Monatshefte für Chemie - Chemical Monthly
https://doi.org/10.1007/s00706-020-02677-4

ORIGINAL PAPER

A silver-coated copper wire as inexpensive drug eluting stent model: determination of the relative releasing properties of leoligin and derivatives

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Received: 13 June 2020 / Accepted: 22 August 2020

Abstract

Cardiovascular diseases are overall the leading cause of mortality and morbidity worldwide. Therefore, treating and preventing coronary heart disease are of high scientific interest. Among several percutaneous coronary intervention procedures, coronary artery stenting displayed potent activity against restenosis, often observed using other invasive therapies. Nowadays, drug eluting stents' superiority over bare metal stents is increasingly recognizable, since drug eluting stents are able to overcome problems encountered with bare metal stent technology. Within this study, we developed a novel method for performing drug-releasing experiments utilizing an affordable stent model made from a readily available silver-coated copper wire, which was further coated with poly(*n*-butyl methacrylate). Leoligin, previously reported to inhibit intimal hyperplasia and the regrowth of endothelial cells, was exploited along with several structural analogs in drug-releasing experiments. It was found that compounds exhibiting similar biological activity can have significantly different releasing properties, a crucial parameter to know for the selection of compounds for in vivo studies.

Keywords Drug eluting stent · Cardiovascular disease · Leoligin · Vascular smooth muscle cell proliferation inhibition

In memoriam Prof. Fritz Sauter.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00706-020-02677-4) contains supplementary material, which is available to authorized users.

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Introduction

Cardiovascular diseases (CVDs) are the main cause of death in Austria [1] with a mortality of 38.9% (2018), and according to WHO also worldwide (17.9 million deaths in 2016 – newest data available). Heart attack and stroke are estimated to lead to 85% of these deaths [2]. The term atherosclerosis describes a condition in which plaque builds up in the inner walls of the arteries causing their narrowing (stenosis) that subsequently hampers the blood flow through the blood vessel [3]. Atherosclerotic plaques are consisting of fibrous tissue, foam cells, and accumulated modified lipids and are often unstable [4]. Although, atherosclerosis itself is rarely a relatively stable and slow-progressing condition, plaque rupture (structural failure of the fibrocellular cap that separates an atheromatous core from the lumen of an atherosclerotic artery) is more dangerous as it can potentially cause fatal thrombosis [5].

Treatment for stenosis includes several invasive methods such as percutaneous transluminal coronary angioplasty (PTCA) and coronary artery stenting (CAS). PTCA, is a minimal invasive procedure that widens blocked coronary

Published online: 05 November 2020

arteries, utilizing an inflatable balloon, thus allowing the blood to flow through the vessel [6]. However, this popular treatment option of the late 1980s had a significant drawback that is restenosis, the re-narrowing of the treated artery observed in 4–8% of patients while more than 20% required urgently a coronary bypass surgery. These complications led to the development of the bare metal stent (BMS) implantation technology, that proved to be superior over balloon angioplasty with respect to the reduction of the elastic recoil of the vessel. Unfortunately, thrombosis and neointimal hyperplasia emerged as the major limitations of the new treatment method [7].

To minimize the problems encountered with bare metal stenting and to avoid a second surgical intervention, drug eluting stents (DES) were designed. The first DES was launched in the European market in 2002 and since then they have been developed through several generations [8]. First generation DES consisted of stainless steel coated with paclitaxel (Taxus) or sirolimus (Cypher) [9]. Subsequently, studies proved that rapamycin agents could be superior to already applied solutions and thus, they were carried in the second-generation stents, made out of cobalt-chromium and platinum-chromium alloys. Improved eluting profiles, flexibility and promising re-endothelialisation were also observed [8, 10]. These new generation DES aim to provide long-term results while minimizing renewed vasoconstriction in the treated area [11]. The drug is either directly applied on the stent or impregnated within polymer matrices. Typically, biocompatible polymers are used already applied in several medical applications [12]. The polymer coating layer included poly(ethylene-*co*-vinyl acetate) (PEVA) [13] and poly(*n*-butyl methacrylate) (PBMA) [13, 14] which were used as a platform for drug-loading and drug-releasing purposes. These two polymers are easily soluble in organic solvents such as THF or halogenated solvents, which is essential for control studies. PBMA is available as transparent bead with high hydrophobicity, has good biocompatibility and durability at high molecular weight (200000–320000 Daltons) with slow drug-release kinetics which is able to adhere well to both the underlying stent and the drug coating. Within the framework of this project, PBMA was preferably used instead of PEVA, given that PEVA coatings' durability was significantly inferior.

Although there are several known techniques used to coat stents, dip-coating and spray-coating are the two most commonly used methods due to their general applicability [10]. The air-brush spray coating technique utilizes a special apparatus to deposit a drug in several solvents and polymer layers onto the stent. Thus, the drug release deposition onto the stent surface could be readily optimized, but the apparatus cost is rather high to perform introductory studies. The major benefit of the dip coating is that no special equipment is required, nor extensive time is needed. The stent is submerged in a solution of the polymer and drug in an appropriate solvent and then is allowed to dry either naturally or in an oven. Despite, the lack of the techniques' uniformity when considering the relative concentrations of drug and polymer (coating thickness) that may vary, the method is reliable for studying purposes [10].

The sustained release of drugs from polymer-coated stents is a key point for the DES overall applicability and performance. The physical mechanism that determines the transport of the drug from the majority of DES currently investigated is mainly molecular diffusion through a polymer layer. That is, dispersion of the anti-proliferative agent in the polymer matrix and its release to the corresponding medium through it. However, there are several parameters that govern the release profile such as polymer concentration, drug loading, drug-polymer interactions, and dissolution media [15].

Although, paclitaxel and sirolimus are the main drugs used to inhibit VSMCs proliferation, further drug development is always desirable. In the context of a multi-disciplinary project on the identification of natural products with anti-inflammatory properties, the stereoselective synthesis of the naturally occurring compound leoligin (**1**) and structurally modified analogs was recently developed in our group [16]. Leoligin was shown to increase macrophage cholesterol efflux [17], to inhibit the NF-κB (nuclear factor kappa-light-chain enhancer of activated B cells) signaling pathway and intimal hyperplasia [16–18]. Due to these interesting properties we considered it important utilizing this molecular scaffold along with its derivatives in context of the DES technology. The development of an affordable model system to make release studies of larger compound sets avoiding the utilization of the too expensive stents available in the market, was the main objective of this work.

