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Synthesis of (2*E*)-2-methyl-3-(4-{[4-(quinolin-2-ylmethoxy)phenyl]sulfanyl}phenyl)prop-2-enoic acid (VUFB 20609) and 2-methyl-3-(4-{[4-(quinolin-2-ylmethoxy)phenyl]sulfanyl}phenyl)propanoic acid (VUFB 20584) as potential antileukotrienic agents

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Abstract

The syntheses of (2*E*)-2-methyl-3-(4-{[4-(quinolin-2-ylmethoxy)phenyl]sulfanyl}phenyl) prop-2-enoic acid (VUFB 20609) and racemic 2-methyl-3-(4-{[4-(quinolin-2-ylmethoxy) phenyl]sulfanyl}phenyl)propanoic acid (VUFB 20584) as new potential antileukotrienic drugs are described. Due to a low reactivity of the 4-substituted aryl bromides (coupling of the 4-substituted aryl bromides do not provide an activating functional group with 4-methoxybenzene-1-thiol), special conditions, in particular specific heterogeneous copper catalysts, were used. Catalytic hydrogenation of the conjugated double bond on Pd/C in the presence of the sulfanyl group is discussed. In-vitro cytotoxicity testing was performed using a microplate colorimetric acid phosphatase assay. Antiplatelet activity was evaluated using an in-vitro test in human platelet-rich plasma. Some substances inhibited arachidonic acid-induced platelet aggregation.

Introduction

Leukotrienes are generated from arachidonic acid as a result of 5-lipoxygenase action (Samuelsson et al 1987). Leukotrienes play an important role in the inflammatory process accompanying allergic diseases of the respiratory, gastrointestinal and dermatological systems. Peptidoleukotrienes C_4 and D_4 were characterized (Samuelsson 1983) as the components of the slowly reacting substance of anaphylaxis, SRS-A (Bocklehurst 1960). SRS-A has been found to be the inflammation mediator of bronchial constriction; this substance increases vascular permeability in diseases associated with acute hypersensitivity. Hence, antileukotrienic agents could be of importance in the treatment of asthma, where they may represent complementary or alternative therapies to corticosteroids. Leukotriene B_4 (LTB_4) is another important mediator, stimulating aggregation and degranulation of human neutrophils, supporting chemotaxis of leucocytes and triggering pro-inflammatory processes in various other cells. For example, LTB_4 induces inflammatory changes in psoriasis, atopic eczema, ulcerative colitis and rheumatoid arthritis (Henderson 1994). Antileukotrienic agents can be classified according to their mechanism of action as: 5-lipoxygenase inhibitors (non-specific inhibitors, arachidonic acid substrate analogues, redox inhibitors — antioxidants, iron chelating compounds, specific substrate inhibitors, 5-lipoxygenase activating protein (FLAP) inhibitors); leukotriene A_4 (LTA_4)-hydrolase inhibitors; leukotriene receptor antagonists; compounds with dual activity (Musser & Kreft 1992; Opletalová et al 1997).

Research on potential nonsteroidal anti-inflammatory drugs (arylalkanoic acids) has been performed in the Research Institute for Pharmacy and Biochemistry (VUFB a.s.) in Prague for 45 years (Kuchař 2001). At the beginning of the 1990s, the research was focused on antileukotrienic agents with potential multiple mechanisms of action. Several series of derivatives were synthesized (Kuchař et al 1998), and the results of antileukotrienic

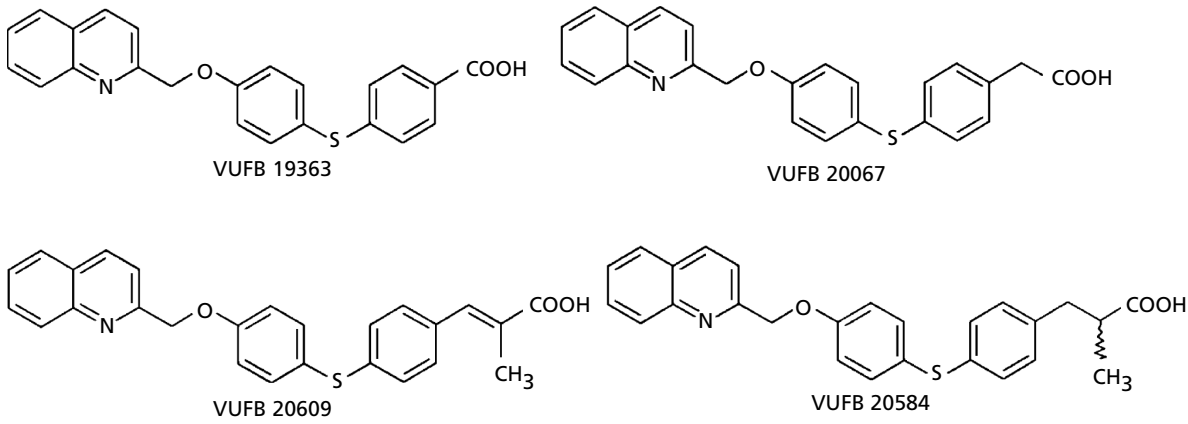

Figure 1 Patented VUFB 19363 and VUFB 20067 tested as potential antileukotrienic agents and prepared C_4-homologues: VUFB 20609, VUFB 20584.

bioassays were interpreted using a standard QSAR approach (Kuchař et al 1999a). Quinoline derivatives of arylsulfanylphenylcarboxylic acids were found and their structural parameters further optimized. As a result, antileukotrienic compounds VUFB 19363 and VUFB 20067 (Figure 1) were prepared and patented (Kuchař et al 1999b). Our work aimed at preparing higher homologues (C_4), with anticipated improvement of biological activity (Figure 1). The main reason for the prolongation of the aliphatic linker between the carboxylic group and the aromatic part was the higher antileukotrienic activity of VUFB 20067 (acetic acid derivative) compared with VUFB 19363 (benzoic acid derivative) (Kuchař et al 1999a; Kuchař 2001). Moreover, prolongation of the chain reduces the lipophilicity of both patented compounds. Finally, the introduction of a chiral centre might have an interesting effect on biological activity.

Materials and Methods

Chemistry

All organic solvents used for the synthesis were of analytical grade. The solvents were dried and freshly distilled under an argon atmosphere. Copper catalyst Cu_2O (powder, 97% purity) and Cu_2S (powder, 99% purity) were purchased from Sigma-Aldrich and 10% Pd/$BaSO_4$ and 10% Pd/C were from Fluka. Kieselgel 60, 0.040–0.063 mm (Merck, Germany) was used for flash chromatography (F_c). TLC was performed on Silufol UV 254 plates (Kavalier, Votice, Czech Republic) and HPTLC-Fertigplatten CN F_{254}s (Merck, Germany) in the following solvent systems: Et_2O–petroleum ether (1:4, A); Et_2O–petroleum ether (1:3, B); Et_2O–petroleum ether (1:1, C); Et_2O–petroleum ether (6:4, D); Et_2O–petroleum ether (3:1, E); Et_2O–petroleum ether (1:2, F); Et_2O–petroleum ether (3:7, G). The plates were illuminated under UV (254 nm) and the spots were then visualized using a solution of Bromothymol Blue in NaOH. Melting points were determined on Boetius PHMK 05 (VEB Kombinat Nagema, Radebeul, Germany) and are uncorrected. Elemental analyses were carried out on an automatic microanalyser EA1110CE (CE Instruments, Milano, Italy). Infrared spectra were recorded with neat oils (for non-crystalline materials) and in KBr pellets (for crystalline materials) on an IR-spectrometer Nicolet Impact 400. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury – Vx BB 300 (299.95 MHz for ^{1}H and 75.43 MHz for ^{13}C) (Bruker Comp., Karlsruhe, Germany). Chemical shifts are given relative to internal $Si(CH_3)_4$.

*Methyl 4-[(4-methoxyphenyl)sulfanyl]benzoate (**2**)*

Method A. $SOCl_2$ (15 mL) was added drop-wise at −10°C to methanol (100 mL) under stirring. After 1 h, acid **1** (3.0 g, 11.5 mmol) (Figure 2) was added. The cooling bath was removed and the reaction mixture was then stirred for 24 h. The solvent was removed at reduced pressure. F_c on silica gel (eluted with Et_2O–petroleum ether, 1:4) provided a white crystalline compound. Yield: 3.00 g (95%).

Method B. Thiol **3** (2.0 g, 14.3 mmol, 1.7 mL) was added slowly to an ice-cool suspension of NaH (60% dispersion in mineral oil, 0.4 g, 16.7 mmol) in dry dimethylformamide (DMF, 50 mL). The mixture was stirred for a few minutes until the evolution of hydrogen gas stopped. Ester **4** (2.15 g, 10.0 mmol) and copper(I) oxide (0.35 g, 2.5 mmol) were then added, and the mixture was refluxed under argon for 3 h. The cooled mixture was poured onto ice and extracted with Et_2O. The combined organic extracts were washed with aqueous ammonia (35%) and water, dried over anhydrous $MgSO_4$ and filtered. The solvent was removed at reduced pressure. F_c on silica gel (eluted with Et_2O–petroleum ether, 1:9) gave a white crystalline compound. Yield: 2.50 g (91%).

