Bioorganic & Medicinal Chemistry 18 (2010) 1507–1515

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

11β-Hydroxysteroid dehydrogenase 1 inhibiting constituents from *Eriobotrya japonica* revealed by bioactivity-guided isolation and computational approaches

Judith M. Rollinger [a,*], Denise V. Kratschmar [b], Daniela Schuster [c], Petra H. Pfisterer [a], Christel Gumy [b], Evelyne M. Aubry [b], Sarah Brandstötter [a], Hermann Stuppner [a], Gerhard Wolber [c], Alex Odermatt [b,*]

[a] Institute of Pharmacy/Pharmacognosy and Center for Molecular Biosciences Innsbruck, University of Innsbruck, Innrain 52c, A-6020 Innsbruck, Austria
[b] Division of Molecular and Systems Toxicology, Department of Pharmaceutical Sciences, University of Basel, Klingelbergstrasse 50, CH-4056 Basel, Switzerland
[c] Institute of Pharmacy/Pharmaceutical Chemistry and Center for Molecular Biosciences Innsbruck, University of Innsbruck, Innrain 52c, A-6020 Innsbruck, Austria

ARTICLE INFO

Article history:
Received 28 November 2009
Revised 4 January 2010
Accepted 5 January 2010
Available online 11 January 2010

Keywords:
Eriobotrya japonica
11β-HSD1
Diabetes
Glucocorticoid receptor
Metabolic syndrome

ABSTRACT

The inhibition of 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1), which catalyzes the conversion of inactive 11-ketoglucocorticoids to active 11β-hydroxyglucocorticoids, emerged as promising strategy to treat symptoms of the metabolic syndrome, including obesity and type 2 diabetes. In this study the leaves of the anti-diabetic medicinal plant loquat (*Eriobotrya japonica*) were phytochemically investigated following hints from a pharmacophore-based virtual screening and a bioactivity-guided approach. Determination of the 11β-HSD1 and 11β-HSD2 inhibitory activities in cell lysates revealed triterpenes from the ursane type as selective, low micro-molar inhibitors of 11β-HSD1, that is, corosolic acid (**1**), 3-epicorosolic acid methyl ester (**4**), 2-α hydroxy-3-oxo urs-12-en-28-oic acid (**6**), tormentic acid methyl ester (**8**), and ursolic acid (**9**). Importantly, a mixture of loquat constituents with moderate activities displayed a pronounced additive effect. By means of molecular modeling studies and the identification of the 11β-HSD1-inhibiting 11-keto-ursolic acid (**17**) and 3-acetyl-11-keto-ursolic acid (**18**) a structure–activity relationship was deduced for this group of pentacyclic triterpenes. The mechanism of action elucidated in the present work together with the previously determined pharmacological activities provides these natural products with an astonishing multi-targeted anti-diabetic profile.

1. Introduction

Obesity and its related metabolic diseases, including type 2 diabetes, dyslipidemia, hypertension, and cardiovascular complications, represent a major health problem in the industrialized world. There is a great need for novel, improved therapeutic strategies to combat the consequences of these diseases.

Glucocorticoids play a central role in the modulation of carbohydrate and lipid metabolism, and the prolonged exposure to elevated glucocorticoids has been associated with metabolic disturbances such as visceral obesity, insulin and leptin resistance, hyperglycemia, elevated triglyceride and cholesterol levels, and elevated blood pressure. In recent years, it became evident that inappropriately elevated local glucocorticoid activity rather than systemic levels contribute to the adverse metabolic effects observed in obese individuals.[1] On a tissue-specific level, the enzyme 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) catalyzes the conversion of inactive 11keto-glucocorticoids (cortisone in human, 11-dehydrocorticosterone in rodents) to active 11β-hydroxyglucocorticoids (cortisol in human, corticosterone in rodents). This enzyme is highly expressed in metabolically relevant tissues such as liver, adipose and skeletal muscles. A second enzyme, 11β-HSD2, is expressed in kidney cortical collecting ducts, distal colon and placenta, and catalyzes the reverse reaction.

The consequences of elevated glucocorticoid activation by 11β-HSD1 have been well demonstrated in transgenic mice overexpressing the enzyme in adipose tissue.[2,3] These mice present typical features of metabolic syndrome. In addition, several clinical studies described the negative impact of elevated 11β-HSD1 activity on various metabolic functions.[4] Based on these observations, inhibition of 11β-HSD1 is considered a promising strategy to treat metabolic syndrome, and many potent synthetic inhibitors have been described recently.[5,6] In contrast, only few natural compounds that inhibit 11β-HSD1 have been identified at present. Because 11β-HSD1, in addition to its role in glucocorticoid metabolism, has other functions such as metabolism of 7-oxysterols, 7-oxy-neurosteroids and xenobiotics,[7] full inhibition of this

* Corresponding authors. Tel.: +43 512 507 5308; fax: +43 512 507 2939 (J.M.R.); tel.: +41 61 267 1530; fax: +41 61 267 1515 (A.O.).
E-mail addresses: Judith.rollinger@uibk.ac.at (J.M. Rollinger), alex.odermatt@unibas.ch (A. Odermatt).

0968-0896/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2010.01.010

enzyme may interfere with these alternative functions, and the use of moderate inhibitors may be a preferred strategy.

Therefore, we recently initiated a search for natural compounds inhibiting 11β-HSD1. We scrutinized extracts of six medicinal plants used as traditional anti-diabetic medicines for their potential to inhibit 11β-HSD1 activity and glucocorticoid receptor activation in transfected HEK-cells.[8] In addition, the selectivity for 11β-HSD1 was determined by measuring the effects on the 11β-HSD2-dependent oxidation of cortisol to cortisone, since inhibition of this enzyme induces a cortisol-dependent activation of the mineralocorticoid receptor and results in increased blood pressure.[1]

Among the tested extracts *Eriobotrya japonica* (Thunb.) Lindl. showed promising effects. Loquat or *E. japonica* (Thunb.) Lindl. from the Rosaceae family is not only famous for its delicious fruits, but also the leaves are well known in traditional Chinese medicine for their beneficial effect in the treatment of diabetic patients.[9] A number of chemical and pharmacological studies using animal tests confirmed the hypoglycaemic action of Folium Eriobotryae.[10–15] However, there is a lack of information in respect to the mechanism/s and site/s of action of the investigated extracts, fractions and individual loquat constituents. In our recent study we found that the methanol and the dichloromethane (DCM) leaf extracts of *E. japonica* showed both a dose-dependent inhibition of 11β-HSD1, and a preferential inhibition of 11β-HSD1 versus 11β-HSD2. In cell lysates, the DCM extract exerted an IC_{50} of 24 ± 3 μg/ml against 11β-HSD1 activity and an approximately threefold weaker inhibition towards 11β-HSD2. The effects measured in cell lysates could be confirmed by the results obtained from intact cells, that is, stably transfected HEK-293 cells.[8] Following these results and virtual hits from a previously established pharmacophore model,[16] the aim of this study was to identify those secondary metabolites responsible for the inhibitory effects of the 11β-HSD1 activity observed for the extracts of the medicinal plant *E. japonica*.