Results and discussion

In this study, particular emphasis has been put into the development of a cheaper model system, since the high cost for a single stent (~ 1000 $) is recognized as the main limiting factor in the studies of the DES technology. A cheap stent model would enable the investigation of the releasing properties of larger sets of drug candidates in an academic environment, in our case leoligin and analogs thereof. For this reason, a simple readily available silver coated copper (costs as low as 5 €/100 m) wire was used for our in vitro studies. The excellent mechanical properties and its corrosion resistance along with its high elastic modulus and tensile strength render it capable of creating a spiral profile [19]. Additionally, silver ions possess excellent antimicrobial and antibacterial activity against various microorganisms, and thus, they are utilized for medical purposes [20]. For each model stent 60 cm of silver wire (0.25 mm diameter) was

bend and wrapped to an approximately 20 mm long spiral stent (around a Pasteur pipette) and was cleaned by dipping it in tetrachloroethylene. Removing the rest of the solvent in a vacuum oven for 1 h at 40 °C under ca 20 mbar pressure yielded a self-made silver stent which was comparable to the original stent but much cheaper (~ 0.03 €/stent) and easily available [20] (see Fig. 1).

The weight of the stent was determined in µg and the length in mm. Next, a stock solution of the desired polymer and the respective compound was prepared. For that purpose, 80.0 mg of PBMA were dissolved in 4.0 cm^3 of dry THF, at room temperature and shaken for 24 h. 100 mm^3 of this polymer stock solution were used for the coating mixture. The stock solution of the desired therapeutic agent was prepared by placing 2.0 mg of the compound, weighed with microgram accuracy into a 0.2 cm^3 micro vial and then dissolved in 100 mm^3 of dry THF at room temperature.

A novel HPLC method was developed, enabling the accurate detection of up to 1.0 ng of the released material. For the HPLC calibration, 5 mm^3 of the respective compound's stock solution were used after undergoing a dilution of 1:1000. The remaining 95 mm^3 were mixed with the 100 mm^3 polymer stock solution above. According to the literature, this 1:1 ratio between the polymer and the substance, along with the optimal viscosity of the solution, render it ideal for coating purposes [13]. Thus, the self-made spiral stent was dipped into the polymer/substance mixture using tweezers without touching the surface of the stent (see Fig. 1), the vial was closed and shaken for 1–2 min. The stent was removed from the solution using tweezers, allowed to dry in air and then placed into a 1.5 cm^3 glass vial. The drying process was completed in a vacuum oven at 40 °C and approximately 20 mbar for 24 h.

Releasing experiments were carried out with the following compounds (Fig. 2) previously prepared in our group [17, 21, 22].

Determination of the relative releasing amount

For further evaluation of the stent releasing properties, we developed a new method (see below) with the aim to determine the amount of the loaded compound in the loaded polymer/compound mixture. Given that, gravimetrically only the sum of the compound and the polymer can be determined, the amount of the substance that remains on the stent surface is unknown. Since every stent has a different surface, the amount of compound loaded on the stents depends on several parameters such as the concentration, the temperature but mostly the stent production. By a calibrated HPLC method we could prove the stents' releasing property and also the already released amount of the compound, however, we were not able to determine the relative amount released and the ratio between released and loaded compound. Thus, it was important to implement another method that would enable our calculations.

To overcome this problem, two stents were prepared and placed into the same THF solution which contained the substance and the polymer in a completely dissolved form. The solvent was removed, and the sum of loaded compound and polymer was determined for each stent gravimetrically. Hence, two stents are created under identical conditions and it can be assumed that they have identical properties. Thus, we were able to calculate also the weight of compound plus the weight of polymer (Fig. 1).

The first releasing studies with a stent were carried out in pure THF which immediately dissolved the polymer enabling the exact determination of the loaded amount of the compound in µg of loaded compound using a calibration curve. From this value the actual loading of the second stent in µg/stent could be calculated considering the different weight of the stents. The second stent was released in pure water, therefore allowing for the calculation of the relative released amount of the compound.

For the evaluation of the stent properties we filled the glass vials up 1.5 cm^3 volume, to ensure efficient shaking condition. The probes were always measured in pairs, one in THF and the other one in water. Sampling was always

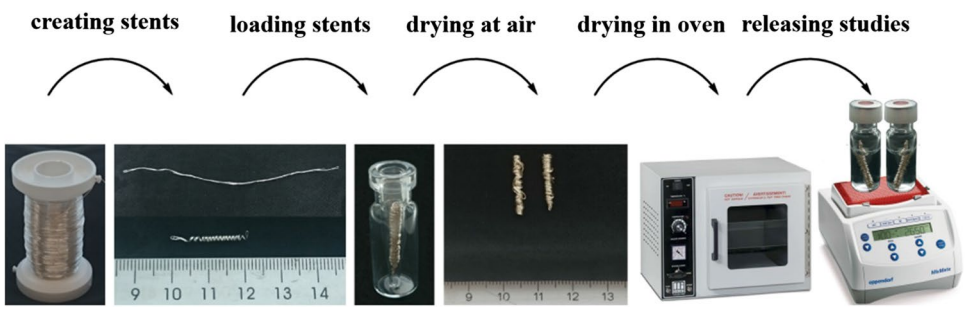


Fig. 1 In-house preparation of stents and the experimental setup, being used for releasing experiments

