,3}$ =6 Hz, 1H, 3-H), 4.55 (t, 2H, OCH_2), 4.1 (q, 2H, $COOCH_2$), 2.35 (t, 2H, CH_2COO), 2.20–1.35 (m, 6H, $3CH_2$), 1.15 (t, 3H, CH_3).[e]
2b	H	OCH_3	$C_{18}H_{23}NO_4$	103–105 (acetone)	27[f] 48[g]	1720 (COO)	8.75 (d, $J_{2,3}$ = 6 Hz, 1H, 2-H), 7.80 (dd, $J_{5,6}$ = 8 Hz, $J_{5,7}$ = 1.5 Hz, 1H, 5-H), 7.40 (t, $J_{5,6}$ = $J_{6,7}$ = 8 Hz, 1H, 6-H), 7.05 (dd, $J_{6,7}$ = 8 Hz, $J_{5,7}$ = 1.5 Hz, 1H, 7-H), 6.70 (d, $J_{2,3}$ = 6 Hz, 1H, 3-H), 4.40–4.00 (m, 7H, OCH_3, OCH_2, $COOCH_2$), 2.30 (t, 2H, CH_2COO), 2.10–1.35 (m, 6H, $3CH_2$), 1.20 (t, 3H, CH_3).
2c	OCH_3	H	$C_{18}H_{23}NO_4$[h]	121–128[i] (acetone)[i]	26[f] 22[g]	1720 (COO)	8.65 (d, $J_{2,3}$ = 5 Hz, 1H, 2-H), 8.00 (d, $J_{7,8}$ = 9 Hz, 1H, 8-H), 7.60–7.30 (m, 2H, 5-H, 7-H), 6.65 (d, $J_{2,3}$ = 5 Hz, 1H, 3-H), 4.40–4.00 (m, 4H, OCH_2, $COOCH_2$), 3.90 (s, 3H, OCH_3), 2.40 (t, 2H, CH_2COO), 2.20–1.40 (m, 6H, $3CH_2$), 1.20 (t, 3H, CH_3).
						2800–2300 (NH), 1700 (COO)[i]	8.86 (d, $J_{2,3}$ = 6.7 Hz, 1H, 2-H), 8.50 (d, $J_{7,8}$ = 9.2 Hz, 1H, 8-H), 7.72 (dd, $J_{7,8}$ = 9.2 Hz, $J_{5,7}$ = 2.6 Hz, 1H, 7-H), 7.64 (d, $J_{5,7}$ =2.6 Hz, 1H, 5-H), 7.48 (d, $J_{2,3}$ = 6.7 Hz, 1H, 3-H), 4.60 (t, 2H, OCH_2), 4.15–4.05 (m, 5H, $COOCH_2$, OCH_3), 2.39 (t, 2H, CH_2COO), 2.17–1.50 (m, 6H, $3CH_2$), 1.15 (t, 3H, CH_3).[i]
2d	OCH_3	OCH_3	$C_{19}H_{25}NO_5$	66–69 (Et_20)	61[f] 22[g]	1710 (COO)	8.70 (d, $J_{2,3}$ = 5 Hz, 1H, 2-H), 7.10 (d, $J_{5,7}$ = 3 Hz, 1H, 5-H), 6.80 (m, 2H, 3-H, 7-H), 4.35–3.85 (m, 10H, $2OCH_3$, OCH_2, $COOCH_2$), 2.40 (t, 2H, CH_2COO), 2.15–1.60 (m, 6H, $3CH_2$), 1.25 (t, 3H, CH_3).
2e	H	OBzl	$C_{24}H_{27}NO_4$	62–64 (*n*-hexane)	54[f]	1715 (COO)	8.90 (d, $J_{2,3}$ = 6 Hz, 1H, 2-H), 7.80 (d, $J_{5,6}$ = 9 Hz, 1H, 5-H), 7.70–7.25 (m, 6H, 6-H, Ph), 7.05 (d, $J_{6,7}$ = 9 Hz, 1H, 7-H), 6.80 (d, $J_{2,3}$ = 6 Hz, 1H, 3-H), 5.45 (s, 2H, OCH_2Ph), 4.40–4.00 (m, 4H, OCH_2, $COOCH_2$), 2.35 (t, 2H, CH_2COO), 2.15–1.35 (m, 6H, $3CH_2$), 1.20 (t, 3H, CH_3).