Twelve constituents from the chemical class of triterpenes have been isolated and identified. Together with further naturally derived triterpenic acids the isolated constituents have been tested for their potential to inhibit the activity of 11β-HSD1 and 11β-HSD2. The results of the compounds tested in this study were used for computational analysis (i) to rationalize the binding interactions in the 11β-HSD1 binding site and (2) to derive a structure–activity relationship for this class of compounds.

2. Results and discussion

2.1. In silico screening of a natural products database

In a previous study, we have generated a ligand-based pharmacophore model for 11β-HSD1 inhibitors.[16] Using this model as query, our 3D-multiconformational molecular database DIOS consisting of approximately 10,000 reported constituents from medicinal plants described in Dioscorides' *De materia medica*,[17] was virtually screened returning 172 hits. With 28 members, the chemical class of triterpenoids was one of the chemical scaffolds dominating this virtual hit list. Among the highest scored triterpenes was corosolic acid (**1**; Fig. 1).

This natural product is a constituent of different herbal remedies and plant derived nutritionals, particularly from the Rosaceae family; for example, it is described as constituent in almond hulls,[18] blackberries,[19] and apple peels.[20] Furthermore, it is known as the prominent ingredient from the leaf extract of *E. japonica*,[21,22] which in our previous study showed a distinct potential to inhibit the activity of 11β-HSD1.[8]

Commercially available corosolic acid (**1**) was therefore tested for its potential to inhibit recombinant human 11β-HSD1 and

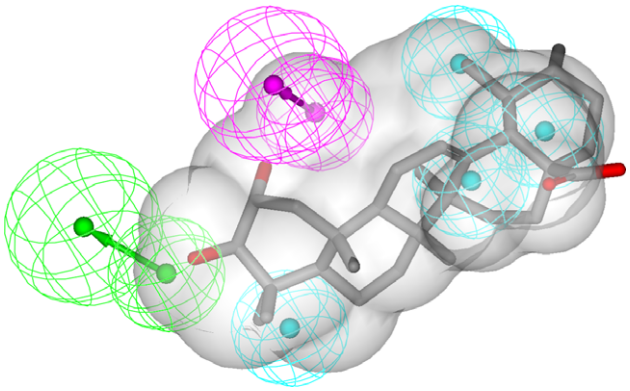


Figure 1. Corosolic acid (**1**) mapped into the pharmacophore model for 11β-HSD1 inhibitors. Chemical features are color-coded: magenta—hydrogen bond donor, green—hydrogen bond acceptor, cyan—hydrophobic, grey—shape (size constraint).

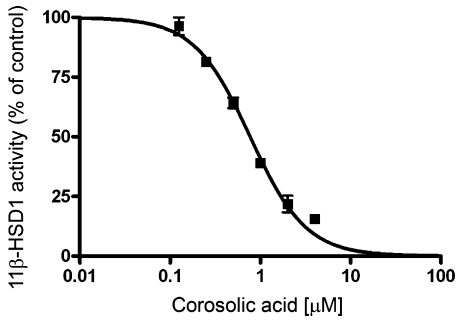


Figure 2. Concentration-dependent 11β-HSD1 inhibitory activity of corosolic acid (**1**) measured in lysates of cells expressing recombinant human 11β-HSD1. Data were normalized to control, that is, in the presence of 0.1% DMSO, and represent mean ± S.D. from three independent experiments.

11β-HSD2 activities in lysates of stably transfected HEK-293 cells. As shown in Figure 2, **1** exhibited a concentration-dependent 11β-HSD1 inhibitory activity with an IC_{50} of 0.81 ± 0.06 μM. The effect of **1** was selective as no activity against 11β-HSD2 was detected at concentrations up to 20 μM.

2.2. Phytochemical studies and bioassay-guided isolation

Using LC–MS and in comparison with commercial corosolic acid (**1**), this bioactive compound was confirmed as prominent constituent in the methanol leaf extract of *E. japonica*. This is in accordance with the recently determined quantification of triterpene acids from this plant material.[21] However, in the 11β-HSD1 inhibiting DCM extract, compound **1** could only be detected in traces. In order to identify the further secondary metabolite/s, responsible for the previously measured effect, bioassay-guided phytochemical investigations were performed with the DCM leaf extract from *E. japonica*.

Fractionation of 10.2 g of the DCM extract by silica gel column chromatography resulted in 13 fractions (A1–A13), which were tested in cell lysates on their inhibitory activity on 11β-HSD1 (Fig. 3A) and 11β-HSD2 (Fig. 3B).

At concentrations of 25 μg/ml, fractions A11–A13 diminished the activity of 11β-HSD1 to below 35% (Fig. 3A), whereas the activity of 11β-HSD2 was scarcely influenced by these fractions (Fig. 3B). Thus, A11–A13 were analysed using TLC, DAD-ELSD-HPLC and LC–MS. Repeated chromatographic separation and purification steps afforded 12 triterpenoid constituents. By using mass spectrometry, extensive 1D and 2D NMR experiments, optical rotation