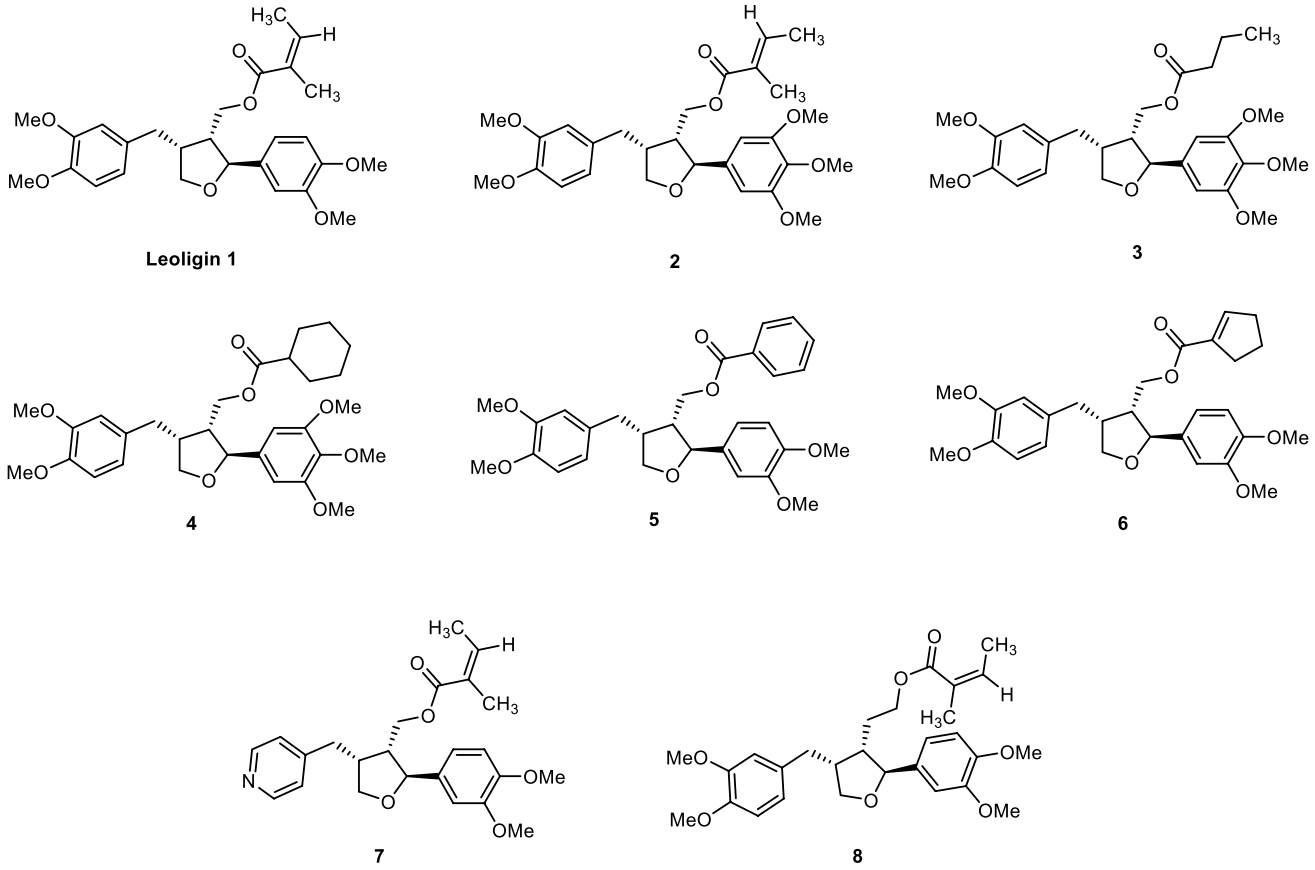


Fig. 2 Leoligin (**1**) and analogs thereof, being tested in releasing experiments

performed after shaking was stopped and through the septum without opening of the vials.

To obtain a precise calibration curve, a 5-point calibration method was utilized to determine the peak area vs the injected amount of the compound in ng as mentioned before the determination of the relative released amount of the desired compound, two loaded stents were placed in a 1.5 cm^3 GC vial each, after removing the solvent in the oven. The first vial was adjusted with 1.5 cm^3 of dry THF and the second one with 1.5 cm^3 of water. The sealed vials were then shaken on a shaker engine at room temperature with 200 rpm. For the HPLC analysis, 3×10 mm^3 of the solution were injected and the area value was calculated.

In the next step, it was crucial to determine the relative (released ng/loaded ng)*100) released amount of the desired compounds in 1.5 cm^3 of sample volume (Table 1 and Fig. 3).

Our measured ratios loaded compound/loaded polymer are comparable with literature examples which published 30% rapamycin in coating formulation using PBMA as polymer agent [23].

The set of compounds selected for the releasing studies is on purpose structurally relatively similar to see whether also marginal differences in structure have a significant effect on the releasing properties. Indeed, releasing studies of the parent leoligin and closely related derivatives proved that despite the structurally similar set of compounds being tested, the releasing properties of each one differs significantly over a wide range. The best releasing compound is **3** with 49.7% relative release, whereas **5** is at the other end of the scale with only 4.5% relative release. Hence, the difference spans a whole order of magnitude. Additionally, there is also an example of a compound **7** which is not released at all. Comparing the structures of **3** and **5**, it can be seen that the only differences are an additional methoxy-group in the aryl ring in position 2 of the THF-core, and a different ester group in position 3.

These promising preliminary findings should be considered when selecting compounds for in vivo testing in combination with proper stents, which are much more cost intensive for more sophisticated studies in the future. For example, in our set of compounds **4** and **6** showed a very similar potency for the inhibition of vascular smooth muscle cell (VSMC) proliferation with IC_{50} values of 14.4 µM and 15.9 µM, respectively. Inhibition of VSMC proliferation in vivo reduces intimal hyperplasia, an undesired result of surgical interventions

Table 1 Loaded and released amounts of the compounds and the polymer

Entry	Loaded mix /µg	Loaded compound /µg	Loaded polymer /µg	Loaded compound/loaded polymer	Released compound/ng/stent	Relative releasing /%
1 Leoligin	242	53.7	188.3	0.3	4.2	7.7
2	190	21.8	168.2	0.1	6.2	28.4
3	80	19.7	60.3	0.3	9.8	49.7
4	160	36.0	124.0	0.3	2.4	6.7
5	245	35.7	209.3	0.2	16.2	4.5
6	290	42.4	247.6	0.2	12.2	28.9
7	250[a]	250[a]	250[a]	b	0	0.0
8	230	31.0	199.0	0.2	12.2	39.5

PBMA has been used as the polymer for all releasing studies. For compound **7** [a]only the sum of the compound and the polymer was determined, [b]unknown value.