2f	H	NO_2	$C_{17}H_{20}N_2O_5$	74–76 (cyclohexane)	55[f]	1720 (COO); 1520, 1310 (NO_2)	8.90 (d, $J_{2,3}$ = 6 Hz, 1H, 2-H), 8.45(dd, $J_{6,7}$ = 9 Hz, $J_{5,7}$ = 2Hz, 1H, 7-H), 8.00 (dd, $J_{5,6}$ = 9 Hz, $J_{5,7}$ = 2Hz, 1H, 5-H), 7.55 (t, $J_{5,6}$ = $J_{6,7}$ = 9 Hz, 1H, 6-H), 6.80 (d, $J_{2,3}$ = 6 Hz, 1H, 3-H), 4.35–4.00 (m, 4H, OCH_2, $COOCH_2$), 2.35 (t, 2H, CH_2COO), 2.20–1.40 (m, 6H, $3CH_2$), 1.25 (t, 3H, CH_3).
2g	OCH_3	NO_2	$C_{18}H_{22}N_2O_6$	94–97 (EtOH)	62[f]	1705 (COO); 1520, 1350 (NO_2)	8.75 (d, $J_{2,3}$ = 5 Hz, 1H, 2-H), 7.70 (s, 2H, 5-H, 7-H), 6.80 (d, $J_{2,3}$ = 5 Hz, 1H, 3-H), 4.45–4.10 (m, 4H, OCH_2, $COOCH_2$), 4.00 (s, 3H, OCH_3), 2.40 (t, 2H, CH_2COO), 2.20–1.40 (m, 6H, $3CH_2$), 1.25 (t, 3H, CH_3).
2h	H	OH	$C_{17}H_{21}NO_4$	111–113 (EtOH)	60	3440–3100 (OH), 1725 (COO)	8.75 (d, $J_{2,3}$ = 5 Hz, 1H, 2-H), 7.70–6.85 (m, 5H, 3-H, 5-H-7-H, OH), 4.40–3.85 (m, 4H, OCH_2, $COOCH_2$), 2.30 (t, 2H, CH_2COO), 2.05–1.35 (m, 6H, $3CH_2$), 1.15 (t, 3H, CH_3).
2i	H	NH_2	$C_{17}H_{22}N_2O_3$	80–83 (MeOH)	95	3440, 3340 (NH_2); 1700 (COO)	8.60 (d, $J_{2,3}$ = 6 Hz, 1H, 2-H), 7.55(dd, $J_{5,6}$ = 8 Hz, $J_{5,7}$ = 2 Hz, 1H, 5-H), 7.30 (t,$J_{5,6}$ = $J_{6,7}$ = 8 Hz, 1H, 6-H), 6.90 (dd, $J_{6,7}$ = 8 Hz, $J_{5,7}$ = 2 Hz, 1H, 7-H), 6.65 (d, $J_{2,3}$ = 6 Hz, 1H, 3-H), 5.05–4.40 (bs, 2H, NH_2), 4.30–4.00 (m, 4H, OCH_2, $COOCH_2$), 2.30 (t, 2H, CH_2COO), 2.10–1.40 (m, 6H, $3CH_2$), 1.20 (t, 3H, CH_3).
2l	OCH_3	NH_2	$C_{18}H_{24}N_2O_4$	124–127 (acetone)	92	3440, 3340 (NH_2); 1700 (COO)	8.55 (d, $J_{2,3}$ = 5 Hz, 1H, 2-H), 6.90 (d, $J_{5,7}$ = 2 Hz, 1H, 5-H), 6.70 (d, $J_{2,3}$ = 5 Hz, 1H, 3-H), 6.60 (d, $J_{5,7}$ = 2 Hz, 1H, 7-H), 5.20–4.50 (bs, 2H, NH_2), 4.35–4.00 (m, 4H, OCH_2, $COOCH_2$), 3.90 (s, 3H, OCH_3), 2.35 (t, 2H, CH_2COO), 2.20–1.40 (m, 6H, $3CH_2$), 1.25 (t, 3H, CH_3).