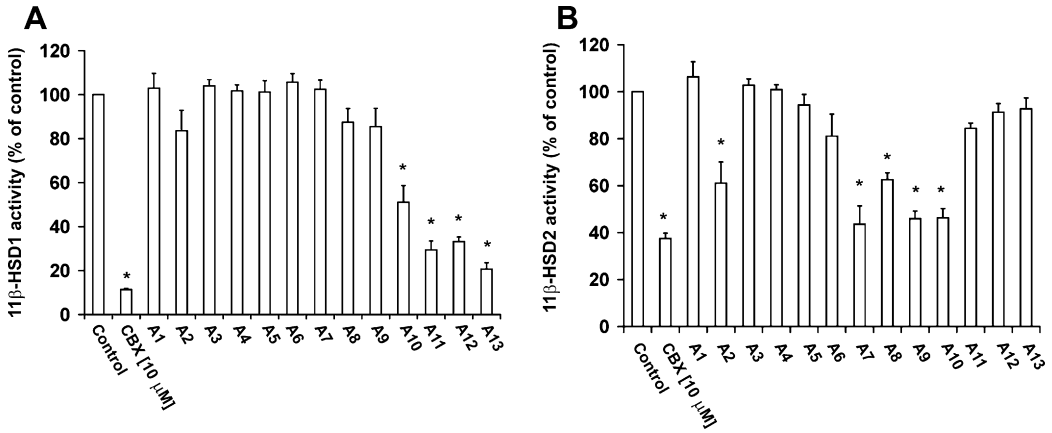


Figure 3. Inhibition of 11β-HSD1-dependent reduction of cortisone to cortisol (A) and 11β-HSD2-dependent oxidation of cortisol to cortisone (B) by fractions A1 to A13 of the DCM extract of *E. japonica* ($c = 25$ μg/ml) measured in lysates of HEK-293 cells stably expressing recombinant human 11β-HSD1 (A) or 11β-HSD2 (B). The non-selective inhibitor carbenoxolone (CBX) was used as a positive control. Data were normalized to control, that is, in the presence of 0.1% DMSO, and represent mean ± S.D. from three independent experiments. $^*p < 0.01$.

and comparison with data from the literature, the isolates were identified as ursolic acid methyl ester (**2**),[23] β-sitosterol (**3**),[24] 3-epicorosolic acid methyl ester (**4**),[25] uvaol (**5**),[26] 2-α hydroxy-3-oxo urs-12-en-28-oic acid (**6**),[27] corosolic acid methyl ester (**7**),[28] tormentic acid methyl ester (**8**),[29] ursolic acid (**9**),[22] maslinic acid methyl ester (**10**),[30] 3-*O*-*trans*-*p*-coumaroyltormentic acid (**11**), 3-*O*-*cis*-*p*-coumaroyltormentic acid (**12**), and tormentic acid (**13**; Scheme 1).[31] All the isolated triterpenes are known natural compounds; **1**–**3** and **9**–**13** have already been described as secondary metabolites from *E. japonica*; **4**, **7** and **8** were isolated as methyl esters of the known loquat constituents 3-epicorosolic acid,[23] corosolic acid,[21,22] and tormentic acid,[11] respectively. The pentacyclic triterpenoids **5** and **6** belonging to the ursane type have not been previously reported from this natural source.

2.3. Determination of 11β-HSD1 and 11β-HSD2 inhibitory activities

The isolated triterpene metabolites (**2**–**13**) were screened for their inhibitory activity on 11β-HSD1 and 11β-HSD2, respectively, at 20 μM. At this concentration, none of the tested compounds reduced the 11β-HSD2 activity; however, **4**, **6**, **8**, and **9** distinctly inhibited the activity of 11β-HSD1. Their IC_{50} values against 11β-HSD1 were determined to be 5.2 ± 0.9 μM (**4**), 17 ± 4 μM (**6**), 9.4 ± 0.8 μM (**8**), and 1.90 ± 0.25 μM (**9**; Fig. 4).

In order to evaluate potentially additive effects of active metabolites from *E. japonica*, compounds **4**, **6**, **8** and **9** were tested for their individual 11β-HSD1 activities using concentrations of 2, 3, 4 and 1.5 μM, respectively, which result in only partial inhibition. The obtained effects were compared with that achieved in a mixture containing the same concentration of each compound (Fig. 5). Importantly, whereas each individual compound inhibited 11β-HSD1 only partially, the mixture showed a significantly increased inhibitory potential (Fig. 5).

2.4. Evaluation of further triterpenic acids

Based on the findings from the loquat constituents, further triterpenic acids well known as plant constituents were selected to evaluate their effects on 11β-HSD1 and 11β-HSD2 (**14**–**18**; Scheme 2). Ganoderic acid A (**14**) was chosen as representative of a lanostane type triterpene from the famous TCM fungus *Ganoderma lucidum* P. Karst.[32] The pentacyclic triterpene acids 11-keto-β-boswellic acid (**15**) and 3-acetyl-11-keto-β-boswellic acid (**16**) are prominent ingredients from incense, that is, the resin of *Boswellia serrata* Roxb.[33] Guided by the 11β-HSD1 inhibiting activity of ursolic acid (**9**), its derivatives 11-keto-ursolic acid (**17**) and 3-acetyl-11-keto-ursolic acid (**18**) were also selected for a screening on 11β-HSD1 and 11β-HSD2. They were reported as constituents from Indian linaloe, that is, the resin from *Bursera delpechiana* Poiss.[34]