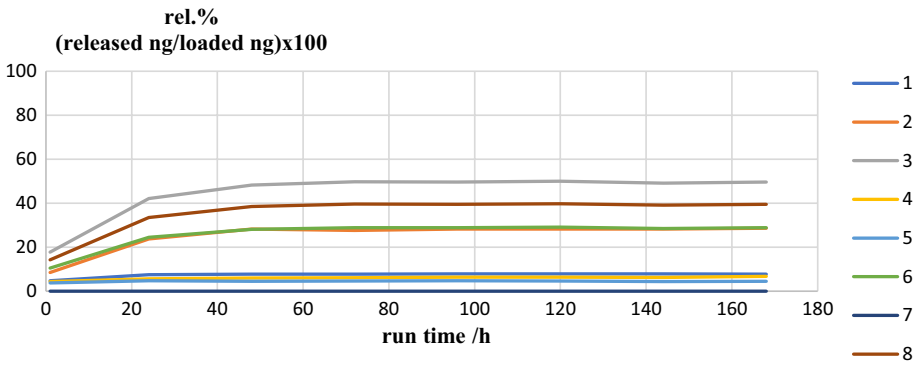


Fig. 3 Relative releasing of leoligin and derivatives

such as PTCA [18]. Another biological activity that was also observed was NF-κB inhibition. Nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB) is a transcription factor that promotes the expression of pro-inflammatory target genes. Hence, it is considered a promising pharmacological target in (neo)atherosclerosis and restenosis [24, 25]. Compounds **4** and **6** showed again a similar potency, with IC_{50} values of 7.3 µM and 6.5 µM, respectively. However, the releasing properties differ significantly, with **4** being released poorly (6.7% relative releasing) and **6** being released from the stent much better (28.9% relative release). Hence, **6** would be the better candidate for clinical testing. This example illustrates nicely why a simple and inexpensive stent model is important: To judge which compounds should be prioritized for in vivo studies it is not possible to opt for the ideal compound based exclusively on its biological profile, but it is important to expand the research on compounds that can also be released sufficiently in the system. An extreme case is compound **7**, which was not released at all from the stent (Table 2).

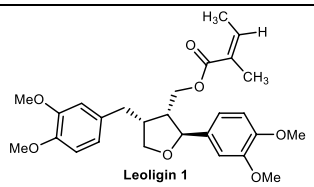
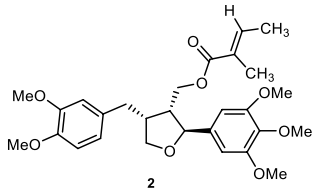
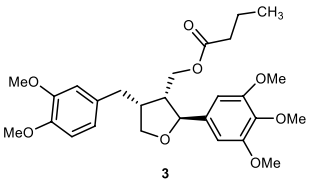
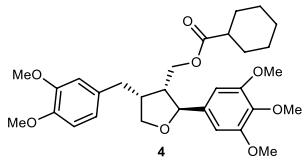
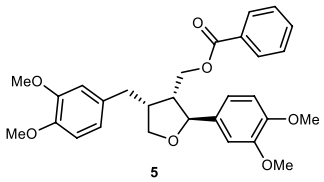
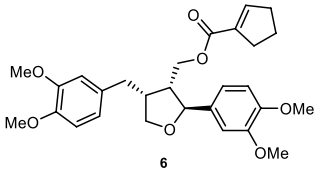
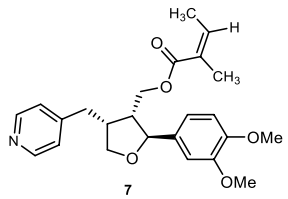
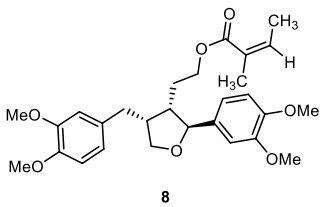
Conclusion

In conclusion, we have presented an inexpensive stent model made from a silver coated copper wire. This model was coated with PBMA and loaded with leoligin (**1**) or derivatives thereof. The releasing properties were evaluated, and it was found that structurally similar compounds can have significantly different releasing properties, which is important for selecting compounds for in vivo studies. This was illustrated in an example of two compounds with basically identical biological activity profile, but significantly different releasing properties.

Experimental

Unless noted otherwise, reactants and reagents were purchased from commercial sources and used without further purification. Dry CH_2Cl_2 and THF were obtained from a

Table 2 VSMC and NF-κB inhibition of tested compounds [16, 26]

Chemical Structure	VSMC inhibition: IC_{50} /μM	NF-κB inhibition: IC_{50} /μM
 Leoligin 1	27.7 (natural) 32.1 (synthetic)	22.7 (natural) 19.7 (synthetic)
 2	24.2	≥20
 3	≥30	≥20
 4	14.4	7.3
 5	60.3	≥20 12.7
 6	15.9	6.5
 7	≥30	≥20
 8	≥30	not determined

dispensing system by passing commercial material through a cartridge containing activated alumina (PURESOLV, Innovative Technology), stored under dry nitrogen and then used as such without further drying unless specified. Molecular sieves were activated by heating them to 200 °C for approximately 6 h in high vacuum and were then stored under argon.

Melting ranges were determined using a Kofler-type Leica Galen III micro hot stage microscope or an SRS OptiMelt Automated Melting Point System. Temperatures are reported in intervals of 0.5 °C. Aluminum-backed Merck silica gel 60 with fluorescence indicator F_{254} was used for thin layer chromatography (TLC). Spots were visualized under UV light (254 nm) and by staining with cerium ammonium molybdate (CAM) solution (20 g of ammonium pentamolybdate, 0.8 g of cerium(IV) ammonium sulfate, 400 cm^3 of 10 v/v % sulfuric acid) as a general purpose reagent. Alcohols were also visualized with *p*-anisaldehyde solution (3.5 g *p*-anisaldehyde, 1.5 cm^3 acetic acid, 5 cm^3 sulfuric acid, 120 cm^3 ethanol), and compounds pertaining double bonds were visualized with potassium permanganate solution (1.5 g potassium permanganate, 10 g potassium carbonate, 1 cm^3 10 w/w % NaOH, 200 cm^3 water). Specific rotation was measured using an Anton Parr MCP500 polarimeter and HPLC grade solvents under conditions as specified individually. Values are reported in the form + or − specific rotation (concentration in terms of g/100 cm^3, solvent).

Analytical chromatography–spectroscopy

Gas chromatography–mass spectroscopy (GC–MS) was used to analyze samples of reaction products with sufficient volatility. Instrument: Thermo Scientific Finnigan Focus GC/Quadrupole DSQ II device using a helium flow of 2.0 cm^3/min, analyzing an *m/z* range from 50–650. Column: BGB 5 (0.25 µm film; 30 m × 0.25 mm ID). Temperature gradients are as follows: Method A: 100 °C (2 min), to 280 °C in 4.5 min, 41.5 min hold at 280 °C (48 min). Column: TR-5 MS (0.50 µm film; 30 m × 0.25 mm ID). Temperature gradients are as follows: Method B: 100 °C (2 min), to 280 °C in 4.5 min (40 °C/min), 51.5 min hold at 280 °C (58 min).

Data is reported in the form retention time; m/z_1 (relative intensity in %), m/z_2 (relative intensity in %), … Only signals with $m/z \geq 90$ and relative intensity ≥ 15 % are given, except for the signal at 100 % relative intensity which is always given. Also, the molecular ion signal M^+ is given regardless of its intensity or *m/z*; in cases where M^+ was not visible due to excessive fragmentation, a characteristic fragment signal is identified instead.