R_F (A) 0.41; mp: 76–77 °C. For $C_{15}H_{14}O_3S$ (274.50) calculated: 65.67% C, 5.14% H, 11.69% S; found: 65.77% C, 5.10% H, 11.70% S. IR (KBr, cm^{-1}): 1724 (ester), 1580 (Ph), 1462 (OCH_3), 1110 (S-Ph). ^{1}H NMR (300 MHz, $CDCl_3$), δ: 7.90–7.80 (m AA'BB',2H, H2', H6'), 7.52–7.43

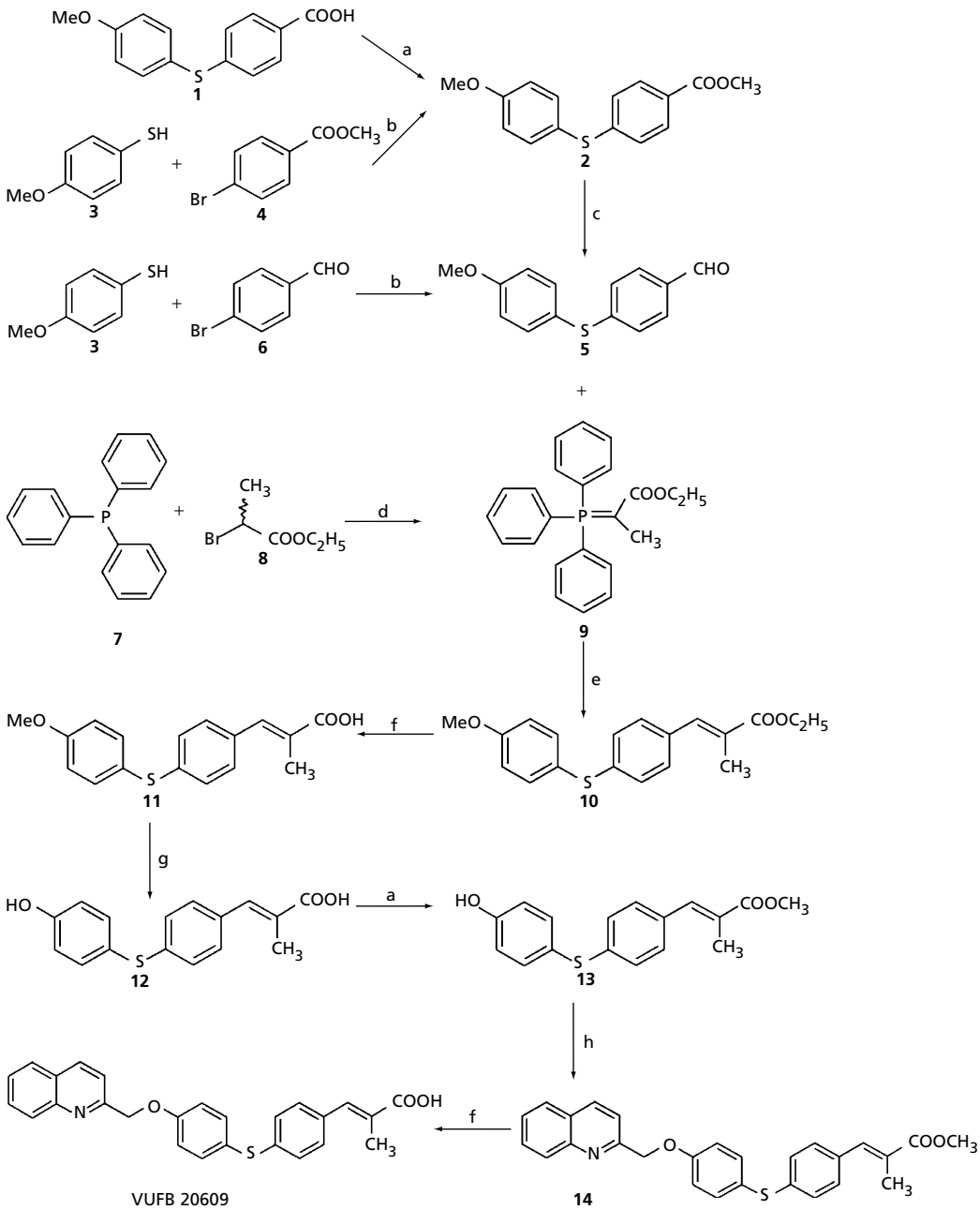

Figure 2 Synthesis of compound VUFB 20609. Reagents: a, MeOH, $SOCl_2$; b, NaH, Cu_2O, DMF; c, DIBAL, toluene; d, MeCN; e, toluene; f, NaOH (i), HCl (ii); g, BBr_3, CH_2Cl_2; h, Cl-quinaldine, K_2CO_3, KI, DMF.

(m AA'BB', 2H, H2'', H6''), 7.12–7.04 (m AA'BB', 2H, H3', H5'), 7.00–6.91 (m AA'BB', 2H, H3'', H5''), 3.87 (s, 3H, OCH_3), 3.85 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$), δ: 166.8, 160.6, 146.4, 136.7, 129.9, 126.6, 125.7, 121.5, 115.3, 55.4, 52.0.

*4-[(4-Methoxyphenyl)sulfanyl]benzaldehyde (**5**)*

Method A. The ester **2** (2.0 g, 7.3 mmol) in dry toluene (50 mL) was cooled to −70°C under argon and then DIBAL (1.5 mol solution in toluene, 10.0 mmol) was added drop-wise to the stirring reaction mixture at such a rate that the temperature did not exceed −60°C. When all DIBAL had been added, the mixture was kept at −45°C for an additional 4 h under argon, and then methanol (2 mL) was added slowly, maintaining the temperature at −45°C for a further 30 min. A mixture of EtOAc and of saturated aqueous sodium-potassium tartrate solution was added to the mixture, and the temperature was allowed to rise to room temperature. The reaction mixture

was transferred to a separation funnel and very strongly extracted using additional EtOAc and saturated tartrate solution (sufficient to dissolve all of the aluminium salts that precipitated during workup). The EtOAc extracts were combined, dried over anhydrous $MgSO_4$, and evaporated in-vacuo to give a yellow oil. F_c on silica gel (eluted with Et_2O–petroleum ether, 1:4) gave a white crystalline compound. Yield: 1.70 g (96%).

Method B. For the coupling of thiol **3** and aldehyde **6**, conditions were as for the synthesis of compound **2**, method B (see above). F_c on silica gel (eluted with Et_2O–petroleum ether, 1:9) gave a white crystalline compound. Yield: 3.70 g (96%).

R_F (B) 0.22; mp: 46–46.5°C. For $C_{14}H_{12}O_2S$ (244.31) calculated: 68.83% C, 4.95% H, 13.12% S; found: 68.91% C, 4.99% H, 13.09% S. IR (KBr, cm^{-1}): 2836, 1697 (CHO), 1591 (Ph), 1461 (OCH_3), 1099 (S-Ph). ^{1}H NMR (300 MHz, $CDCl_3$), δ: 9.88 (s, 1H, CHO), 7.72–7.65 (m AA'BB', 2H, H2', H6'), 7.52–7.45 (m AA'BB', 2H, H2'', H6''), 7.16–7.10 (m AA'BB', 2H, H3', H5'), 7.01–6.94 (m AA'BB', 2H, H3'', H5''), 3.86 (s, 3H, OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$), δ: 191.2, 160.8, 149.0, 137.0, 133.2, 130.0, 125.8, 120.7, 115.4, 55.4.

*Ethyl (2E)-3-{4-[(4-methoxyphenyl)sulfanyl]phenyl}-2-methylprop-2-enoate (**10**)*

Method A. Aldehyde **5** (0.5 g, 2.1 mmol) and phosphorane **9** (Kuchař et al 1973) (2.0 g, 3.2 mmol) were dissolved in toluene (80 mL) and refluxed under argon for 10 h. The solvent was removed under reduced pressure, and the residue suspended in 30 mL of Et_2O–petroleum ether (1:3). After 12 h the solid was filtered off, the filtrate evaporated in-vacuo and the residue subjected to F_c on silica gel. Elution with Et_2O–petroleum ether (1:9) provided a white crystalline compound. Yield: 0.59 g (88%).

Method B. For the coupling of thiol **3** and ethyl ester **15** (Figure 3), conditions were as for the synthesis of compound **2**, method B (see above). F_c on silica gel (eluted with Et_2O–petroleum ether, 1:9) provided a white crystalline compound. Yield: 7.80 g (64%).