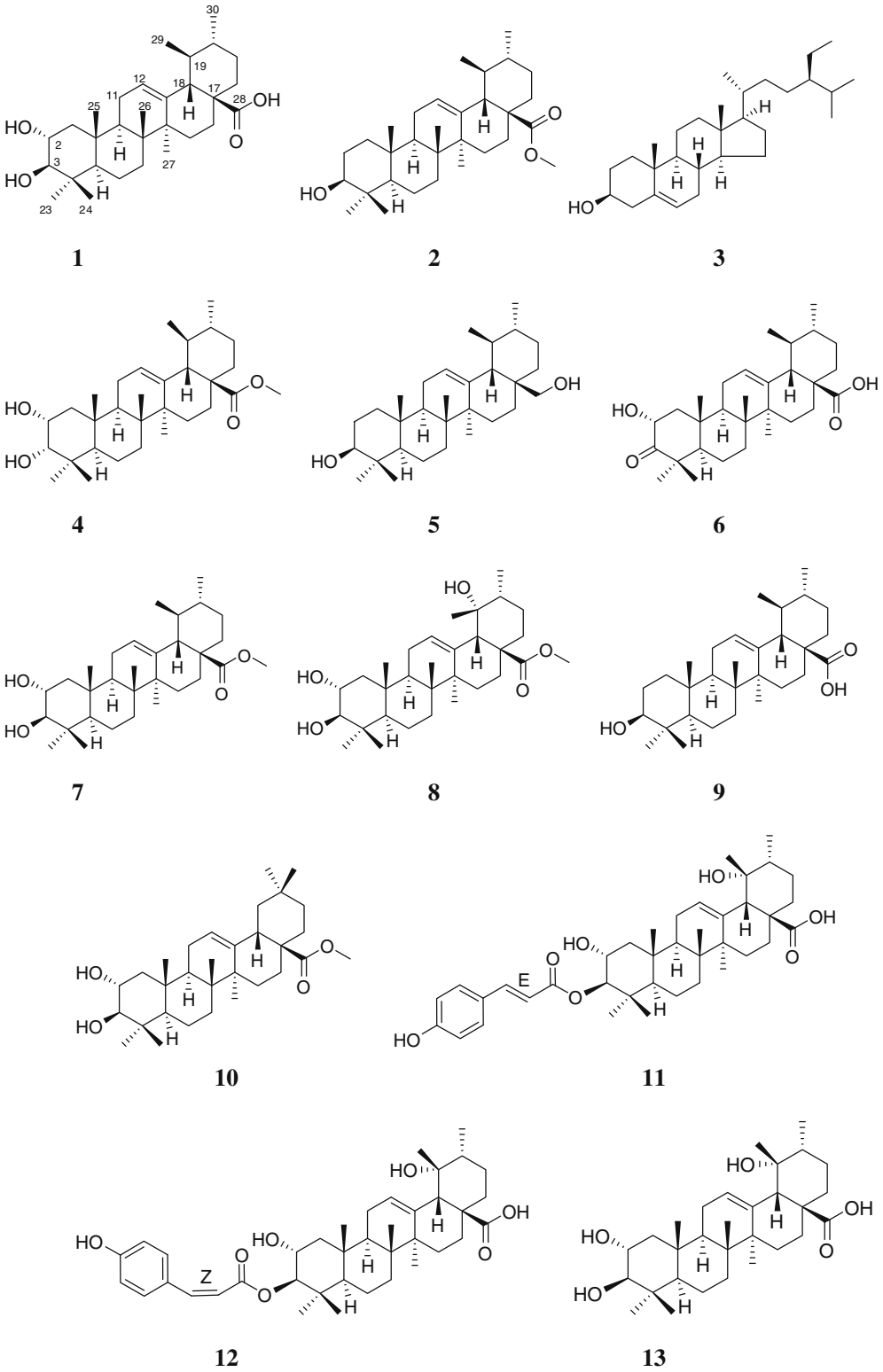
At 20 μM the triterpene acids **14**, **15**, and **16** moderately inhibited the activities of 11β-HSD1 by 46%, 52% and 63%, and that of 11β-HSD2 by 76%, 68% and 52%, respectively. Due to their unfavorable missing selectivity towards 11β-HSD1, they were not further investigated. However, the ursolic acid derivatives **17** and **18** exerted a significant and selective 11β-HSD1 inhibitory activity with IC_{50} values of 2.06 ± 0.44 μM and 1.35 ± 0.52 μM, respectively.

2.5. Structure–activity relationship (SAR) of triterpene inhibitors of 11β-HSD1

When analyzing our results, the crucial role of the carboxylic group position and derivatization became obvious. While compounds with 4-subsituted carboxylic acids were inactive, carboxylic groups in position 17 seemed advantageous for ligand binding. In some cases, methylation led to a complete loss of activity (compare **1**/**7** and **9**/**2**). However, other methyl esters still showed activity. In addition, stereochemistry of the hydroxyl group at position 3 seemed important (compare **4**/**7**). For a deeper understanding of these observations, we docked all compounds into the active site of 11β-HSD1 (PDB[35] entry 2bel chain A).

When analyzing the docking poses of corosolic acid (**1**) (Fig. 6A and B), a possible interaction of the 28-carboxylic group with the Tyr177 hydroxyl group was observed. It seems that ester formation at this position rather leads to unfavorable steric clashes with the protein than to disrupt this interaction. Carboxylic acid substituents on other positions, for example, position 4 in 11-keto-boswellic acid (**15**), were not observed to form interactions with the protein. The hydroxyl groups at positions 2 and 3 form hydrogen bonds with the backbone oxo group of Thr124. The stereochemistry and presence or absence of hydroxyl groups at these positions alters the ligands' interaction with Thr124 and thereby the anchoring of the whole molecule in the ligand binding site. From the data obtained so far, the 2*S* and 3*R* configuration leads to an optimal interaction geometry.

Several X-ray crystal structures of 11β-HSD1 in complex with potent inhibitors are reported in the literature and available in the PDB (e.g., 2irw, 2rbe, 3byz, or 3fco). With no exception, they are observed to form hydrogen bonds with the catalytically ac-



Scheme 1. Constituents from the leaves of *E. japonica*.

tive amino acid residues Ser170 and Tyr183. Although the triterpenoid carbenoxolone is also anchored with its 11-keto group between those residues in the crystal structure (PDB code 2bel), mutational analysis studies by Kim et al. show that a different amino acid—Tyr177—is essential for binding of the highly potent triterpenoid inhibitor glycyrrhetinic acid—but not the substrate cortisone.[36] This points towards a different binding mode for the triterpenoid compound class in comparison to the published synthetic inhibitors. Therefore, interactions with the catalytically active residues may not play an important role in triterpenoid inhibitor binding. The observation that ursolic acid (**9**) and 11-keto ursolic acid (**17**) do not show significant differences in IC_{50} values for 11β-HSD1 inhibition underlines this assumption.

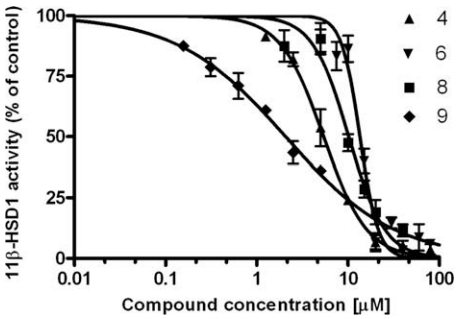


Figure 4. Concentration-dependent 11β-HSD1 inhibitory activities of compounds **4**, **6**, **8**, and **9** measured in lysates of HEK-293 cells stably expressing recombinant human 11β-HSD1. Data were normalized to control, that is, in the presence of 0.1% DMSO, and represent mean ± S.D. from three independent experiments.