High Pressure Liquid Chromatography (HPLC) was used to determine enantiomeric excess of reaction products, using a Dionex UltiMate 3000 device (RS Diode Array Detector). Chiral separation columns and analysis conditions are specified individually. In all cases, retention times include appropriate guard cartridges containing the same stationary phase as the separation column.

The HPLC analyses performed for the releasing studies were carried on a Shimadzu Nexera UHPLC instrument equipped with two LC-30AD pumps, CTO-20A column oven, DGU-20A5 degasser, CMB-20A controller, SPD-M20A UV detector, and SIL-30AC auto injector. Column: Phenomenex Kinetex PFP 100x 4.6 mm, 2.6 µm, equipped with a pre-column; mobile phase A: water; mobile phase B: acetonitrile; gradient: 0.00–0.2 min 60 % B, 0.21–3.0 min 100 % B, 3.01–4.8 min 100 % B, 4.81–5.0 min 60 % B, 7.0 min controller stop; Flow rate: 0.8 cm^3/min; column oven: 50 °C; injection volume: 10 mm^3; runtime: 7 min; UV detection: 270 nm. For processing of the measured HPLC chromatograms was used the software LabSolution v. 5.72 provided by Shimadzu.

Liquid Chromatography-High Resolution Mass Spectroscopy (LC-HRMS) was used to confirm exact molecular mass of reaction products by their quasi-molecular ions ($M+H^+$ or $M+Na^+$). The following two instruments were used:

Instrument 1

Shimadzu Prominence HPLC device (DGU-20 A3 degassing unit, 2 × LC-20AD binary gradient pump, SIL-20 A auto injector, CTO-20AC column oven, CBM-20A control module, and SPD-M20A diode array detector). Samples were eluted through a Phenomenex Kinetex precolumn (5 µm core shell ODS(3) phase; 4 mm x 2 mm ID) at 40 °C under conditions comprising gradients of H_2O/MeOH containing formic acid (0.1 v/v %), and then detected using a Shimadzu IT-TOF-MS by electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI), as indicated individually. Analyses were performed by E. Rosenberg (CTA, VUT) and L. Czollner (IAS, VUT).

Instrument 2

Agilent 1100/1200 HPLC device (degassing unit, 1200SL binary gradient pump, column thermostat, and CTC Analytics HTC PAL autosampler). Samples were eluted through a silica-based Phenomenex C-18 security guard cartridge (1.7 µm PD; 2.1 mm ID) at 40 °C under isocratic conditions comprising H_2O containing formic acid (0.1 v/v %)/MeOH containing formic acid (0.1 v/v %) in a ratio of 30:70 at a flow rate of 0.5 cm^3/min, and then detected using an Agilent 6230 LC-TOF-MS equipped with an Agilent Dual AJS ESI source by electrospray ionization (ESI). Analyses were performed by L. Czollner (IAS, VUT).

Preparative chromatography

Flash column chromatography was carried out on Merck silica gel 60 (40–63 µm), and separations were performed using a Büchi Sepacore system (dual Pump Module C-605, Pump Manager C-615, Fraction Collector C-660, and UV Monitor C-630 or UV Photometer C-635).

Preparative High Performance Liquid Chromatography (preparative HPLC) was carried out on a Phenomenex Luna reversed-phase column (10 µm C18(2) phase, 100 A; 250 mm × 21.20 mm ID), and separations were performed using a Shimadzu LC-8A device (SIL-10AP autosampler, SPD-20 detector, and FRC-10A fraction collector).

Reaction temperatures were measured externally (electronic thermometer connected to heater-stirrer or low temperature thermometer in case of cryogenic reactions) unless otherwise noted.

Nuclear Magnetic Resonance spectra were recorded from $CDCl_3$ solutions on a Bruker AC 200 (200 MHz proton resonance frequency) or a Bruker Avance UltraShield (400 MHz) spectrometer (as indicated individually), and chemical shifts are reported in ascending order in ppm relative to the nominal residual solvent signals, i.e. ^{1}H: $\delta = 2.50$ ppm (DMSO-d_6); ^{13}C: $\delta = 77.16$ ppm ($CDCl_3$), $\delta = 39.52$ ppm (DMSO-d_6). For all ^{1}H spectra in $CDCl_3$, however, shifts are reported relative to TMS as internal standard ($\delta = 0$ ppm) due to the interference of aromatic signals of many samples with the residual solvent signal of $CDCl_3$. For ^{13}C spectra, *J*-modulated (APT) or DEPT-135 pulse sequences were used to aid in the assignment. The numbering used for signal assignment is given in Fig. 4.

The syntheses of leoligin (**1**) [16] and compounds **2**–**5** were previously reported in literature [33].