[a]C, H, and N analyses were within ±0.4% of the theoretical value. [b]KBr or film for oil; [c]$CDCl_3$, or [D_6]DMSO (**2a·HCl**, **2h**), or CD_3OD (**2c·HCl**). [d]Elemental analysis was performed on the hydrochloride, it crystallized with 1.00 H_2O. [e]as hydrochloride · 1.00 H_2O. [f]Method A. [g]Method B. [h]Elemental analysis was performed on the hydrochloride. [i]as hydrochloride.

Table 3: Chemical and physical data of ethyl 6-(1,4-dihydro-4-oxo-1H-quinolin-1-yl)hexanoates (**3a–e, h**).

Cpd	R	R′	Formula[a]	Mp(°C) (solvent)	Yield (%)	IR: cm^{-1}[b]	^1H NMR: δ[c]
3a	H	H	$C_{17}H_{21}NO_3$[d]	100–103[e] (acetone)	35[f]	1715 (COO), 1610 (C=O)[g]	8.50 (d, $J_{5,6}$ = 7 Hz, 1H, 5-H), 7.80–7.20 (m, 4H, 2-H, 6-H, 7-H, 8-H), 6.20 (d, $J_{2,3}$ = 8 Hz, 1H, 3-H), 4.30–3.85 (m, 4H, NCH_2, $COOCH_2$), 2.25 (t, 2H, CH_2COO), 2.00–1.30 (m, 6H, $3CH_2$), 1.20 (t, 3H, CH_3)[g].
						2600–2080, 2000–1800 (NH); 1710 (COO), 1600 (C=O)[e]	8.96 (d, $J_{2,3}$ = 7.0 Hz, 1H, 2-H), 8.53 (dd, $J_{7,8}$ = 8.4 Hz, $J_{6,8}$ = 1.0 Hz, 1H, 8-H), 8.30 (d, $J_{5,6}$ = 8.9 Hz, 1H, 5-H), 8.22–8.12 (m, $J_{5,6}$ = 8.9 Hz, $J_{6,7}$ = 7.0 Hz, $J_{6,8}$ = 1.0 Hz, 1H, 6-H), 7.93–7.81 (m, $J_{7,8}$ = 8.4 Hz, $J_{6,7}$ = 7.0 Hz, $J_{5,7}$ = 1.0 Hz, 1H, 7-H), 7.20 (d, $J_{2,3}$ = 7.0 Hz, 1H, 3-H), 4.80 (t, 2H, NCH_2), 4.07 (q, 2H, $COOCH_2$), 2.33 (t, 2H, CH_2COO), 2.14–1.94 (m, 2H, CH_2), 1.80–1.59 (m, 2H, CH_2), 1.59–1.35 (m, 2H, CH_2), 1.20 (t, 3H, CH_3)[e].
3b	H	OCH_3	$C_{18}H_{23}NO_4$[h]	149–153[i] (acetone)	23[l]	1715 (COO), 1615 (C=O)[g]	8.06 (dd, $J_{5,6}$ = 8.0 Hz, $J_{5,7}$ = 1.3 Hz, 1H, 5-H), 7.53 (d, $J_{2,3}$ = 7.6 Hz, 1H, 2-H), 7.31 (t, $J_{5,6}$ = 8.0 Hz, $J_{6,7}$ = 8.0 Hz, 1H, 6-H), 7.14 (dd, $J_{6,7}$ = 8.0 Hz, $J_{5,7}$ = 1.3 Hz, 1H, 7-H), 6.27 (d, $J_{2,3}$ = 7.6 Hz, 1H, 3-H), 4.23–4.11 (m, 4H, NCH_2, $COOCH_2$), 3.60 (s, 3H, OCH_3), 2.30 (t, 2H, CH_2COO), 2.78–1.30 (m, 6H, $3CH_2$), 1.25 (t, 3H, CH_3)[g].
						2600–2000, 1980–1830 (NH); 1710 (COO), 1600 (C=O)[i]	8.82 (d, $J_{2,3}$ = 7.0 Hz, 1H, 2-H), 8.11 (dd, $J_{5,6}$ = 8.0 Hz, $J_{5,7}$ = 1.6 Hz, 5-H) 7.79 (t, $J_{6,7}$ = 8.0 Hz, $J_{5,6}$ = 8.0 Hz, 1H, 6-H), 7.71 (dd, $J_{6,7}$ = 8.0 Hz, $J_{5,7}$ = 1.6 Hz, 1H, 7-H), 7.16 (d, $J_{2,3}$ = 7.0 Hz, 1H, 3-H), 5.01 (t, 2H, NCH_2) 4.21–4.16 (m, 5H, $COOCH_2$, OCH_3), 2.35 (t, 2H, CH_2COO), 2.00–2.20 (m, 2H, CH_2), 1.78–1.60 (m, 2H, CH_2), 1.60–1.31 (m, 2H, CH_2), 1.24 (t, 3H, CH_3)[i].