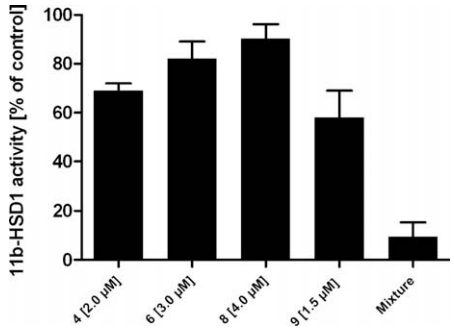


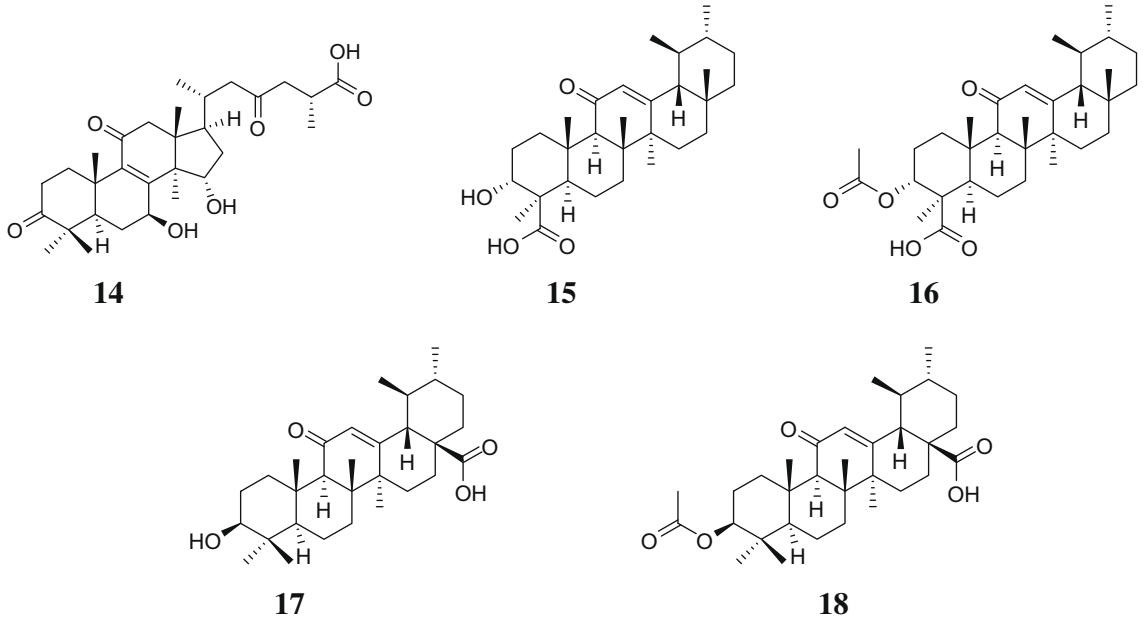
Figure 5. Inhibition of 11β-HSD1 activity by compound **4** (2 μM), **6** (3 μM), **8** (4 μM), **9** (1.5 μM), and a mixture containing the respective concentration of each compound. Data were normalized to control, that is, in the presence of 0.1% DMSO, and represent mean ± S.D. from three independent experiments.

In order to further explore the properties that are favorable for 11β-HSD1 inhibition, the four most active compounds **1**, **9**, **17**, and **18** were flexibly aligned by pharmacophoric points using LigandScout 3.0.[37] From this overlay a ligand-based 3D pharmacophore model consisting of the chemical feature set shared by all four compounds was calculated. The resulting model contains 11 hydrophobic features, representing the triterpene core structure, a negatively ionizable feature and two hydrogen bond acceptors placed on the carboxylic acid structure on C28, and one hydrogen bond acceptor on the 3*R* position (Fig. 7).

The elucidation of these important substructures was in line with the observations from the docking experiments. We additionally investigated the discriminatory power of this model by screening a database consisting of all 18 compounds using LigandScout. The model retrieved 13 compounds as hits. The ranking based on the geometric fit showed a clear enrichment of active compounds among the highly-ranked hits (Table 1).

3. Conclusion

Growing evidence suggests that selective inhibition of 11β-HSD1 lowers blood glucose concentrations, counteracts the accumulation of visceral fat and ameliorates related metabolic abnormalities in type 2 diabetes.[38] In the search for natural compounds selectively inhibiting 11β-HSD1, the traditionally used anti-diabetic medicinal plant *E. japonica* emerged as promising starting material.[8] By using this knowledge and a previously established pharmacophore model, we identified the loquat ingredients corosolic acid (**1**) and ursolic acid (**9**) as well as the ursolic acid derivatives **17** and **18**, as plant constituents able to selectively inhibit 11β-HSD1 with IC_{50} values between 0.8 and 2 μM. Moreover, several additional compounds with moderate activity and IC_{50} values in the low micro-molar range were isolated from the loquat DCM leaf extract and their structures determined. Importantly, a pronounced additive effect was observed in a mixture of constituents with moderate activity. In a recently published study, Li et al. performed an HPLC-UV quantification of Eriobotrya constituents from 11 leaf samples collected from different regions in China. Therein, pentacyclic triterpene acids revealed as the most considerable percentage of secondary metabolites in the samples.[21] It could be shown that the seven major triterpene constituents account for 10–16 mg per g crude drug. Among them, the most abundant metabolites were assigned to corosolic acid (**1**) and ursolic acid (**9**) which amount to more than 50% of the quantified triterpene fraction. This fact combined with our findings that several triterpene acids of the ursane type from loquat are endowed with a



Scheme 2. Further natural triterpenic acids.

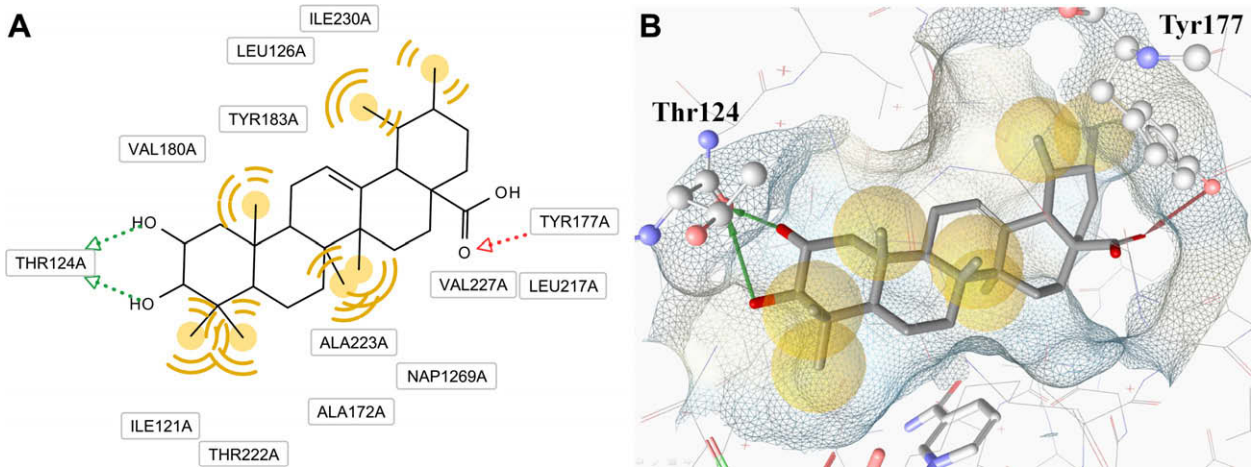


Figure 6. Predicted binding orientation of corosolic acid (**1**) in the 11β-HSD1 active site. Chemical features are color-coded: yellow—hydrophobic, green—hydrogen bond donor, red—hydrogen bond acceptor. (A) 2D view of chemical interactions observed between **1** and the protein. (B) Docked binding pose and interaction pattern of **1** bound to 11β-HSD1. At the bottom of the binding site, the cofactor molecule NADPH is located.