R_F (B) 0.47; mp: 56.5–57.5°C. For $C_{19}H_{20}O_3S$ (328.42) calculated: 69.49% C, 6.16% H, 9.76% S; found: 69.52% C, 5.98% H, 9.73% S. IR (KBr, cm^{-1}): 2971 (CH_3), 1734 (ester), 1639 (C=C), 1591 (Ph), 1462 (OCH_3), 1110 (S-Ph). ^{1}H NMR (300 MHz, $CDCl_3$), δ: 7.60 (bs, 1H, CH), 7.49–7.43 (m AA'BB', 2H, Ar), 7.30–7.24 (m AA'BB', 2H, Ar), 7.15–7.08 (m AA'BB', 2H, Ar), 6.97–6.90 (m AA'BB', 2H, Ar), 4.26 (q, 2H, J = 7.14 Hz, OCH_2), 3.84 (s, 3H, OCH_3), 2.09 (d, 3H, J = 1.37 Hz, CH_3), 1.34 (t, 3H, J = 7.14 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$), δ: 168.7, 160.2, 139.9, 138.0, 136.1, 133.0, 130.2, 128.1, 126.9, 122.9, 115.1, 60.9, 55.4, 14.3.

*(2E)-3-{4-[(4-Methoxyphenyl)sulfanyl]phenyl}-2-methylprop-2-enoic acid (**11**)*

Ethyl ester **10** (1.0 g, 3.05 mmol) was suspended in a 10% solution of NaOH and refluxed for 1 h. Charcoal was added to the hot solution and then filtered off. Cooled filtrate was acidified with 15% HCl to pH 2 and filtered. A pure white crystalline product was obtained. Yield: 0.84 g (92%). R_F (C) 0.55; mp: 178–179°C. For $C_{17}H_{16}O_3S$ (300.38) calculated: 67.98% C, 5.37% H, 10.67% S; (found): 68.19% C, 5.35% H, 10.63% S. IR (KBr, cm^{-1}): 3085 (acid), 2969 (CH_3), 1643 (C=C), 1580 (Ph), 1462 (OCH_3), 1107 (S-Ph). ^{1}H NMR (300 MHz, $CDCl_3$), δ: 7.74 s, 1 H (CH); 7.51–7.43 (m AA'BB', 2H, H2'', H6''), 7.35–7.27 (m AA'BB', 2H, H2', H6'), 7.16–7.08 (m AA'BB', 2H, H3', H5'), 6.98–6.90 (m AA'BB', 2H, H3'', H5''), 3.84 (s, 3H, OCH_3), 2.12 (d, 3H, J = 1.37 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$), δ: 174.1, 160.3, 140.8, 140.4, 136.3, 132.6, 130.5, 126.8, 122.6, 115.2, 55.4, 13.8.

*(2E)-3-{4-[(4-Hydroxyphenyl)sulfanyl]phenyl}-2-methylprop-2-enoic acid (**12**)*

Acid **11** (1.0 g, 3.3 mmol) was solved in dry CH_2Cl_2 under argon atmosphere and cooling to −50°C and then BBr_3 (1.0 mol solution in CH_2Cl_2, 20 mL) was added drop-wise. The cooling bath was removed and the reaction mixture was stirred for 3 h. To stop the reaction, the mixture was poured to water and the CH_2Cl_2 layer was separated in a separating funnel. The water layer was extracted with Et_2O, the combined organic extracts were dried over anhydrous $MgSO_4$, filtered and the solvent was removed under reduced pressure. F_c on silica gel (eluted with Et_2O–petroleum ether, 6:4) provided a white crystalline compound. Yield: 0.86 g (91%). R_F (D) 0.42; mp: 176–177°C. For $C_{16}H_{14}O_3S$ (286.35) calculated: 67.11% C, 4.93% H, 11.20% S; found: 67.19% C, 4.95% H, 11.19% S. IR (KBr, cm^{-1}): 3330 (acid), 2969 (CH_3), 1640 (C=C), 1580 (Ph), 1219 (Ph-OH), 1111 (S-Ph). ^{1}H NMR (300 MHz, DMSO-d_6), δ: 12.49 (bs, 1H, OH), 9.95 (s, 1H, OH), 7.52–7.47 (m, 1H, CH), 7.42–7.32 (m, 4H, H2', H6', H2'', H6''), 7.10–7.01 (m AA'BB', 2H, H3', H5'), 6.90–6.82 (m AA'BB', 2H, H3'', H5''), 1.98 (d, 3H, J = 1.37 Hz, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6), δ: 169.6, 158.9, 140.1, 137.2, 136.8, 132.7, 130.7, 128.3, 126.3, 119.3, 117.2, 14.2.

*Methyl (2E)-3-{4-[(4-hydroxyphenyl)sulfanyl]phenyl}-2-methylprop-2-enoate (**13**)*

For the esterification of acid **12**, conditions were as for the synthesis of compound **2**, method A (see above). F_c on silica gel (eluted with Et_2O–petroleum ether, 3:1) provided a white crystalline compound. Yield: 0.99 g (95%). R_F (E) 0.61; mp: 174–175°C. For $C_{17}H_{16}O_3S$ (300.38) calculated: 67.98% C, 5.37% H, 10.67% S; found: 67.99% C, 5.35% H, 10.64% S. IR (KBr, cm^{-1}): 2973 (CH_3), 1734 (ester), 1640 (C=C), 1580 (Ph), 1220 (Ph-OH), 1110 (S-Ph). ^{1}H NMR (300 MHz, $CDCl_3$), δ: 7.61 (bs, 1H, CH), 7.44–7.38 (m AA'BB', 2H, H2'', H6''), 7.30–7.24 (m AA'BB', 2H, H2', H6'), 7.15–7.09 (m AA'BB', 2H, H3', H5'), 6.92–6.86 (m AA'BB', 2H, H3'', H5''), 3.82 (s, 3H, OCH_3), 2.10 (d, 3H, J = 1.37 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$), δ: 169.5, 156.6, 140.1, 138.6, 136.4, 132.8, 130.3, 127.6, 126.9, 122.8, 116.7, 52.2, 14.1.

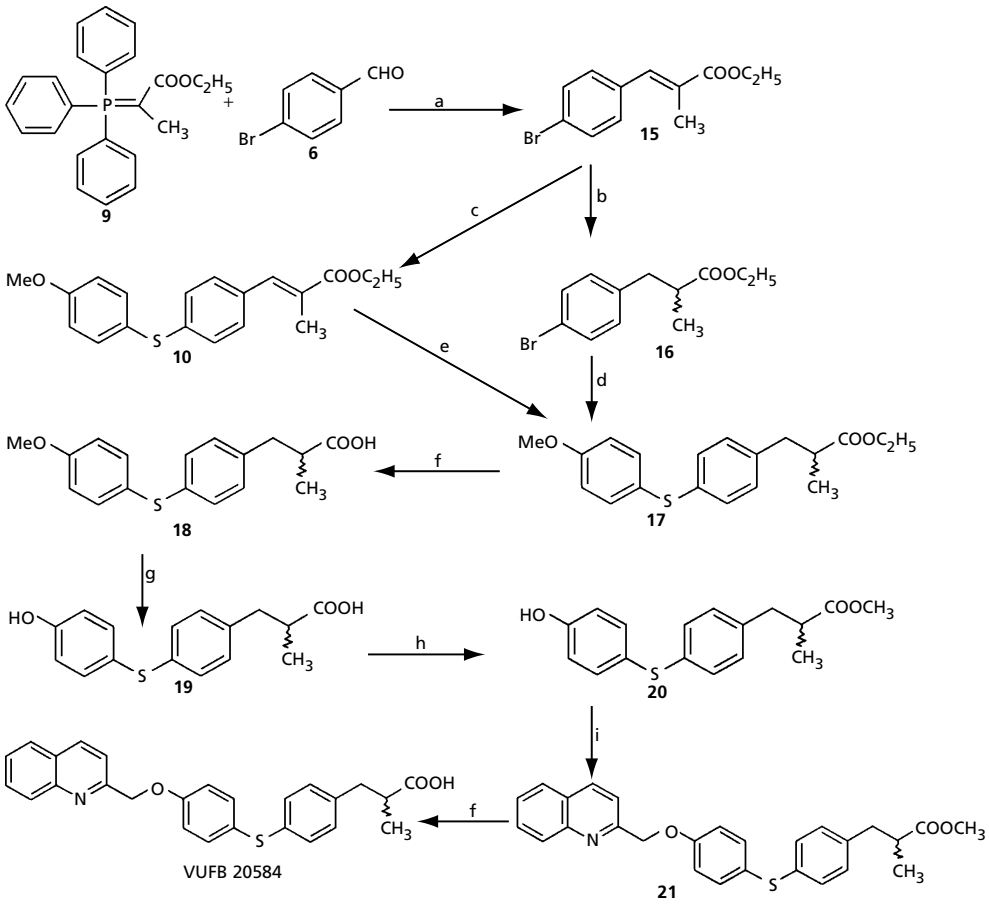

Figure 3 Synthesis of compound VUFB 20584. Reagents: a, toluene; b, H_2, $Pd/BaSO_4$, $AcONa.3H_2O$, AcOH; c, $4\text{-}CH_3OC_6H_4SH$, NaH, Cu_2O, DMF; d, $4\text{-}CH_3OC_6H_4SH$, NaH, Cu_2S, DMF; e, H_2, Pd/C, AcOH; f, NaOH (i), HCl (ii); g, Pyr.HCl; h, MeOH, $SOCl_2$; i, Cl-quinaldine, K_2CO_3, KI, DMF.