3c	OCH_3	H	$C_{18}H_{23}NO_4$[h]	147–149[i] (acetone/ Et_2O)	12[l]	1710 (COO), 1615 (C=O)[g]	7.80 (d, $J_{5,7}$ = 2.7 Hz, 1H, 5-H), 7.67 (d, $J_{2,3}$ = 7.4 Hz, 1H, 2-H) 7.36 (d, $J_{7,8}$ = 9.2 Hz, 1H, 8-H), 7.20 (dd, $J_{5,7}$ = 2.7 Hz, $J_{7,8}$ = 9.2 Hz, 1H, 7-H), 6.30 (d, $J_{2,3}$ = 7.4 Hz, 1H, 3-H), 4.30–4.10 (m, 4H, NCH_2, $COOCH_2$), 3.70 (s, 3H, OCH_3), 2.28 (t, 2H, CH_2COO), 2.10–1.30 (m, 6H, $3CH_2$), 1.22 (t, 3H, CH_3)[g].
						2600–2140, 2100–1800 (NH); 1700 (COO), 1600 (C=O)[i]	8.80 (d, $J_{2,3}$ = 6.9 Hz, 1H, 2-H), 8.25 (d, $J_{7,8}$ = 8.6 Hz, 1H, 8-H), 7.84–7.72 (m, 2H, 5-H, 7-H), 7.14 (d, $J_{2,3}$ = 6.9 Hz, 1H, 3-H), 4.77 (t, 2H, NCH_2), 4.15–3.94 (m, 5H, $COOCH_2$, OCH_3), 2.33 (t, 2H, CH_2COO), 2.10–1.91 (m, 2H, CH_2), 1.75–1.56 (m, 2H, CH_2), 1.56–1.34 (m, 2H, CH_2), 1.20 (t, 3H, CH_3)[i].
3d	OCH_3	OCH_3	$C_{19}H_{25}NO_5$[d]	114–116[e] (acetone)	14[l]	1720 (COO), 1610 (C=O)[g]	7.53 (d, $J_{2,3}$ = 7.5 Hz, 1H, 2-H), 7.45 (d, $J_{5,7}$ = 2.7 Hz, 1H, 5-H), 6.74 (d, $J_{5,7}$ = 2.7 Hz, 1H, 7-H), 6.22 (d, $J_{2,3}$ = 7.5 Hz, 1H, 3-H), 4.10 (q, 2H, $COOCH_2$), 3.92 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 3.62 (t, 2H, NCH_2) , 2.29 (t, 2H, CH_2COO), 1.81–1.24 (m, 6H, $3CH_2$), 1.22 (t, 3H, CH_3)[g].
						2600–2100, 2000–1800 (NH); 1710 (COO), 1600 (C=O)[e]	8.67 (d, $J_{2,3}$ = 6.9 Hz, 1H, 2-H), 7.40 (d, $J_{5,7}$ = 2.6 Hz, 1H, 5-H), 7.27 (d, $J_{5,7}$ = 2.6 Hz, 1H, 7-H), 7.11 (d, $J_{2,3}$ = 6.9 Hz, 1H, 3-H), 4.96 (t, 2H, NCH_2), 4.11 (s, 3H, OCH_3), 4.09 (q, 2H, $COOCH_2$), 3.99 (s, 3H, OCH_3), 2.35 (t, 2H, CH_2COO), 2.02–1.85 (m, 2H, CH_2), 1.76–1.59 (m, 2H, CH_2), 1.52–1.35 (m, 2H, CH_2), 1.22 (t, 3H, CH_3)[e].
3e	H	OBzl	$C_{24}H_{27}NO_4$	104–105 (acetone)	11[f]	1710 (COO), 1610 (C=O)	8.20 (dd, $J_{5,6}$ = 6 Hz, $J_{5,7}$ = 3 Hz, 1H, 5-H), 7.45 (s, 5H, Ph), 7.40–7.10 (m, 3H, 2-H, 6-H, 7-H), 6.20 (d, $J_{2,3}$ = 8 Hz, 1H, 3-H), 5.10 (s, 2H, OCH_2Ph), 4.45–3.95 (m, 4H, NCH_2, $COOCH_2$), 2.10 (t, 2H, CH_2COO), 1.90–1.35 (m, 6H, $3CH_2$), 1.20 (t, 3H, CH_3).
3h	H	OH	$C_{17}H_{21}NO_4$	198–199 (EtOH)	90	2800–2300 (OH), 1710 (COO), 1610 (C=O)	8.20 (dd, $J_{5,6}$ = 6 Hz, $J_{5,7}$ = 3 Hz, 1H, 5-H), 7.45–7.10 (m, 4H, 2-H, 6-H, 7-H, OH), 6.20 (d, $J_{2,3}$ = 8 Hz, 1H, 3-H), 4.30–4.00 (m, 4H, NCH_2, $COOCH_2$), 2.20 (t, 2H, CH_2COO), 2.00–1.30 (m, 6H, $3CH_2$), 1.25 (t, 3H, CH_3).