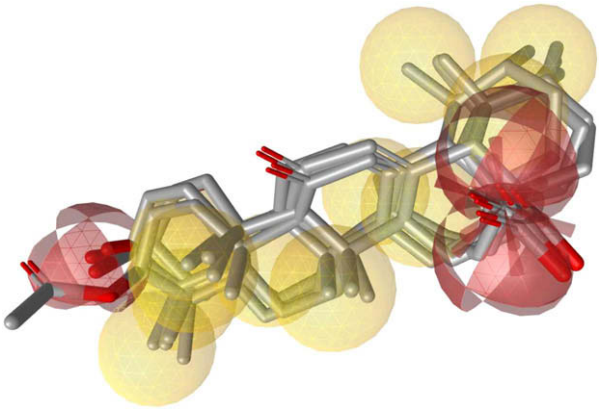


Figure 7. Merged features pharmacophore model from LigandScout derived from compounds **1**, **9**, **17**, and **18**. Chemical features are color-coded: hydrophobic—yellow; hydrogen bond acceptors—red.

Table 1
Pharmacophore-based ranking of triterpene 11β-HSD1 inhibitors

Compd	Hit list rank	In vitro IC_{50} [μM]
1	1	0.81
13	2	Inactive
4	3	5.2
9	4	1.9
17	5	2.06
18	6	1.35
8	7	9.4
5	8	Inactive
7	9	Inactive
11	10	Inactive
8	11	Inactive
10	12	Inactive
12	13	Inactive

low micro-molar inhibitory potential on 11β-HSD1 might explain the activity of the whole extract measured not only in cell lysates, but also in intact, stably transfected HEK-cells.[8]

Pentacyclic triterpenes represent a promising class of multi-target agents.[39] Corosolic acid (**1**), which widely exists in traditionally used medicinal herbs, has attracted much attention as anti-diabetic agent with hypoglycemic effects being proved on animal experiments[40] and in clinical trials.[41] A number of mechanisms have already been attested to this anti-diabetic compound.[42] Corosolic acid was identified as the most active phosphorylase A inhibiting constituent from *Lagerstroemia speciosa* leaf extract,[43,44] was able to stimulate glucose uptake by enhancing the insulin receptor phosphorylation,[45] was reported as low micro-molar inhibitor of protein tyrosine phosphatase 1B[46] and as an inhibitor of the hydrolysis of sucrose.[47] Also, ursolic acid (**9**) was shown recently to exhibit potential anti-diabetic and immunomodulatory properties in type 1 diabetic mice fed a high-fat diet.[48] The underlying mechanisms refer as well to tyrosine phosphatase 1B[46] and phosphorylase A.[49] Additionally, **9** was identified as one of the constituents from the hexane extract of *Phyllanthus amarus* causing a significant inhibition of the α-amylase, suggesting to contribute at least partly by this mechanism to the anti-diabetic effect of the investigated medicinal plant extract.[50]

Recently, a high hypoglycemic and hypolipidemic potential on normal, alloxan-diabetic, and streptozotocin-induced diabetic mice has been attested to the triterpene acid fraction yielded from the leaves of *E. japonica*.[13] In our study, among the constituents isolated from the *E. japonica* leaf extracts, we identified some potent inhibitors of 11β-HSD1 belonging to pentacyclic triterpenes of the ursane type. Intriguingly, these compounds, unlike glycyrrhetinic acid, did not inhibit 11β-HSD2, which is associated with cortisol-induced mineralocorticoid receptor activation and an elevation of blood pressure. Such inhibitors do have a considerable potential as drugs directed against glucocorticoid-related metabolic disorders. The mechanism of action elucidated in the present work together with the previously determined pharmacological activities provides these natural products with an astonishing multi-targeted anti-diabetic profile. Furthermore, clinical trials with corosolic acid (**1**),[41] and a long-term proven efficacy of Folium Eriobotryae as famous traditional Chinese medicine with hypoglycemic effect[10–15] contribute to a safe and well-approved herbal remedy.

4. Methods

4.1. Virtual screening of a natural products database

Pharmacophore models represent the three-dimensional (3D) arrangement of chemical functionalities that are essential for the interaction of a ligand with a specific pharmacological target structure (proteins, RNA, or DNA).[51] These models can be used to virtually screen multiconformational 3D databases of compounds in

order to find other molecules that fulfil the requirements for binding to the respective target. In this study, the natural products database DIOS was screened, which consists of 9676 reported constituents from medicinal plants described in Dioscorides' *De materia medica*.[17] A previously reported pharmacophore model for 11β-HSD1 inhibitors was used as search query.[16] The screening was performed within Catalyst 4.11 (Accelrys Software Inc., San Diego, CA) using the best flexible search algorithm.

4.2. Structure–activity relationship (SAR) of triterpene inhibitors of 11β-HSD1

For a better understanding of the differences in compound activity, all molecules discussed in this study were fitted into the 11β-HSD1 ligand binding site using docking. Generally, docking methods intend to predict the 3D structure of small molecules interacting with a protein binding site. Docking studies on all reported compounds evaluated in this study were carried out using GOLD SUITE (version 1.0.1; Cambridge Crystallographic Data Centre, Cambridge, UK). This program employs a genetic algorithm for finding accurate docking solutions. Furthermore, the program allows for full ligand flexibility, partial protein flexibility, and a distinct treatment of water molecules that are present in the ligand binding domain. Overall, the default parameters of the program were used. Protein and ligand atom types were determined by GOLD. Cocrystallized water from the ligand binding site was included in the docking process by setting those molecules to 'toggle' and 'spin'. The 'toggle' option lets the program decide whether the water should be present or absent (i.e., displaced by the ligand) during docking. Allowing the water molecules to 'spin' allows for the automatic optimization of the orientation of the hydrogen atoms.