*Methyl (2E)-2-methyl-3-(4-{[4-(quinolin-2-yl-methoxy)phenyl]sulfanyl}phenyl)prop-2-enoate (**14**)*

A stirred mixture of methyl ester **13** (1.0 g, 3.33 mmol), 2-chloromethylquinoline (0.65 g, 3.5 mmol), anhydrous K_2CO_3 (3.0 g), KI (0.1 g) and DMF (100 mL) was refluxed for 8 h. The hot mixture was filtered, the filtration cake was washed with boiling DMF and the solution was evaporated under reduced pressure. F_c on silica-gel-impregnated Et_3N (eluted with Et_2O–petroleum ether, 3:1) provided a white crystalline compound. Yield: 0.60 g (40%). R_F (E) 0.41; mp: 105–106°C. For $C_{27}H_{23}NO_3S$ (441.55) calculated: 73.45% C, 5.25% H, 3.17% N, 7.26% S; found: 73.40% C, 5.28% H, 3.15% N, 7.23% S. IR (KBr, cm^{-1}): 2972 (CH_3), 1735 (ester), 1640 (C=C), 1627 (quinoline), 1591 (Ph), 1270 (C-O-C), 1109 (S-Ph). ^{1}H NMR (300 MHz, DMSO-d_6), δ: 8.38 (d, 1H, J = 8.51 Hz, H8'''), 8.15–8.05 (m, 2H, H5''', H6'''), 7.87–7.79 (m, 1H, H7'''), 7.71 (d, 1H, J = 8.52 Hz, H4'''), 7.68–7.61 (m, 1H, H3'''), 7.59–7.51 (m, 1H, CH), 7.49–7.43 (m AA'BB', 2H, H2'', H6''), 7.43–7.38 (m AA'BB', 2H, H2', H6'), 7.19–7.15 (m AA'BB', 2H, H3', H5'), 7.15–7.05 (m AA'BB', 2H, H3'', H5''), 5.41 (s, 2H, OCH_2), 3.81 (s, 3H, OCH_3), 2.10 (d, 3H, J = 1.37 Hz, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6), δ: 165.0, 158.9, 157.3, 147.5, 138.2, 137.2, 136.7, 135.9, 133.1, 130.3, 130.0, 128.8, 128.5, 128.1, 127.6, 127.2, 126.7, 123.8, 119.4, 116.0, 71.3, 52.0, 14.1.

(2E)-2-Methyl-3-(4-{[4-(quinolin-2-ylmethoxy) phenyl]sulfanyl}phenyl) prop-2-enoic acid (VUFB 20609)

Ester **14** (0.9 g, 2.04 mmol) was suspended in a 10% solution of NaOH and refluxed for 1 h. Charcoal was added to the hot solution and then filtered off. Cold solution was acidified with AcOH to pH 4, the mixture was cooled to 5°C, and the formed precipitate was filtered. A crude product was purified by crystallization from EtOH/H_2O, and a white crystalline compound was obtained. Yield: 0.85 g (97%). R_F (D) 0.2; mp: 170–171°C. For $C_{26}H_{21}NO_3S$ (427.53) calculated: 73.05% C, 4.95% H, 3.28% N, 7.05% S; found: 72.95% C, 4.86% H, 3.27% N, 7.01% S. IR (KBr, cm^{-1}): 3232 (acid), 2969 (CH_3), 1638 (C=C), 1630 (quinoline), 1591 (Ph), 1270 (C-O-C), 1110 (S-Ph). ^{1}H NMR (300 MHz, DMSO-d_6), δ: 12.46 (bs, 1H, OH), 8.43 (d, 1H, J = 8.51 Hz, H8'''), 8.05–7.97 (m, 2H, H5''', H6'''), 7.82–7.75 (m, 1H, H7'''), 7.68 (d, 1H, J = 8.52 Hz, H4'''), 7.65–7.58 (m, 1H, H3'''), 7.52–7.49 (m, 1H, CH),

7.49–7.43 (m AA'BB', 2H, H2'', H6''), 7.42–7.36 (m AA'BB', 2H, H2', H6'), 7.20–7.14 (m AA'BB', 2H, H3', H5'), 7.14–7.08 (m AA'BB', 2H, H3'', H5''), 5.41 (s, 2H, OCH_2), 1.98 (d, 3H, J = 1.37 Hz, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6), δ: 169.5, 159.1, 157.4, 147.2, 139.0, 137.4, 137.1, 136.1, 133.2, 130.7, 130.2, 128.8, 128.6, 128.2, 127.4, 127.2, 126.9, 122.8, 119.8, 116.6, 71.2, 14.2.

*Ethyl (2E)-3-(4-bromophenyl)-2-methylprop-2-enoate (**15**)*

For the Wittig reaction of aldehyde **6** and phosphorane **9**, conditions were as for the synthesis of compound **10**, method A (see above). F_c on silica gel (eluted with Et_2O–petroleum ether, 1:3) provided a colourless oil. Yield: 10.90 g (94%). R_F (F) 0.63. For $C_{12}H_{13}BrO_2$ (269.14) calculated: 53.55% C, 4.87% H; found: 53.60% C, 4.82% H. IR (neat, cm^{-1}): 2982 (CH_3), 1728 (ester), 1635 (C=C), 1580 (Ph). ^{1}H NMR (300 MHz, $CDCl_3$), δ: 7.58 (s, 1H, CH), 7.55–7.45 (m AA'BB', 2H, Ar), 7.30–7.20 (m AA'BB', 2H, Ar), 4.26 (q, 2H, J = 7.14 Hz, CH_2), 2.08 (s, 3H, CH_3), 1.35 (t, 3H, J = 7.14 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$), δ: 168.4, 137.3, 134.8, 131.5, 131.1, 129.3, 122.4, 61.0, 14.3, 14.0.

*Ethyl 3-(4-bromophenyl)-2-methylpropanoate (**16**)*

The cinnamate **15** (2.0 g, 7.4 mmol) was dissolved in 99% AcOH (30 mL) and 10% Pd/$BaSO_4$ (0.63 g) and AcONa. $3H_2O$ (3.58 g) were added. The mixture was bubbled with nitrogen and then kept under hydrogen, while stirring (1000 rev min^{-1}), at 0.1 MPa and 20°C for 8 h. After filtration of the catalyst the solvent was evaporated. F_c on silica gel (eluted with Et_2O–petroleum ether, 1:3) gave a colourless oil. Yield: 1.89 g (95%). R_F (G) 0.63. For $C_{12}H_{15}BrO_2$ (271.16) calculated: 53.16% C, 5.58% H; found: 53.15% C, 5.60% H. IR (neat, cm^{-1}): 2980 (CH_3), 2929 (CH_2), 1724 (ester), 1580 (Ph). ^{1}H NMR (300 MHz, $CDCl_3$), δ: 7.30–7.12 (m, 4H, Ar), 4.07 (q, 2H, J = 7.14 Hz, CH_2), 3.06–2.95 (m, 1H, CH), 2.76–2.60 (m, 2H, CH_2), 1.17 (t, 3H, J = 7.14 Hz, CH_3), 1.14 (d, 3H, J = 7.14 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$), δ: 176.1, 139.4, 128.9, 128.3, 126.2, 60.2, 41.5, 39.7, 16.7, 14.1.

*Ethyl 3-{4-[(4-methoxyphenyl)sulfanyl]phenyl}-2-methylpropanoate (**17**)*

Method A. Thiol **3** (8.4 g, 60.0 mmol) was added slowly to an ice-cool suspension of NaH (60% dispersion in mineral oil, 1.68 g, 70.0 mmol) in dry DMF (150 mL). The mixture was stirred for a few minutes until the evolution of hydrogen gas stopped. Ester **16** (11.0 g, 40.0 mmol) and copper(I) sulfide (1.5 g, 10.0 mmol) were then added, and the mixture was refluxed under argon for 3 h. The cooled mixture was poured onto ice and extracted with Et_2O. The combined organic extracts were washed with aqueous ammonia (35%) and water, dried over anhydrous $MgSO_4$ and filtered. The solvent was removed at reduced pressure. F_c on silica gel (eluted with Et_2O–petroleum ether, 1:9) provided a colourless oil. Yield: 5.00 g (38%). R_F (B) 0.3.