[a]C, H, and N analyses were within ±0.4% of the theoretical value. [b]KBr or film for oil; [c]$CDCl_3$, or CD_3OD (hydrochlorides), or $[D_6]$DMSO (**3h**). [d]Elemental analysis was performed on the hydrochloride, it crystallized with 1.00 H_2O. [e]as hydrochloride · 1.00 H_2O. [f] Method A. [g]as free bases. [h]Elemental analysis was performed on the hydrochloride.[i]as hydrochloride. [l] Method B.

Table 4: Chemical and physical data of 6-[(4-quinolinyl)oxy]hexanoic acids (**4a–i**).

Cpd	R	R′	Formula[a]	Mp (°C) (solvent)	Yield (%)	IR (KBr): cm^{-1}	^1H NMR ([D$_6$]DMSO): δ
4a	H	H	$C_{15}H_{17}NO_3$	192–194 (DMF)	77	2600–2200, 2000–1800, 1670 (NH); 1570 (COO^-)	12.25–11.80 (bs, 1H, COOH), 8.75 (d, $J_{2,3}$ = 5 Hz, 1H, 2-H), 8.40–7.50 (m, 4H, 5-H-8-H), 7.05 (d, $J_{2,3}$ = 5 Hz, 1H, 3-H), 4.25 (d, 2H, OCH_2), 2.25 (t, 2H, CH_2COO), 2.05–1.35 (m, 6H, $3CH_2$).
4b	H	OCH_3	$C_{16}H_{19}NO_4$[b]	178–180[c] (dil HCl)[c]	71	3000–2200 (NH, OH), 2020–1850 (NH), 1700 (*COOH*)[c]	9.10 (d, $J_{2,3}$ = 7 Hz, 1H, 2-H), 8.00–7.60 (m, 4H, 3-H, 5-H-7-H), 4.60 (t, 2H, OCH_2), 4.15 (s, 3H, OCH_3), 2.30 (t, 2H, CH_2COO), 2.15–1.35 (m, 6H, $3CH_2$)[c].
4c	OCH_3	H	$C_{16}H_{19}NO_4$[d]	165–167[e] (DMF/ acetone)[e]	55	3000–2200 (NH, OH), 2030–1850 (NH), 1700 (*COOH*)[e]	12.15–11.85 (bs, 1H, COOH), 9.15 (d, $J_{2,3}$ = 6 Hz, 1H, 2-H), 8.40 (d, $J_{7,8}$ = 9 Hz, 1H, 8-H), 7.85 (dd, $J_{7,8}$ = 9 Hz, $J_{5,7}$ = 3 Hz, 1H, 7-H), 7.70–7.55 (m, 2H, 3-H, 5-H), 4.55 (t, 2H, OCH_2), 4.00 (s, 3H, OCH_3), 2.30 (t, 2H, CH_2COO), 2.15–1.35 (m, 6H, $3CH_2$)[e].
4d	OCH_3	OCH_3	$C_{17}H_{21}NO_5$[f]	186–188 (EtOH)	85	2600–2200, 2100–1800, 1680 (NH); 1580 (COO^-)	8.55 (d, $J_{2,3}$ = 6 Hz, 1H, 2-H), 7.15–6.90 (m, 2H, 3-H, 5-H), 6.80 (d, $J_{5,7}$ = 1.5 Hz, 1H, 7-H), 4.25 (t,2H, OCH_2), 3.95 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 2.25 (t, 2H, CH_2COO), 2.10–1.35 (m, 6H, $3CH_2$).