[(2S,3R,4R)-4-(3,4-Dimethoxybenzyl)-2-(3,4- dimethoxyphenyl)tetrahydrofuran-3-yl]methyl cyclopent-1-enecarboxylate (6, $C_{28}H_{34}O_7$) A reaction vessel was charged with a stirring bar, 40.4 mg cyclopent-1-enecarboxylic acid (0.360 mmol, 4.0 equiv.) and 1.1 mg 4-DMAP (9.0 µmol, 0.1 equiv.), and then evacuated and back-filled with argon using standard Schlenk technique. Dry CH_2Cl_2 (1.0 cm^3) was then added via syringe and the solution was cooled to 0 °C in an ice bath. The vessel was briefly opened, 63.8 mg EDCI•HCl (0.333 mmol, 3.7 equiv.) added in one go and the mixture was stirred for 3 h at 0 °C. Meanwhile, a second vessel was charged with a stirring bar and starting material, 35.0 mg [(2*S*,3*R*,4*S*)-4-(3,4-dimethoxybenzyl)-2-(3,4-dimethoxyphenyl)tetrahydrofuran-3-yl]methanol (8'-*epi*-dimethylalariciresinol, 0.090 mmol, 1.00 equiv.), evacuated, and back-filled with argon (3×), and 78 mm^3 DIPEA (0.45 mmol, 5.0 equiv.) was added via syringe. After 3 h, the solution containing the activated carboxylic acid was transferred to the second vial via syringe and stirred for 24 h at room temperature. The reaction solution was used directly for flash column chromatography (9 g silica, flow rate 20 cm^3/min, EtOAc/LP, 10 : 90 to 22 : 78 in 9 min, then 22 : 78 isocratically for 6 min, then to 62 : 38 in 30 min) to afford the title compound **6** (39.4 mg, 91 %) as a colorless oil. R_f (PE: EtOAc = 1:1) = 0.43; $[\alpha]_D^{20} = +20.0°$ (MeOH, $c = 3.49$); LC-HRMS (ESI):exact mass calculated for M+Na$^+$: 505.2197, found: 505.2200, Δ: 0.59 ppm; GC–MS (EI, 70 eV): RT: 37.45 min (method B), *m/z* (rel. int.) = 482.2 (M$^+$, 2), 219.1 (28), 207.1 (17), 189.1 (16), 177.1 (16), 165.1 (81), 152.1 (15), 151.1 (100), 107.1 (17), 95.1 (73); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 1.95$ (quint, $^3J = 7.4$ Hz, 2H, H5'''), 2.40-2.85 (m, 7H, H3, H4, C4-CH, 2 x H4''', 2 x H6'''), 2.89 (dd, $^2J = 12.4$ Hz, $^3J = 4.3$ Hz, 1H, C4–CH), 3.76 (dd, $^2J = 8.6$ Hz, $^3J = 6.4$ Hz, 1H, H5), 3.86 (s, 3H, Ar–OCH$_3$), 3.87 (s, 6H, Ar–OCH$_3$), 3.88 (s, 3H, Ar–OCH$_3$), 4.09 (dd, $^2J = 8.6$ Hz, $^3J = 6.3$ Hz, 1H, H5), 4.27 (dd, $^2J = 11.2$ Hz, $^3J = 7.0$ Hz, 1H, C3–CH), 4.43 (dd, $^2J = 11.3$ Hz, $^3J = 6.7$ Hz, 1H, C3–CH), 4.83 (d, $^3J = 6.3$ Hz, 1H, H2), 6.63-6.91 (m, 7H, 6 x Ar-H, H3'''') ppm; ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 23.2$ (t, C5'''), 31.4 (t, C4'''*), 33.3 (t, C4–C), 33.5 (t, C6'''*), 42.7 (d, C4), 49.2 (d, C3), 56.0 (q, 4 x Ar-OCH$_3$), 62.5 (t, C3–C), 72.9 (t, C5), 83.2 (d, C2), 109.0 (d, C2'), 111.1 (d, C5'), 111.4 (d, C5''*), 112.0 (d, C2''*), 118.2 (d, C6'), 120.5 (d, C6''), 132.8 (s, C1''), 135.1 (s, C1'), 136.3 (s, C2'''), 144.5 (d, C3'''), 147.6 (s, C4''), 148.5 (s, C4'), 149.0 (s, C3''), 149.1 (s, C3'), 165.2 (s, C1''') ppm.

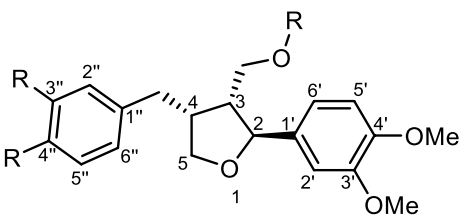


Fig. 4 Numbering scheme for compounds **6**–**8**

(Z)-[(2S,3R,4R)-2-(3,4-Dimethoxyphenyl)-4-(pyridine-4-ylmethyl)tetrahydrofuran-3-yl]methyl 2-ethylbut-2-enoate (7, $C_{24}H_{29}NO_5$) A reaction vessel was charged with a stirring bar, starting material, 31.2 mg [(2*S*,3*R*,4*R*)-2-(3,4-dimethoxyphenyl)-4-(pyridin-4-ylmethyl)tetrahydrofuran-3-yl]methanol (0.095 mmol, 1.00 equiv.), 8.6 mg angelic acid (0.086 mmol, 1.5 equiv.), and 52.7 mg PPh_3 (0.201 mmol, 3.5 equiv.), and then evacuated and back-filled with argon using standard Schlenk technique. Dry THF (0.75 cm^3) was then added and the solution cooled to 0 °C in an ice bath. To the stirred mixture was then added a solution of 50.7 mg ADD (0.201 mmol, 3.5 equiv.) in 1.0 cm^3 dry THF via syringe over approximately 1 min, and the reaction stirred for 22 h while being kept away from light and allowed to warm slowly to room temperature (first leg). Then

the reaction was cooled in an ice bath again, and there was added more angelic acid (4.3 mg, 0.043 mmol, 0.8 equiv.) and PPh_3 (26.3 mg, 0.100 mmol, 1.8 equiv.) in 0.75 cm^3 dry THF via syringe, followed by the addition of more ADD (25.3 mg, 0.100 mmol, 1.8 equiv.) in 1.0 cm^3 dry THF via syringe over approximately 1 min, and the reaction stirred for 24 h while being kept away from light and allowed to warm slowly to room temperature again (second leg). Et_2O (5 cm^3) was added to the reaction content, which was then filtered and rinsed with more Et_2O (15 cm^3). The solvents were evaporated and flash column chromatography was performed (9 g silica, flow rate 20 cm^3/min, EtOAc/LP, 50 : 50 to 100 : 0 in 30 min), followed by preparative HPLC (flow rate 20.0 cm^3/min, MeOH/water, 54 : 46 to 58 : 42 in 40 min, then to 60 : 40 in 12 min, 63 : 37 in 9 min) to afford the title compound **7** (26.5 mg, 68%) as a colourless oil. R_f (EtOAc) = 0.32; $[\alpha]_D^{23}$= +36.5° (MeOH, c = 1.15); LC-HRMS (ESI): exact mass calculated for M+H^+: 412.2118, found: 412.2100, Δ: − 4.37 ppm; GC–MS (EI, 70 eV): RT: 15.34 min (method A), *m/z* (rel. int.) = 411.2 (M^+, 3), 219.1 (24), 192.1 (15), 166.1 (40), 165.0 (100), 146.1 (19), 93.1 (36); ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.85-1.90 (m, 3H, H5'''), 2.00 (dq, ^3J = 7.2 Hz, ^5J = 1.5 Hz, 3H, H4'''), 2.56–2.90 (m, 3H, H3, H4, C4-CH), 2.95 (dd, ^2J = 12.6 Hz, ^3J = 4.0 Hz, 1H, C4-CH), 3.72 (dd, ^2J = 8.7 Hz, ^3J = 6.0 Hz, 1H, H5), 3.87 (s, 3H, Ar'-OCH_3), 3.88 (s, 3H, Ar'–OCH_3), 4.09 (dd, ^2J = 8.8 Hz, ^3J = 6.1 Hz, 1H, H5), 4.27 (dd, ^2J = 11.4 Hz, ^3J = 6.6 Hz, 1H, C3–CH), 4.38 (dd, ^2J = 11.5 Hz, ^3J = 7.2 Hz, 1H, C3-CH), 4.83 (d, ^3J = 6.4 Hz, 1H, H2), 6.12 (qq, ^3J = 7.2 Hz, ^4J = 1.4 Hz, 1H, H3'''), 6.80-6.89 (m, 3H, Ar'-H), 7.12 (d, ^3J = 5.8 Hz, 2H, H2'', H6''), 8.53 (d, ^3J = 4.2 Hz, 2H, H3'', H5'') ppm; ^{13}C NMR (50 MHz, $CDCl_3$): δ = 16.0 (q, C4'''), 20.7 (q, C5'''), 33.2 (t, C4–C), 41.6 (d, C4), 49.2 (d, C3), 56.0 (q, Ar'–OCH_3), 56.1 (q, Ar'–OCH_3), 62.0 (t, C3–C), 72.5 (t, C5), 82.8 (d, C2), 108.9 (d, C2'), 111.2 (d, C5'), 118.1 (d, C6'), 124.1 (d, C2'', C6''), 127.3 (s, C2'''), 134.6 (s, C1'), 139.4 (d, C3'''), 148.7 (s, C4'), 149.2 (s, C3'), 150.1 (d, C3'', C5''), 167.7 (s, C1''') ppm.