Method B. The ester **10** (Figure 2) (0.33 g, 1.0 mmol) was dissolved in 99% AcOH (30 mL) and 10% Pd/C (2.0 g) was added. The mixture was bubbled with nitrogen and then kept under hydrogen, while stirring (1000 rev min^{-1}), at 0.1 MPa and 20°C for 7 h. After filtration of the catalyst the solvent was evaporated. F_c on silica gel (eluted with Et_2O–petroleum ether, 1:3) gave a colourless oil. Yield: 0.15 g (45%). R_F (F) 0.59. For $C_{19}H_{22}O_3S$ (330.45) calculated: 69.06% C, 6.71% H, 9.70% S; found: 69.05% C, 6.76% H, 9.71% S. IR (neat, cm^{-1}): 2971 (CH_3), 2936 (CH_2), 1730 (ester), 1580 (Ph), 1462 (OCH_3), 1110 (S-Ph). ^{1}H NMR (300 MHz, $CDCl_3$), δ: 7.49–7.43 (m AA'BB', 2H, Ar), 7.30–7.24 (m AA'BB', 2H, Ar), 7.15–7.07 (m AA'BB', 2H, Ar), 6.96–6.90 (m AA'BB', 2H, Ar), 4.25 (q, 2H, J = 7.14 Hz, OCH_2), 3.84 (s, 3H, OCH_3), 3.06–2.95 (m, 1H, CH), 2.76–2.53 (m, 2H, CH_2), 1.34 (t, 3H, J = 7.14 Hz, CH_3); 1.13 (d, 3H, J = 6.87 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$), δ: 176.0, 160.2, 138.0, 136.1, 134.9, 130.2, 128.6, 122.9, 115.1, 60.8, 55.4, 41.4, 39.1, 16.8, 14.1.

*3-{4-[(4-Methoxyphenyl)sulfanyl]phenyl}-2-methylpropanoic acid (**18**)*

For the hydrolysis of ester **17**, conditions were as for the synthesis of compound **11** (see above). A pure white crystalline product was obtained. Yield: 1.15 g (90%). R_F (C) 0.43; mp: 103–104°C. For $C_{17}H_{18}O_3S$ (302.40) calculated: 67.53% C, 6.00% H, 10.60% S; found: 67.49% C, 6.10% H, 10.58% S. IR (KBr, cm^{-1}): 2993 (acid), 2973 (CH_3), 2931 (CH_2), 1580 (Ph), 1462 (OCH_3), 1109 (S-Ph). ^{1}H NMR (300 MHz, DMSO-d_6), δ: 7.41–7.36 (m, 2H, Ar), 7.16–6.92 (m, 6H, Ar), 3.76 (s, 3H, OCH_3), 2.92–2.72 (m, 1H, CH), 2.64–2.42 (m, 2H, CH_2), 1.02 (d, 3H, J = 6.87 Hz, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6), δ: 177.2, 160.2, 138.2, 136.0, 135.1, 130.3, 128.7, 123.9, 115.9, 55.7, 41.7, 39.3, 16.9.

*3-{4-[(4-Hydroxyphenyl)sulfanyl]phenyl}-2-methylpropanoic acid (**19**)*

Acid **18** (0.81 g, 2.9 mmol) and pyridine hydrochloride (2.3 g, 20.1 mmol) were stirred at 240°C under argon atmosphere for 30 min. After cooling to 80°C, water was added, the mixture was cooled to 5°C and extracted with Et_2O. The combined Et_2O extracts were dried over anhydrous $MgSO_4$, filtered and the solvent was removed under reduced pressure. F_c on silica gel (eluted with Et_2O–petroleum ether, 6:4) provided a white crystalline compound. Yield: 0.77 g (98%). R_F (D) 0.36; mp: 88–90°C. For $C_{16}H_{16}O_3S$ (288.37) calculated: 66.64% C, 5.59% H, 11.12% S; found: 66.70% C, 5.64% H, 11.10% S. IR (KBr, cm^{-1}): 3328 (acid), 2970 (CH_3), 2925 (CH_2), 1580 (Ph), 1222 (Ph-OH), 1110 (S-Ph). ^{1}H NMR (300 MHz, $CDCl_3$), δ: 7.38–7.29 (m AA'BB;, 2H, Ar), 7.15–6.98 (m, 4H, Ar), 6.86–6.77 (m AA'BB', 2H, Ar), 3.05–2.92 (m, 1H, CH), 2.78–2.58 (m, 2H, CH_2), 1.18 (d, 3H, J = 6.87 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$), δ: 181.7, 155.8, 136.8, 136.1, 135.3, 129.6, 128.5, 124.6, 116.5, 41.1, 38.7, 16.6.

*Methyl 3-{4-[(4-hydroxyphenyl)sulfanyl]phenyl}-2-methylpropanoate (**20**)*

For the esterification of acid **19**, conditions were as for the synthesis of compound **2**, method A (see above). F_c on silica gel (eluted with Et_2O–petroleum ether, 3:1) provided a white crystalline compound. Yield: 0.76 g (94%). R_F (E) 0.56; mp: 75–77°C. For $C_{17}H_{18}O_3S$ (302.40) calculated: 67.52% C, 6.00% H, 10.60% S; found: 67.57% C, 5.95% H, 10.58% S. IR (KBr, cm^{-1}): 2974 (CH_3), 2928 (CH_2), 1732 (ester), 1580 (Ph), 1222 (Ph-OH), 1111 (S-Ph). ^{1}H NMR (300 MHz, $CDCl_3$), δ: 7.38–7.29 (m AA'BB', 2H, Ar), 7.15–6.98 (m, 4H, Ar), 6.86–6.77 (m AA'BB', 2H, Ar), 3.64 s, 3H, OCH_3), 3.01–2.89 (m, 1H, CH), 2.78–2.55 (m, 2H, CH_2), 1.14 (d, 3H, $J = 6.87$ Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$), δ: 176.8, 156.0, 137.1, 136.1, 135.3, 129.6, 128.5, 124.5, 116.4, 51.8, 41.3, 39.1, 16.7.

*Methyl 2-methyl-3-(4-{[4-(quinolin-2-ylmethoxy)phenyl]sulfanyl}phenyl)propanoate (**21**)*

For the etherification of ester **20**, conditions were as for the synthesis of compound **14** (see above). F_c on silica-gel-impregnated Et_3N (eluted with Et_2O–petroleum ether, 3:1) provided a viscous light yellow oil. Yield: 0.38 g (34%). R_F (E) 0.3. For $C_{27}H_{25}NO_3S$ (446.57) calculated: 73.11% C, 5.68% H, 3.16% N, 7.23% S; found: 73.15% C, 5.66% H, 3.17% N, 7.21% S. IR (neat, cm^{-1}): 2972 (CH_3), 2926 (CH_2), 1734 (ester), 1625 (quinoline), 1600 (Ph), 1268 (C-O-C), 1105 (S-Ph). ^{1}H NMR (300 MHz, $CDCl_3$), δ: 8.21 (d, 1H, $J = 8.51$ Hz, Ar), 8.08 (d, 1H, $J = 8.52$ Hz, Ar), 7.87–7.81 (m, 1H, Ar), 7.78–7.70 (m, 1H, Ar), 7.69–7.64 (m AA'BB', 2H, Ar), 7.60–7.52 (m, 1H, Ar), 7.43–7.35 (m AA'BB', 2H, Ar), 7.23–7.09 (m, 3H, Ar), 7.05–6.98 (m AA'BB', 2H, Ar), 5.39 (s, 2H, OCH_2), 3.61 (s, 3H, CH_3), 3.01–2.90 (m, 1H, CH), 2.75–2.52 (m, 2H, CH_2), 1.12 (d, 3H, $J = 6.60$ Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$), δ: 174.8, 158.6, 157.5, 147.7, 138.3, 137.1, 135.2, 135.2, 129.9, 128.7, 128.4, 128.1, 127.7, 126.6, 126.3, 125.1, 119.1, 115.9, 71.2, 52.0, 41.7, 39.2, 16.7.

2-Methyl-3-(4-{[4-(quinolin-2-ylmethoxy)phenyl]sulfanyl}phenyl)propanoic acid (VUFB 20584)

For the hydrolysis of ester **21**, conditions were as for the synthesis of VUFB 20609 (see above). A crude product was purified by crystallization from $EtOH/H_2O$ and a white crystalline compound was obtained. Yield: 0.43 g (89%). R_F (D) 0.3; mp: 66–67°C. For $C_{26}H_{23}NO_3S$ (429.50) calculated: 72.70% C, 5.40% H, 3.26% N, 7.46% S; found: 72.31% C, 5.86% H, 3.27% N, 7.21% S. IR (KBr, cm^{-1}): 3243 (acid), 2970 (CH_3), 2921 (CH_2), 1627 (quinoline), 1591 (Ph), 1262 (C-O-C), 1108 (S-Ph). ^{1}H NMR (300 MHz, DMSO-d_6), δ: 8.41 (d, 1H, $J = 8.38$ Hz, Ar), 8.03–7.96 (m, 2H, Ar), 7.82–7.71 (m, 2H, Ar), 7.67 (d, 1H, $J = 8.38$ Hz, Ar), 7.64–7.58 (m, 1H, Ar), 7.40–7.32 (m AA'BB', 2H, Ar), 7.16–7.04 (m, 5H, Ar), 5.38 (s, 2H, OCH_2), 2.89–2.76 (m, 1H, CH), 2.64–2.51 (m, 2H, CH_2), 1.00 (d, 3H, $J = 6.60$ Hz, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6), δ: 177.0, 158.6, 157.5, 147.2, 138.3, 137.4, 134.8, 134.7, 130.2, 130.1, 128.8, 128.8, 128.2, 127.5, 126.9, 124.8, 119.8, 116.4, 71.2, 40.7, 38.6, 16.9.