4e	H	OBzl	$C_{22}H_{23}NO_4$	195–197 (DMF)	85	2600–2200, 2000–1830, 1680 (NH), 1580 (COO^-)	12.30–11.85 (bs, 1H, COOH), 8.75 (d, $J_{2,3}$ = 6 Hz, 1H, 2-H), 7.85–7.20 (m, 8H, 5-H-7-H, Ph), 7.05 (d, $J_{2,3}$ = 6 Hz, 1H, 3-H), 5.30 (s, 2H, OCH_2Ph), 4.25 (t, 2H, OCH_2), 2.25 (t, 2H, CH_2COO), 2.05–1.30 (m, 6H, $3CH_2$).
4f	H	NO_2	$C_{15}H_{16}N_2O_5$	146–149 (EtOH)	60	2600–2200, 1970–1770, 1680 (NH); 1580 (COO^-); 1540, 1370 (NO_2)	12.05–11.90 (bs, 1H, COOH), 8.95 (d, $J_{2,3}$ = 5 Hz, 1H, 2-H), 8.55–8.20 (m, 2H, 5-H, 7-H), 7.75 (t, 1H, 6-H), 7.25 (d, $J_{2,3}$ = 5 Hz, 1H, 3-H), 4.30 (t, 2H, OCH_2), 2.30 (t, 2H, CH_2COO), 2.10–1.25 (m, 6H, $3CH_2$).
4g	OCH_3	NO_2	$C_{16}H_{18}N_2O_6$	165–168 (EtOH)	81	2660–2400, 2000–1800, 1680 (NH); 1580 (COO^-); 1525, 1350 (NO_2)	12.40–11.70 (bs, 1H, COOH), 8.75 (d, $J_{2,3}$ = 5 Hz, 1H, 2-H), 8.05 (d, $J_{5,7}$ = 3 Hz, 1H, 7-H), 7.65 (d, $J_{5,7}$ = 3 Hz, 1H, 5-H), 7.15 (d, $J_{2,3}$ = 5 Hz, 1H, 3-H), 4.30 (t, 2H, OCH_2), 3.95 (s, 3H, OCH_3), 2.25 (t, 2H, CH_2COO), 2.00–1.35 (m, 6H, $3CH_2$).
4h	H	OH	$C_{15}H_{17}NO_4$	187–189 (EtOH)	74	3500–3100 (OH); 2600–2100, 2000–1800, 1670 (NH); 1580 (COO^-)	12.20–11.80 (bs, 1H, COOH), 8.75 (d, $J_{2,3}$ = 5 Hz, 1H, 2-H), 7.75–6.90 (m, 4H, 3-H, 5-H-7-H), 4.25 (t, 2H, OCH_2), 2.30 (t, 2H, CH_2COO), 2.05–1.35 (m, 6H, $3CH_2$).
4i	H	NH_2	$C_{15}H_{18}N_2O_3$	159–161 (MeOH)	81	3440, 3340 (NH_2); 2600–2200, 2000–1800, 1660 (NH); 1580 (COO^-)	12.25–11.70 (bs, 1H, COOH), 8.65 (d, $J_{2,3}$ = 5 Hz, 1H, 2-H), 8.00–6.30 (m, 6H, 3-H, 5-H, 6-H, 7-H, NH_2), 4.10 (t, 2H, OCH_2), 2.30 (t, 2H, CH_2COO), 2.10–1.25 (m, 6H, $3CH_2$).
4l	OCH_3	NH_2	$C_{16}H_{20}N_2O_4$	160–164 (MeOH)	96	3420, 3300 (NH_2); 2600–2200, 2000–1800, 1680 (NH); 1580 (COO^-)	12.40–11.70 (bs, 1H, COOH), 8.55 (d, $J_{2,3}$ = 6 Hz, 1H, 2-H), 7.00 (d, $J_{2,3}$ = 6 Hz, 1H, 3-H), 6.75 (d, $J_{5,7}$ = 3 Hz, 1H, 5-H), 6.65 (d, $J_{5,7}$ = 3 Hz, 1H, 7-H), 6.30–5.75 (bs, 2H, NH_2), 4.30 (t, 2H, OCH_2), 3.90 (s, 3H, OCH_3), 2.35 (t, 2H, CH_2COO), 2.20–1.40 (m, 6H, $3CH_2$).