(Z)-2-[(2S,3S,4R)-4-(3,4-Dimethoxybenzyl)-2-(3,4-dimethoxyphenyl)tetrahydrofuran-3-yl]ethyl 2-methylbut-2-enoate (8, $C_{28}H_{36}O_7$) A reaction vessel was charged with a stirring bar, starting material, 17.5 mg 2-[(2*S*,3*S*,4*R*)-4-(3,4-dimethoxybenzyl)-2-(3,4-dimethoxyphenyl)-tetrahydrofuran-3-yl]ethanol (0.043 mmol, 1.00 equiv.), 6.5 mg angelic acid (0.065 mmol, 1.50 equiv.), and 39.8 mg PPh_3 (0.152 mmol, 3.50 equiv.), and then evacuated and back-filled with argon using standard Schlenk technique. Dry THF (0.75 cm^3) was then added and the solution cooled to 0 °C in an ice bath. To the stirred mixture was then added a solution of 38.3 mg ADD (0.152 mmol, 3.50 equiv.) in 1.0 cm^3 dry THF via syringe over approximately 1 min, and the reaction stirred for 23 h while being kept away from light and allowed to warm slowly to room temperature. Et_2O (5 cm^3) was then added to the reaction content, which was then filtered and rinsed with more Et_2O (15 cm^3). The solvents were evaporated and flash column chromatography was performed (9 g silica, flow rate 20 cm^3/min, EtOAc/heptane, 10 : 90 to 40 : 60 in 30 min), followed by preparative HPLC (flow rate 21.2 cm^3/min, MeOH/water, 63 : 37 to 70 : 30 in 60 min) to afford the title compound **8** (12.0 mg, 47 %) as a colorless oil. R_f (EtOAc) = 0.47; $[\alpha]_D^{20}$= +49.9° (MeOH, c = 1.15); LC-HRMS (ESI): exact mass calculated for M+Na^+: 507.2359, found: 507.2365, Δ: 1.25 ppm; GC-MS (EI, 70 eV): RT: 46.61 min (method B), *m/z* (rel. int.) = 484.2 (M^+, 20), 205.1 (100), 165.1 (14), 151.0 (58); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.78 (dq, ^2J = 13.8 Hz, ^3J = 6.9 Hz, 1H, C3-CH), 1.83-1.86 (m, 3H, H5'''), 1.93 (dq, ^3J = 7.2 Hz, ^5J = 1.5 Hz, 3H, H4'''), 1.99 (dq, ^2J = 13.8 Hz, ^3J = 6.9 Hz, 1H, C3-CH), 2.25-2.35 (m, 1H, H3), 2.49 (dd, ^2J = 13.2 Hz, ^3J = 11.7 Hz, 1H, C4-CH), 2.59-2.69 (m, 1H, H4), 2.89 (dd, ^2J = 13.4 Hz, ^3J = 4.2 Hz, 1H, C4-CH), 3.80 (dd, ^2J = 8.7 Hz, ^3J = 3.9 Hz, 1H, H5), 3.87 (s, 3H, Ar-OCH_3), 3.87 (s, 6H, Ar-OCH_3), 3.88 (s, 3H, Ar-OCH_3), 4.03 (dd, ^2J = 8.7 Hz, ^3J = 5.8 Hz, 1H, H5), 4.19 (dt, ^2J = 11.1 Hz, ^3J = 6.9 Hz, 1H, C3-CH_2-CH), 4.23 (dt, ^2J = 11.1 Hz, ^3J = 6.9 Hz, 1H, C3–CH_2–CH), 4.65 (d, ^3J = 8.0 Hz, 1H, H2), 6.05 (qq, ^3J = 7.2 Hz, ^4J = 1.4 Hz, 1H, H3'''), 6.71 (d, ^4J = 1.9 Hz, 1H, H2''), 6.73 (dd, ^3J = 8.1 Hz, ^4J = 1.9 Hz, 1H, H6''), 6.81 (d, ^3J = 8.1 Hz, 1H, H5''), 6.81-6.88 (m, 3H, H2', H5', H6') ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 15.9 (q, C4'''), 20.7 (q, C5'''), 26.0 (t, C3–C), 33.3 (t, C4–C), 43.0 (d, C4), 47.9 (d, C3), 56.0 (q, Ar–OCH_3), 56.09 (q, Ar–OCH_3), 56.11 (q, Ar–OCH_3), 56.14 (q, Ar–OCH_3), 62.8 (t, C3–CH_2–C), 72.4 (t, C5), 84.8 (d, C2), 109.4 (d, C2'), 111.1 (d, C5'), 111.5 (d, C5''*), 112.3 (d, C2''*), 118.8 (d, C6'), 120.9 (d, C6''), 127.9 (s, C2'''), 132.9 (s, C1''), 135.0 (s, C1'), 138.2 (d, C3'''), 147.6 (s, C4''), 148.8 (s, C4'), 149.1 (s, C3''), 149.3 (s, C3'), 168.1 (s, C1''') ppm.