Biology

Cytotoxicity assay

DLKP, a human poorly differentiated squamous lung carcinoma cell line, was established at the National Institute for Cellular Biotechnology (Dublin, Ireland) (Gilvarry et al 1990). Cells were routinely maintained in DMEM:Ham's F12 (1:1 v/v) culture media supplemented with 5% fetal bovine serum. Antibiotics were not used. Cells were maintained at 37°C.

96-well plate cell toxicity assay. On day one cells were trypsinized, seeded to 96-well plates (Costar) at 1×10^4 cells/mL and left to attach overnight in a 5% CO_2 incubator at 37°C. All test compounds were dissolved in dimethyl sulfoxide (DMSO), filter sterilized and diluted to their working concentrations in culture media (maximum final concentration of DMSO, 1%). The appropriate concentrations were added to 96-well plates at twice the final concentration in 100 μL media. Incubation (total volume 200 μL) with test substance was terminated on day 7 without medium change.

Acid phosphatase assay. At the end of the incubation period the culture media was shaken-off and wells were washed with 100 μL phosphate-buffered saline (PBS). Freshly prepared substrate (100 μL) containing buffer (10 mmol 4-nitrophenylphosphate, 0.1% Triton X-100 in 0.1 mol sodium acetate; pH 5.5) was then added to each well. Incubation proceeded for 2 h (37°C, 5% CO_2) after which the reaction was stopped with addition of 50 μL/well of 1 M NaOH. This causes an electrophilic shift in the product 4-nitrophenol chromophore, producing a yellow colour proportional to the activity of the acid phosphatase enzyme. Plates were further read at 405 nm using 620 nm as a reference wavelength.

Measurement of acid phosphatase activity is a highly effective and reproducible microplate assay based on production of yellow 4-nitrophenol from the acid phosphatase substrate 4-nitrophenylphosphate (Martin & Clynes 1991, 1993). The intensity of colour produced is generally directly proportional to the number of viable cells. The results of cytotoxicity assessment are expressed as EC50 values — the dose that inhibits proliferation of cells by 50% as compared with control, untreated cells. Data are expressed as a mean percentage of cell survival ± standard deviation for a minimum of three determinations. The EC50 values were obtained using Calcusyn, a Windows software package for dose effect analysis from Biosoft (Cambridge, UK). EC50 values of tested compounds were evaluated using a one-way analysis of variance.

Antiplatelet assay

DMSO GR dried was obtained from Merck AG (Darmstadt, Germany). Arachidonic acid was purchased from Chrono-Log Co. (Havertown, PA). Sodium citrate solution was obtained from Biotika (Slovenská Lupča, Slovakia). Acetylsalicylic acid was purchased from Sigma-Aldrich.

Blood samples from healthy, non-smoking subjects who had not taken any drugs for at least 14 days were collected by

venopuncture into a plastic disposable syringe containing 3.8% sodium citrate (1:9, v/v). Platelet-rich plasma (PRP) was obtained as a supernatant by centrifugation of citrated blood at room temperature for 10 min at 1000 rev min^{-1}. Platelet-poor plasma (PPP) was prepared by centrifugation of remaining blood for 10 min at 5000 rev min^{-1}. The platelet count was adjusted to 2.5×10^8 platelets/mL using autologous PPP. The absorbance of PRP was taken as 0% aggregation and the absorbance of PPP as 100%. Platelet aggregation was determined by turbidimetry by means of a Chrono-log 500-Ca aggregometer (Chrono-Log Co.) connected to a computer (Aggro/Link software, Chrono-Log Co.) in agreement with the method described by Born (Born 1962).

Tested substances were dissolved in DMSO. To eliminate the effect of solvent on platelet aggregation, the final concentration of DMSO in tested samples was fixed at 0.5%. PRP (500 μL) was placed into a siliconized glass cuvette and incubated at 37°C for 2 min in the aggregometer well with stirring at 1000 rev min^{-1}. A fixed amount (2.5 μL) of the test substance solution, or only DMSO, was added. Sample was then incubated at 37°C for 3 min. After incubation, platelet aggregation was induced by addition of arachidonic acid (5 μL, final concentration 0.5 mmol). The aggregation process was monitored by Aggro/Link software for 5 min. Acetylsalicylic acid was used as a positive control.

Results are expressed as EC50 values (the molar concentration of tested substance that inhibited platelet aggregation by 50%). The dose–response curve was obtained with PRP from the same donor. The EC50 values were calculated from the dose–response curve (a minimum of three determinations for each concentration) by linear regression analysis using software GraphPad Prism Version 3.02 for Windows (GraphPad Software, San Diego, CA, *www.graphpad.com*). EC50 values of tested compounds were evaluated using a one-way analysis of variance.

Results and Discussion

Chemistry

The methoxy acid **1** (Kuchař et al 1999b) was the starting material (Figure 2). Methyl ester **2** was prepared using methyl chlorosulfinate and further DIBAL reduction (Jampílek et al 2002a) furnished aldehyde **5** in 96% yield. Methyl ester **2** was also prepared by methyl 4-bromobenzoate (**4**) coupling with 4-methoxybenzene-1-thiol (**3**) on powdered copper(I) oxide as a catalyst. In a similar way, aldehyde **5** could be prepared by 4-bromobenzaldehyde (**6**) coupling with thiol **3**, catalysed by powdered copper(I) oxide. The ethyl ester of substituted cinnamic acid **10** was obtained by the reaction of aldehyde **5** and Wittig reagent **9** (mp 157–158°C; ref. (Kuchař et al 1973) mp 156–157°C), prepared from triphenylphosphine (**7**) and ethyl 2-bromopropanoate (**8**). Compound **10** was hydrolysed with 10% aqueous NaOH to acid **11**, which was demethylated with BBr_3 to give product **12** (**11** was treated with pyridinium hydrochloride). Acid **12** was esterified by methanol and thionyl chloride to furnish ester **13**. The phenolic group was then etherified with chloroquinaldine. Alkaline hydrolysis of **14** yielded the first target acid, VUFB 20609, which was obtained over eight steps in 26% total yield (Figure 2).

A different strategy was chosen for VUFB 20584 (and parallel for VUFB 20609) synthesis (Figure 3). Compound **15** was obtained by the reaction of the Wittig reagent **9** (Kuchař et al 1973) and aldehyde **6**. Hydrogenation of the double bond in ester **15** on 10% $Pd/BaSO_4$ under atmospheric pressure gave ester **16**. Both esters **15** and **16** were treated with thiol **3** under catalysis by powdered copper(I) oxide or copper(I) sulfide to give compounds **10** and **17**. The double bond in ester **10** was hydrogenated on 10% Pd/C and the ethyl ester **17** was obtained. The latter was then hydrolysed by 10% aqueous NaOH to acid **18**, which was melted with pyridinium hydrochloride to yield compound **19**. Esterification with methanol and thionyl chloride led to phenolic compound **20**, which was alkylated with chloroquinaldine. Alkaline hydrolysis of **21** furnished the second target acid, VUFB 20584, which was again obtained over eight steps in 9% total yield (Figure 3).

Ester **10** and all compounds obtained from the reaction with the Wittig reagent **9** have the *E* (*trans*) configuration. Approximate chemical shifts for the olefinic proton in both configurations were calculated using the empirical formula $\delta_H = 5.25 + Z_{gem} + Z_{cis} + Z_{trans}$, where Z is a substituent constant for the chemical shifts of substituted ethylenes (Silverstein et al 1991); the constant for the substituted phenyl was approximated with that for phenyl. Experimentally found chemical shifts (7.72, 7.60 ppm) were closer to that calculated for the olefinic proton in (*E*)-alkene (7.28 ppm).

For selective demethylation of the methoxy group, BBr_3 was used in the presence of the ester group according to Greene et al (1991). Unfortunately, a mixture of products (hydrolysed and demethylated) was obtained, although we made a number of optimization attempts. When the deprotection of both groups was performed with BBr_3, large amounts of BBr_3 were necessary for deprotection at low or room temperatures, while substrate **10** was destroyed by BBr_3 on heating. The reaction with BBr_3 at low temperature destroyed compound **18** as well. Melting with pyridinium hydrochloride provided both products as well and, in addition, compound **10** or **11** was destroyed by pyridinium hydrochloride. For these reasons, demethylation of methoxy acids **11** and **18** was carried out using the above-mentioned procedures.