Chemical interactions between the docked binding poses of the ligands and the 11β-HSD1 active site were analyzed using LIGANDSCOUT 3.0. This program automatically interprets chemical interactions observed between a ligand and a protein, based on the chemical functionalities, the geometric distances and angles between neighboring structures.[52]

The model for investigating structure–activity relationships of the discussed compounds was generated using the ligand-based modeling tool 'Espresso', a module in the program LIGANDSCOUT 3.0. This algorithm ranks all compounds according to their flexibility, and subsequently creates cascading pairwise alignments of all conformations producing a flexible overlay of the underlying molecules. This alignment set contains 3D overlays of those conformations that maximize geometric chemical feature overlap. From this set of solutions, the best flexible alignments can either be prioritized (i.e., scored) by steric overlap or pharmacophore feature overlap. For this study 'atom sphere overlap' was used as a scoring function and the highest ranked model was used for the predictions. For all other settings default values were used. Each compound was represented by a comprehensive set of conformers (conformer generation using OpenEye's program OMEGA2 (OpenEye Scientific Software, Santa Fe, NM) using default settings).

4.3. General experimental procedures

Melting points were determined on a Kofler hot-stage microscope and are uncorrected. Optical rotation was measured on a Perkin–Elmer 341 polarimeter (Wellesley, MA) at 25 °C. FTIR spectra were recorded on a Bruker IFS 25 FTIR spectrometer (Bruker Optics, Ettlingen, Germany) in transmission mode (4000–600 cm^{-1}) using ZnSe disks of 2 mm thickness. NMR spectra were recorded on a Bruker-DRX300 (Bruker Biospin, Rheinstetten, Germany) at 300 K in $CDCl_3$ or methanol and calibrated to the residual non-deuterated solvent signals. Upon request, NMR spectra can be obtained from the corresponding author. Column chromatography was performed under TLC monitoring using silica gel flash CC (Silica Gel 60, 40–63 μm; VWR, Darmstadt, Germany) and Sephadex® LH-20 (20–100 μm, Pharmacia Biotech, Uppsala, Sweden). TLC was performed on Silica Gel 60 F_{254} plates (0.25 mm; VWR, Darmstadt, Germany), mobile phase: chloroform/methanol/formic acid, 10:0,5:0,25 (v/v/v), and detected with vanillin/H_2SO_4 (1% w/v and 5% v/v methanolic solutions, respectively). HPLC-data were obtained on a Hewlett–Packard-(HP)-1100 system (Agilent, Waldbronn, Germany), equipped with a photodiode array detector (DAD), column thermostat and auto sampler. The LC was fitted with a Phenomenex Synergi 4 μ Max-RP 80 A column (Torrance, CA; 150 × 4.6 mm id, 4 μm) and a Merck LiChroCART 4–4 guard column with LiChrospher 100 RP18 (5 μm) packing (VWR, Darmstadt, Germany) at a column temperature of 40 °C, flow rate 1.0 ml/min, injection volume 10 μl, using DAD (205, 254, 280) and evaporative light scattering detection Alltech ELSD 2000 (Alltech, Düsseldorf, Germany); tube temperature 97 °C; gas flow 2.5 bar (impactor off). The mobile phases consisted of A: 0.02% TFA (Merck 8.08260.0100) in bidistilled water (v/v), B: methanol (Merck 1.06007.2500); linear gradient: 0 min 60% B; 18 min 98% B; 28 min 98% B. For LC–ESIMS experiments the HPLC was coupled to a Bruker Esquire 3000^{plus} ion trap mass spectrometer (Bruker Daltonics, Bremen, Germany) replacing solvent A with a solution of 0.1% formic acid in bidistilled water (v/v). MS-parameters: split 1:5; ESI positive mode; spray voltage: 4.5 kV; the sheath gas: N_2, 30 psi, the dry gas: N_2, 6 l min^{-1}, 350 °C; scanning range: 50–1000 *m/z*.

All chemicals were analytical grade. Solvents were either analytical grade or puriss. grade and distilled before use.

4.4. Material

Authenticated leaves of *E. japonica* (Thunb.) Lindl. were collected in the Botanical Garden of Innsbruck. A voucher specimen (JR-20061023-A1) was deposited in the Herbarium of the Institute of Pharmacy/Pharmacognosy, Leopold-Franzens University of Innsbruck, Austria.

Corosolic acid (**1**) and ganoderic acid A (**14**) were purchased from Chromadex Inc. (Santa Ana, CA) with a HPLC-purity of ⩾95% and >98%, respectively. 11-keto-β-boswellic acid (**15**), 3-acetyl-11-keto-β-boswellic acid (**16**), 11-keto-ursolic acid (**17**), and 3-acetyl-11-keto-ursolic acid (**18**) were obtained from Phytoplan, Heidelberg, Germany, at a HPLC-purity of >98%.

4.5. Extraction and isolation

Five hundred and fifty grams of the air-dried and milled leaves of *E. japonica* were extracted with 1600 ml DCM three times for 24 h at room temperature. The plant material was filtered off and the solvent was evaporated under reduced pressure to afford 11.9 g DCM extract. In the same way, the remaining plant material was then extracted three times with 1800 ml methanol to gain 45.1 g of the methanol crude extract. 10.2 g of the DCM extract were fractionated using a flash silica gel CC (400 g; 40 × 5.0 cm) with 500 ml step gradients from petrol ether to DCM and from DCM to methanol to yield 13 fractions (A1–A13). Fraction A11 (3880 mg) was subjected to Sephadex CC (75 × 3.5 cm) and eluted with DCM: acetone (85:15) yielding 17 fractions (B1–17). B4 (182 mg) was separated via flash silica gel CC (80 g; 42 × 2.2 cm) with 200 ml step gradients from DCM to acetone to afford nine subfractions (C1–9). C2–4 (29.6 mg) and C5–6 (28.7 mg) were purified over Sephadex CC with DCM: acetone (85:15), respectively, to afford 4.3 mg of a white, microcrystalline powder (**2**; optical rotation $[\alpha]_D^{20}$ +48.4 (methanol, *c* 0.43); MS (ESI): *m/z* 471 $[M+H]^+$, 493 $[M+Na]^+$, 963 $[2M+Na]^+$; 1D and 2D NMR data in accordance with literature)[53] and 18.7 mg of white, microcrystalline compound **3** (optical rotation $[\alpha]_D^{20}$ −24.2 ($CHCl_3$, *c* 0.89); MS