Acknowledgements This project was supported by the Austrian Wirtschaftsservice AWS (Austrian Promotional Bank) within the PRIZE Prototype Development Program (Z090391) and the Austrian Science Fund (FWF) within research grants S10710 and S10704 (NFN 'Drugs from Nature Targeting Inflammation'). Financial support by the Austrian Agency for International Cooperation in Education & Research (OeAD-GmbH) to E.P. is gratefully acknowledged; E.P. is currently as associate fellow of the graduate school program Molecular Drug Targets MolTag (FWF Project W1232).

References

1. Statistik Austria. Die Informationsmanager Todesursachen. https://www.statistik.at/web_de/statistiken/menschen_und_gesellschaft/gesundheit/todesursachen/todesursachen_im_ueberblick/index.html. Accessed 2019, September 5

2. World Health Organization; Cardiovascular diseases (CVDs).https://www.who.int/en/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds). Accessed 2017, May 17
3. Falk E (2006) J Am Coll Cardiol 47 (8, Suppl. C):C7
4. Zheng XX, Zhou T, Wang XA, Tong XH, Ding JW (2015) Atherosclerosis 240:355
5. Schaar JA, Muller JE, Falk E, Virmani R, Fuster V, Serruys PW, Colombo A, Stefanadis C, Ward Casscells S, Moreno PR, Maseri A, van der Steen AF (2004) Eur Heart J 25:1077
6. Scott RA, Panitch A (2014) Biomacromol 15:2825
7. Savage MP, Fischman DL, Rake R, Leon MB, Schatz RA, Penn I, Nobuyoshi M, Moses J, Hirshfeld J, Heuser R, Baim D, Cleman M, Brinker J, Gebhardt S, Goldberg S (1998) J Am Coll Cardiol 31:307
8. Strohbach A, Busch R (2015) Int J Polym Sci 2015:1
9. Lee D-H, Torre Hernandez JMdl (2018) Eur Cardiol Rev 13:54
10. Livingston M, Tan A (2016) J Med Device 10:010801
11. Ho M-Y, Chen C-C, Wang C-Y, Chang S-H, Hsieh M-J, Lee C-H, Wu V, Hsieh IC (2016) Metals 6:168
12. Bernard M, Jubeli E, Pungente MD, Yagoubi N (2018) Biomater Sci 6:2025
13. Taylor C, DeYoung J, McClain J (2015) Stent with polymer coating containing amorphous rapamycin. US2016015537A1, WO2007011708A8. Chem Abstr 146:169475
14. Ding N, Roorda W (2003) Purified polymers for coatings of implantable medical devices. EP523343B1, WO2004009145A1. Chem Abstr 140:117478
15. Freire M, Alexandrino F, Marcelino HR, Picciani PHS, Silva K, Genre J, Oliveira AG, Egito E (2017) Materials (Basel) 10:651
16. Linder T, Liu R, Atanasov AG, Li Y, Geyrhofer S, Schwaiger S, Stuppner H, Schnurch M, Dirsch VM, Mihovilovic MD (2019) Chem Sci 10:5815
17. Wang L, Ladurner A, Latkolik S, Schwaiger S, Linder T, Hosek J, Palme V, Schilcher N, Polanský O, Heiss E, Stang H, Mihovilovic M, Stuppner H, Dirsch V, Atanasov A (2016) J Nat Prod 79:1651
18. Reisinger U, Schwaiger S, Zeller S, Messner B, Stigler R, Wiedemann D (2009) Cardiovasc Res 82:542
19. Mani G, Feldman MD, Patel D, Agrawal CM (2007) Biomaterials 28:1689
20. Yamabe A, Irisawa A, Wada I, Shibukawa G, Fujisawa M, Sato A, Igarashi R, Maki T, Hoshi K (2016) Endosc Int Open 4:E1090
21. Linder T, Geyrhofer S, Papaplioura E, Wang L, Atanasov AG, Stuppner H, Dirsch VM, Schnurch M, Mihovilovic MD (2020) Molecules 25:662
22. Mihovilovic M, Linder T, Dirsch V, Atanasov A, Bernhard D, Stuppner H, Schwaiger S (2015) Leoligin derivatives as smooth muscle cell proliferation inhibitors. WO2015196228A1. Chem Abstr 164:148815
23. Regar E, Sianos G, Serruys P (2001) British Med Bull 59:227
24. Dabek J, Kulach A, Gasior Z (2010) Pharmacol Rep 62:778
25. Pamuksu B, Lip GYH, Shantsila E (2011) Thromb Res 128:117
26. Linder T (2016) Synthetic Lignans Targeting Cardiovascular Diseases. PhD Thesis, Vienna University of Technology
27. Fakhrudin N, Waltenberger B, Gabaravdic M, Atanasov AG, Malainer C, Schachner D, Heiss EH, Liu R, Noha SM, Grzywacz AM, Mihaly-Bison J, Awad EM, Schuster D, Breuss JM, Rollinger JM, Bochkov V, Stuppner H, Dirsch VM (2014) Br J Pharmacol 171:1676
28. Tran TV, Malainer C, Schwaiger S, Atanasov AG, Heiss EH, Dirsch VM, Stuppner H (2014) J Nat Prod 77:483
29. Vogl S, Atanasov AG, Binder M, Bulusu M, Zehl M, Fakhrudin N, Heiss EH, Picker P, Wawrosch C, Saukel J, Reznicek G, Urban E, Bochkov V, Dirsch VM, Kopp B (2013) Evid Based Complement Altern Med 2013:1
30. Markasz L, Stuber G, Vanherberghen B, Flaberg E, Olah E, Carbone E, Eksborg S, Klein E, Skribek H, Szekely L (2007) Mol Cancer Ther 6:644
31. Liu R, Heiss EH, Waltenberger B, Blazevic T, Schachner D, Jiang B, Krystof V, Liu W, Schwaiger S, Pena-Rodriguez LM, Breuss JM, Stuppner H, Dirsch VM, Atanasov AG (2018) Mol Nutr Food Res 62:e1700860
32. Joa H, Vogl S, Atanasov AG, Zehl M, Nakel T, Fakhrudin N, Heiss EH, Picker P, Urban E, Wawrosch C, Saukel J, Reznicek G, Kopp B, Dirsch VM (2011) J Nat Prod 74:1513
33. Mihovilovic M, Linder T, Dirsch VM, Atanasov A, Bernhard D, Stuppner H, Schwaiger S (2015) Preparation of leoligin derivatives as smooth muscle cell proliferation inhibitors. WO2015196228 A1. Chem Abstr 164:148815