The key step in the synthesis of VUFB 20584 was the nucleophilic substitution of **16** with thiol **3**, due to a low reactivity of the 4-substituted aryl bromides. Aryl thioethers can be synthesized from halides and inactivated halides by several methods (Pinchart et al 1998; Schopfer & Schlapbach 2001; and refs. cited therein): aromatic nucleophilic substitutions under vigorous conditions in polar aprotic solvents at high temperatures; photoinitiation in polar aprotic solvents; catalysis by copper in polar or polar aprotic solvents; mild conditions in the presence of $Pd(PPh_3)_4$ or Pd_2dba_3 and DPEphos.

Heterogeneous copper catalysts were chosen for this coupling. Various types of heterogeneous copper catalysts and various reaction conditions of the coupling were evaluated (Jampílek et al 2003). 4-Methoxybenzene-1-thiol

(**3**) reactivity, especially its oxidation/dehydrogenation on heterogeneous copper catalysts, has been described (Jampílek et al 2002b). We showed that Cu(0) and Cu(II) catalysts gave bis(4-methoxyphenyl)disulfide in high yields. In summary, the use of Cu(I) catalysts, in particular oxide and sulfide in hexan-2-one and DMF, provided good yields of the products. Copper(I) oxide coupled **16** with thiol **3**, and oxidized or dehydrogenated compound **16** as well, so that the reaction gave **10** in only 23% yield. Copper(I) sulfide, which does not possess dehydrogenation properties, was used for the preparation of **17** in dry DMF (Jampílek et al 2002b, 2003). On the basis of these results, excellent heterogeneous copper catalysts, copper(I) oxide and copper(I) sulfide, and suitable reaction conditions were identified. Similar types of nucleophilic substitutions using copper(I) catalysts have been described in other papers (Suzuki et al 1980; Rábai 1989; Pinchart et al 1998; Kwong & Buchwald 2002). The more reactive iodo derivatives (Suzuki et al 1980; Rábai 1989; Pinchart et al 1998; Kwong et al 2002) were not used due to financial and stability concerns.

Another problem was the double bond hydrogenation in compound **10**. Conjugated double bonds in the arylalkanoic acids (prochiral compounds) could be hydrogenated on heterogeneous (Pd/C, Pd/$BaSO_4$, Ra-Ni) or even better, homogeneous (Pd, Rh, Ru, Sm systems) (Mander 1994; Lin et al 2001; Concellon & Rodruguez-Solla 2002) catalysts for enantioselective hydrogenation. Catalytic hydrogenation on palladium was chosen based on our previous experience and easy accessibility. Ester **15** was hydrogenated on 10% Pd/$BaSO_4$ in 99% AcOH containing AcONa.$3H_2O$ at 0.1 MPa; ester **16** was obtained without complications in 96% yield. Double bond hydrogenation in the presence of the sulfanyl group required specific conditions (Jarkas et al 2001). The palladium catalyst was used again. The double bond in ester **10** had to be hydrogenated on 10% Pd/C in 99% AcOH under specific conditions (see Figures 4 and 5). Samples (constant volumes) were withdrawn at regular time intervals, processed (see synthesis of compound **17**, method B) by flash chromatography, and then analysed by ^{1}H NMR. The conversion of **17** is dependent on the amount of 10% Pd/C and time is given in Figures 4 and 5. Compound **17** was obtained (after optimization of reaction conditions) in 45% yield in a mixture with unreacted ester **10** and ethyl (2*E*)-2-methyl-3-phenylprop-2-enoate (**22**) (according to ^{1}H and ^{13}C NMR spectra, Tanaka et al (1979)), which was formed by hydrogenolysis (Figure 6). The by-product, thiol **3**, ended up adsorbed on palladium; a part of the catalyst was thus deactivated. Consequently, the partially poisoned catalyst lost its hydrogenating activity. Ethyl 2-methyl-3-phenylpropanoate was not detected. Figure 1 shows that the conversion is a linear function of the catalyst amount in a certain time interval. A part of the catalyst was deactivated (the deactivated amount, 0.9 g, was determined by the intersection of the linear part of the curve with the x-axis). After correction for the amount of the inactive catalyst, the reaction conversion and rate were found to be proportional to the amount of active catalyst, as expected. Since the reaction rate is, within a broad range of conversions, independent of the degree of conversion, we can conclude that the hydrogenation is a zero-order reaction with respect to the hydrogenated substrate. This may be the case for the substances that strongly adsorb on the catalyst surface. The fact that the reaction conversion stopped at 45% at high amounts of catalyst is obviously connected to the occurrence of hydrogenolysis of the C-S bond with the production of ethyl ester **22**, in which the C=C double bond is no longer hydrogenated.

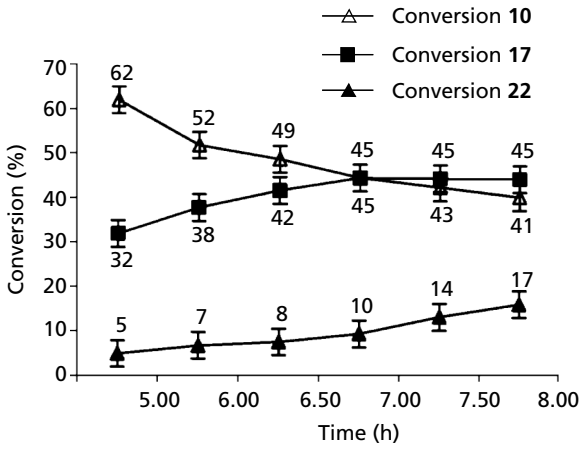

Figure 5 Yields of ethyl (2*E*)-3-{4-[(4-methoxyphenyl)sulfanyl]-phenyl}-2-methylprop-2-enoate (**10**), ethyl 3-{4-[(4-methoxyphenyl)-sulfanyl]phenyl}-2-methylpropanoate (**17**) and ethyl (2*E*)-2-methyl-3-phenylprop-2-enoate (**22**) dependent on time. Conditions: 99% AcOH, 2.0 g 10% Pd/C, 20°C, 0.1 MPa, H_2, 1000 rev min^{-1}.

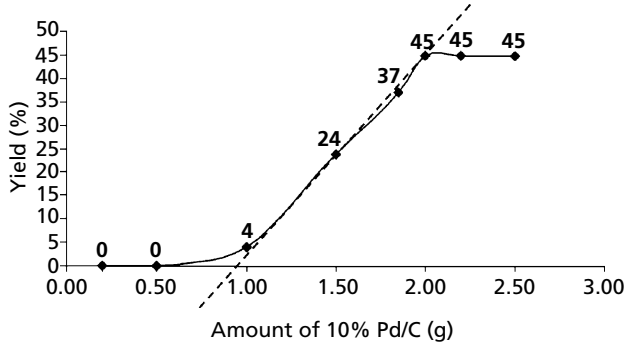

Figure 4 Yield of ethyl 3-{4-[(4-methoxyphenyl)sulfanyl]phenyl}-2-methylpropanoate (**17**) dependent on the amount of 10% Pd/C. Conditions: 99% AcOH, 20°C, 0.1 MPa, H_2, 7 h, 1000 rev min^{-1}.

Biological assay

Cytotoxicity

The inhibition of proliferation was evaluated in the original compound VUFB 19363 first (EC50, 28 μmol L^{-1}). EC50 values of VUFB 20584 and VUFB 20609 were found to be 15 μmol L^{-1} and 24 μmol L^{-1}, respectively (Figure 7). In conclusion, novel compounds VUFB 20584

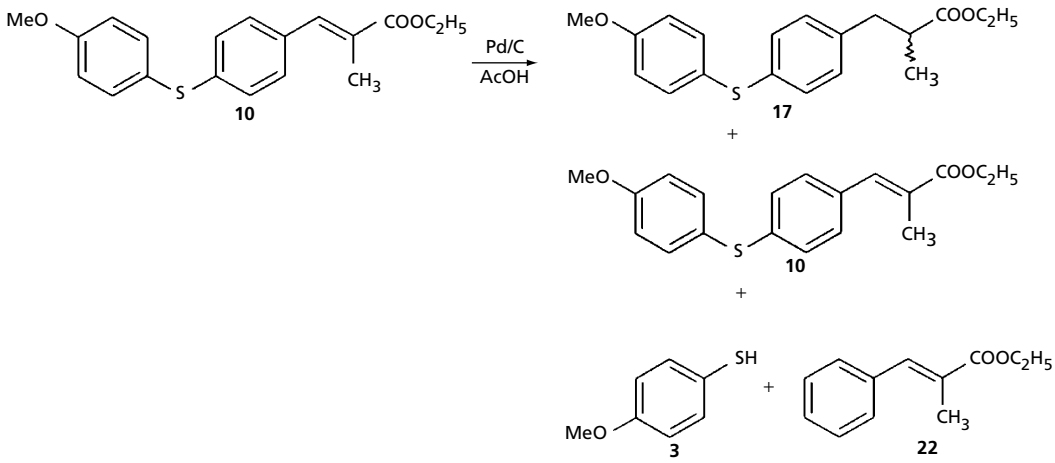

Figure 6 Hydrogenation and hydrogenolysis of compound **13**.

and VUFB 20609, derived from the original VUFB 19363, possess comparable cytotoxicity to the original compound, as determined by the acid phosphatase assay in the DLKP cell line.