[a]C, H, and N analyses were within ±0.4% of the theoretical value. [b]Elemental analysis was performed on the hydrochloride, it crystallized with 0.65 H_2O. [c]as hydrochloride · 0.65 H_2O. [d]Elemental analysis was performed on the hydrochloride. [e]as hydrochloride. [f]It crystallized with 1.00 H_2O.

TxB_2 production at the highest tested concentrations, with 29% and 49% inhibition at 100 and 200 μM, respectively. The selective thromboxane synthase inhibitor dazoxiben completely prevented TxB_2 synthesis at 20 μM [11].

In conclusion, while the inactivity of ethyl 6-[(4-quinolinyl)oxy]hexanoates (**2a–d, 2h–l**), and 6-[(4-quinolinyl)oxy]hexanoic acids (**4a–d, 4h–l**) was disappointing, a new class of leukotriene biosynthesis inhibitors was found in *N*-carbethoxypentyl-4-quinolones (**3a–d, 3h**). Although in comparison to other known inhibitors their activity is quite modest, these new compounds may offer the basis for developing more potent compounds.

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Experimental

Synthesis

Melting points are determined in open glass capillaries on a Büchi SMP-20 apparatus and are uncorrected. IR spectra are recorded on a Perkin-Elmer 1310 instrument and ^1H NMR spectra on a Varian EM-390 or a Bruker AM 200 instrument, using TMS as internal standard.

All compounds were routinely checked by thin-layer chromatography (TLC) and ^1H NMR. TLC was performed using 0.25-mm silica gel or aluminum oxide fluorescent coated plates (Merck, Kieselgel or aluminum oxide 60 F254). Components were visualized by UV light. Column chromatography was performed using silica gel Carlo Erba (0.05–0.20 mm) or aluminum oxide Merck (70–230 mesh). Elemental analyses were performed by the Microanalytical Laboratory of Prof. A. Pietrogrande, University of Padova, Italy, and were within ± 0.4 of theoretical values. The substituted 4-hydroxyquinolines (**1b–g**) were previously described[7,12,13,14], while 4-hydroxyquinoline (**1a**) was commercially available.

*Alkylation of 4-hydroxyquinolines (**1a–g**)*

Method A

The appropriate 4-hydroxyquinoline (0.1 moles) was added to a solution of sodium (0.1 moles) in absolute ethyl alcohol (35 ml) and the mixture was stirred at room temperature until separation of the sodium salt. DMSO was added in the necessary amount to dissolve the salt, and ethyl alcohol was completely removed under reduced pressure. Ethyl 6-bromohexanoate (0.1 moles) was added and the mixture was stirred overnight at room temperature. After dilution with water, the product was filtered off and crystallised (**2f**), or extracted with ethyl acetate (**2a, 2c–e, 2g**) or chloroform (**2b**). The organic layer was dried (Na_2SO_4) and evaporated to dryness. The residue was crystallized (**2g, 2b**) or chromatographed on silica gel eluting with ethyl acetate (**2a, 2c–e**). Chemical and physical data of *O*-alkyl and *N*-alkyl derivatives are reported in Table 2 and Table 3, respectively.