Analysis of variance indicated significant differences in the means of the cytotoxic effects of VUFB 19363, VUFB 20609 and VUFB 20584 ($P > 0.999$). It is apparent from the comparison of the confidence intervals ($P = 0.95$) that the differences among all the three means are significant ($P > 0.95$) (Table 1, Figure 8).

Antiplatelet activity

All the tested methoxy acids **1, 11** and **18** and the target VUFB 20609 and VUFB 20584 show structural similarity with drugs in clinical use (arylalkanoic acids). Structurally similar compounds, hydroxy acids **12** and **19**, were not tested for antiplatelet activity due to their low solubility in the testing medium. Our results showed (Table 2) that substances **1** and VUFB 20584 caused a dose-dependent inhibition of the arachidonic-acid-induced aggregation. Compound **1** had a stronger effect than VUFB 20584 (calculated EC50, 149.8 μmol L^{-1} and 188.6 μmol L^{-1}, respectively). The other four tested compounds, **11**, VUFB 19363, VUFB 20609 and **18**, did not affect the aggregation process (EC50 >1100 μmol L^{-1}). Acetylsalicylic acid was used in this assay as a positive control (EC50, 16.1 μmol L^{-1}). Low or negative antiplatelet activity of all tested compounds was probably caused by low anti-inflammatory activity (low bonding to cyclooxygenase-1 of compounds **1, 11, 18**) or higher selectivity for 5-lipoxygenase (VUFB 19363, VUFB 20609, VUFB 20584). A structure–activity relationship study was not performed due to the small amount of tested compounds.

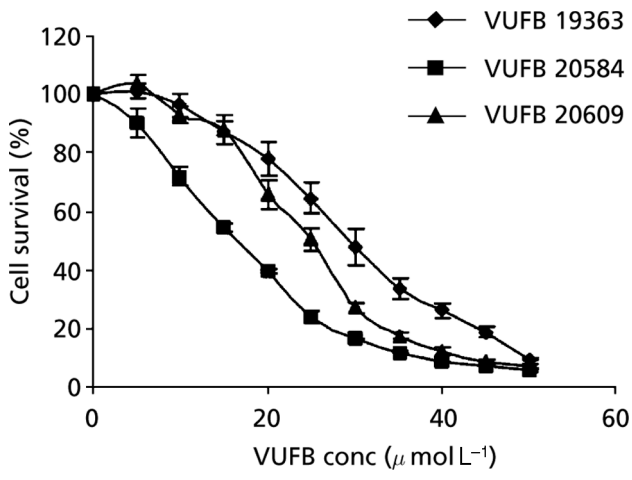

Figure 7 Cytotoxicity assessment of compounds in DLKP cells (a human poorly differentiated squamous lung carcinoma cell line). Data are expressed as a mean percentage of cell survival ± s.d. for a minimum of three determinations.

Table 1 The confidence intervals ($P = 0.95$) of compounds from cytotoxicity assay.

Compound	Lower limit	EC50	Upper limit
VUFB 19363	27.339	28.408	29.518
VUFB 20609	22.393	23.586	24.843
VUFB 20584	14.313	14.999	15.717

EC50 value, the dose that inhibits proliferation of DLKP cells (a human poorly differentiated squamous lung carcinoma cell line) by 50% as compared with control, untreated cells. Data are expressed as a mean ± standard deviation for a minimum of three determinations. The EC50 values were obtained using Calcusyn, a Windows software package for dose effect analysis from Biosoft (Cambridge, UK). EC50 values of tested compounds were evaluated using a one-way analysis of variance.

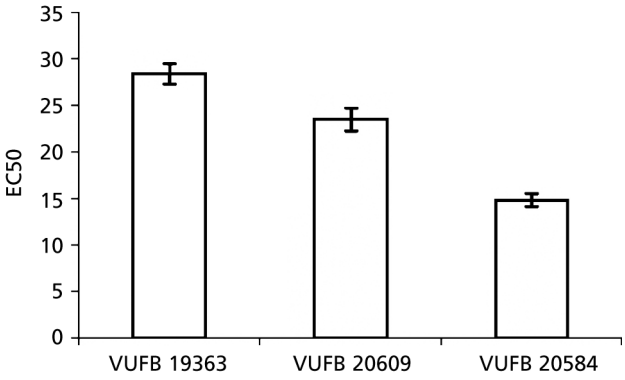

Figure 8 Comparison of EC50 (dose that inhibits proliferation of DLKP cells (a human poorly differentiated squamous lung carcinoma cell line) by 50% as compared with control, untreated cells) for cytotoxic effect. Data are expressed as a mean ± s.d. for a minimum of three determinations. The EC50 values were obtained using Calcusyn, a Windows software package for dose effect analysis from Biosoft (Cambridge, UK). EC50 values of tested compounds were evaluated using a one-way analysis of variance.

Table 2 Antiplatelet activity.

Compound	EC50
1	149.8
11	>1100
VUFB 19363	>1100
VUFB 20609	>1100
18	>1100
VUFB 20584	188.6
Acetylsalicylic acid	16.1

EC50 value, the concentration (μmol L^{-1}) that inhibited arachidonic-acid-induced platelet aggregation in plasma from healthy subjects by 50%, calculated from the dose–response curve (a minimum of three determinations for each concentration) by linear regression analysis. EC50 values were evaluated using a one-way analysis of variance.

Analysis of variance indicated significant differences in the means of the antiplatelet effects of acetylsalicylic, compound **1** and VUFB 20584 ($P > 0.999$). It is apparent from the comparison of the confidence intervals ($P = 0.95$) that the differences among all the three means are significant ($P > 0.95$) (Table 3, Figure 9).

Conclusions

Two potential antileukotrienic compounds, VUFB 20584 and VUFB 20609, were obtained over eight steps — in 26% total yield for VUFB 20609 and in 9% total yield for VUFB 20584. The starting material **1** is described in patent (Kuchař et al 1999b), the reaction intermediate **9** by Kuchař et al (1973) and the hydrogenation by-product **22** by Tanaka et al (1979). The reaction of thiol **3** with esters **15** and **16** were performed using specific copper heterogeneous catalysts. The conditions for double bond hydrogenation on 10% Pd/C were described. All intermediates and the final products (16 compounds) were characterized by ^{1}H and ^{13}C NMR spectra, IR spectra, and by means of CHN analyses. VUFB 20584 and VUFB 20609 have not been tested for their LTB_4 and LTD_4 receptor antagonist activity as appropriate testing facilities are no longer available, therefore this paper deals with chemical aspects of VUFB 20584 and VUFB 20609 syntheses and evaluation of in-vitro cytotoxic and antiplatelet activity.

Table 3 The confidence intervals ($P = 0.95$) of compounds from antiplatelet assay.

Compound	Lower limit	EC50	Upper limit
Acetylsalicylic acid	15.97	16.14	16.32
1	145.98	149.82	153.66
VUFB 20584	186.25	188.58	190.91

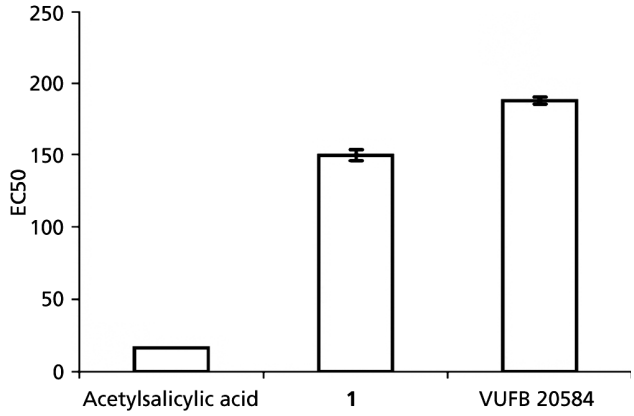

Figure 9 Comparison of EC50 for antiplatelet activity (the concentration (μmol L^{-1}) that inhibited arachidonic-acid-induced platelet aggregation in plasma from healthy subjects by 50%). The EC50 values were calculated from the dose–response curve (obtained with PRP from the same donor) by linear regression analysis. Data are means ± s.d. for a minimum of three determinations. EC50 values were evaluated using a one-way analysis of variance.

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