Method B

A mixture of the appropriate 4-hydroxyquinoline (**1b–d**)(0.10 moles), ethyl 6-bromohexanoate (0.12 moles), and K_2CO_3 (0.14 moles) in absolute ethyl alcohol (200 ml) was refluxed for 6 h under stirring. After cooling, the precipitate was filtered and washed with ethyl alcohol. The filtrate was evaporated to dryness, and the residue was chromatographed on silica gel eluting with ethyl acetate (**2c** and **3c**) or on aluminum oxide eluting with chloroform (**2b** and **3b**, **2d** and **3d**). Chemical and physical data of *O*-alkyl and *N*-alkyl derivatives are reported in Table 2 and Table 3, respectively.

*Synthesis of acids **4a, 4c–g***

A suspension of ester (**2a, 2c–g**) (0.01 moles) in 2N NaOH (100 ml) was refluxed for 2h under stirring. After cooling, the mixture was neutralized with 2N HCl, the precipitate was collected by filtration, washed with water, and crystallized (Table 4).

*6-[(8-methoxy-4-quinolinyl)oxy]hexanoic acid hydrochloride (**4b**)*

A solution of ester **2b** (0.01 moles) in 2N HCl (30 ml) was refluxed for 2h under stirring. After cooling, the precipitate was collected by filtration, washed with water, and crystallized (Table 4).

*Reduction of nitro compounds **2f, 2g, 4f, 4g** to amino compounds **2i, 2l, 4i, 4l***

A mixture of nitro compound (**2f, 2g, 4f, 4g**) (1.5 mmoles) and 5% Pd/C (100 mg) in EtOH (100 ml for **2f, 2g**, 200 ml for **4f, 4g**) was hydrogenated at 45 psi for 3h. After filtration, the filtrate was concentrated and the residue crystallized. Chemical and physical data of esters **2i, 2l** and acids **4i, 4l** are reported in Table 2 and Table 4, respectively.

*Hydrogenolysis of 8-benzyloxyquinolynes **2e, 3e, 4e***

A mixture of the ether (**2e, 3e, 4e**) (1 mmole) and 5% Pd/C (100 mg) in EtOH (100 ml for **2e, 3e**, 250 ml for **4e**) was hydrogenated at 50 psi for 3h. After filtration, the filtrate was concentrated and the residue crystallized. Chemical and physical data of the 8-hydroxy compounds **2h, 3h** and **4h** are reported in Table 2, 3 and 4, respectively.

Biochemical tests

Venous blood was obtained from healthy volunteers who had not taken any drug for at least two weeks and rapidly collected on heparin (10 U/ml). All experiments were carried in duplicate by aliquoting 1 ml of whole blood into polystyrene tubes, 5 μl of the test compound solution or of the solvent (DMSO) were added (final concentrations from 200 to 10 μM), mixed and gently shaken at room temperature for 10 min. Eicosanoid synthesis was triggered by 10 μM Ca ionophore A23187, dissolved in DMSO. Blood samples were mixed for 10 s and incubated at 37 °C for 30 min. The reaction was stopped by adding 10 μl of a solution containing 4-hydroxy-TEMPO 0.5 mmoles, EDTA 0.1 mmoles, indomethacin 0.1 mmoles; 700 μl of blood were transferred for LTB_4 assay and 300 μl centrifuged at 14,000 rpm for 2 min and supernatant stored at –20 °C for TxB_2 assay. Platelet and leukocyte count on whole blood was performed by phase-contrast optical microscopy.

TxB_2 was determined by specific radioimmunoassay on unextracted plasma samples[8]. LTB_4 was measured by HPLC after solvent extraction of samples as previously described[9]. Briefly, after addition of 25 ng of the internal standard PGB_2, the samples were extracted with 7 ml of ethyl acetate, shaken for 10 min, centrifuged at 4 °C for 15 min at 2600 rpm. The transferred upper phase was dried under nitrogen stream, and the residue redissolved in the mobile phase of HPLC: methanol, acetic acid 0.1% in bidistilled water (adjusted to pH 5.6 with NH_4OH) acetonitrile (60, 35, 5) and injected in a reverse phase column Merck (4 μM Superspher 100 RP18 Lichro CART, 250x4.6 mm i.d.) of an HPLC (Beckman, System Gold, Mod 126, equipped with a Diode Array detector, Mod. 168). The flow rate was 0.5 ml/min. LTB_4 peak was recognized on the basis of the retention time and UV spectra compared to authentic standards. Concentrations were calculated from a standard curve of the ratio between the absorbance value of LTB_4 and the absorbance value of the internal standard.

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