(ESI): *m/z* 393 $[M+H]^+$; 1D and 2D NMR data in accordance with literature);[54] B7 (65.1 mg) was fractionated using a Sephadex CC and eluted with DCM: acetone (85:15) to obtain fractions D1–7. Purification of D6 (23.1 mg) via methanol Sephadex CC afforded 5.4 mg of a whitish, microcrystalline powder (**4**; optical rotation $[\alpha]_D^{20}$ −24.1 (methanol, *c* 0.54); MS (ESI): *m/z* 469 $[M+H-H_2O]^+$, 487 $[M+H]^+$, 509 $[M+Na]^+$; 1D and 2D NMR data in accordance with literature).[25] Combined fractions B9 and B10 (552 mg) were separated using a flash silica gel CC (65 g; 41 × 1.6 cm) with 100 ml step gradients from DCM to acetone to obtain eight subfractions (E1–8). Crystallization of E2 (48.0 mg) from methanol yielded whitish crystals (**5**; 34.9 mg; optical rotation $[\alpha]_D^{20}$ +62.6 ($CHCl_3$, c 0.62); MS (ESI): *m/z* 443 $[M+H]^+$; 1D and 2D NMR data in accordance with literature).[53] E4 (23.3 mg) and E5 (19.3 mg) were purified over Sephadex CC with DCM/acetone (85:15), respectively, to afford 4.8 mg of a white, crystalline powder (**6**; optical rotation $[\alpha]_D^{20}$ +42.2 (methanol, *c* 0.48); MS (ESI): *m/z* 471 $[M+H]^+$; 1D and 2D NMR data in accordance with literature);[27] and 4.7 mg of a white, microcrystalline powder (**7**; optical rotation $[\alpha]_D^{20}$ +42.0 (methanol, *c* 0.46); MS (ESI): *m/z* 469 $[M+H-H_2O]^+$, 487 $[M+H]^+$, 509 $[M+Na]^+$; 1D and 2D NMR data in accordance with literature).[28]

E6 (10.1 mg) was purified with methanol Sephadex CC to yield 7.9 mg of white crystalline compound **8** (optical rotation $[\alpha]_D^{20}$ +31.4 (methanol, *c* 0.79); MS (ESI): *m/z* 525 $[M+Na]^+$; 1D and 2D NMR data in accordance with literature).[29]

Combined fractions A12 and A13 (440 mg) were subjected to Sephadex CC (80 × 2.0 cm). Elution with methanol resulted in eight fractions (F1–8). Combined F5 and F6 (61.2 mg) were re-chromatographed on silica gel (15 g; 38 × 1.0 cm) with 100 ml step gradients from DCM to methanol to obtain eight subfractions (G1–8). Recrystallization of G1 and G2 from methanol yielded 2.0 mg of white, microcrystalline compound **9** (optical rotation $[\alpha]_D^{20}$ +71.4 (methanol, *c* 0.20); MS (ESI): *m/z* 479 $[M+Na]^+$; 1D and 2D NMR data in accordance with literature)[55] and 1.7 mg of white microcrystalline compound **10** (optical rotation $[\alpha]_D^{20}$ +61.4 (methanol, *c* 0.17); MS (ESI): *m/z* 509 $[M+Na]^+$; 1D and 2D NMR data in accordance with literature),[56] respectively.

B16 (270 mg) was subjected to silica gel flash CC (45 g; 50 × 2.0 cm) with 100 ml step gradients from petrol ether to DCM and from DCM to acetone (fractions H1–10). For purification, combined fractions H3–5 (49 mg) were re-chromatographed twice by Sephadex CC (50 × 2.0 cm) using methanol as mobile phase to afford 12.5 mg of an inseparable 2:1mixture of **11** and **12**, respectively (MS (ESI): *m/z* 635 $[M+H]^+$; 1D and 2D NMR data in accordance with literature).[31] Hydrolysis of 10 mg of this mixture was performed with 5% sodium hydroxide by incubation at room temperature for 15 h. The reaction mixture was neutralized with 10% H_2SO_4 and extracted with ethyl acetate. The ethyl acetate layer was evaporated to dryness and purified with Sephadex CC (35 × 1.0 cm) using methanol as mobile phase to gain 5.4 mg of white needles of **13** (optical rotation $[\alpha]_D^{20}$ +28.8 (methanol, *c* 0.54); MS (ESI): *m/z* 511 $[M+Na]^+$; 1D and 2D NMR data in accordance with literature).[31]

The purity of all isolated compounds was determined by HPLC to be $\geqslant$95%.

4.6. Pharmacological testing

For measurements of 11β-HSD1 reductase activity, lysates of HEK-293 cells stably expressing human recombinant 11β-HSD1 were incubated for 10 min at 37 °C in a total volume of 22 µl containing 200 nM [1,2-^{3}H]-labelled cortisone (American Radiolabeled Chemicals, St. Louis, MO) and 500 µM NADPH. 11β-HSD2 dependent oxidation of cortisol to cortisone was measured similarly for 10 min at 37 °C in lysates of HEK-293 cells stably expressing human 11β-HSD2 using [1,2,6,7-^{3}H]-cortisol (Amersham Pharmacia, Piscataway, NJ, USA) at a final concentration of 50 nM and 500 µM NAD^+. Extracts of *E. japonica* at 25 µg/ml and pure compounds at final concentrations between 50 nM and 50 µM were diluted from stock solutions in methanol and immediately used for activity assays. The solvent concentration did not exceed 0.5% and had no effect on enzyme activities. Reactions were stopped by adding methanol containing 2 mM unlabeled cortisone and cortisol, followed by separation of steroids by TLC and scintillation counting. Enzyme kinetics was analyzed by non-linear regression using four parameter logistic curve fitting (Sigmaplot, Systat Software Inc.). Data (mean ± SD) were obtained from at least three independent experiments.

Acknowledgements

This work was granted by the Austrian Federal Ministry of Science and Research and the Austrian Federal Ministry of Health within the Project 'Treatment of Metabolic Syndrome by Traditional Chinese Medicine', and the Swiss National Science Foundation, No. 31003A-124912. D.S. acknowledges the financial support of the University of Innsbruck within a young talents grant project and the Erika Cremer Habilitation Program. A.O. has a Chair in Molecular and Systems Toxicology by the Novartis Research Foundation. The authors thank Dr. E.P. Ellmerer for NMR measurements.

References and notes

1. Atanasov, A. G.; Odermatt, A. *Endocr. Metab. Immune Disord.: Drug Targets* **2007**, *7*, 125–140.
2. Masuzaki, H.; Paterson, J.; Shinyama, H.; Morton, N. M.; Mullins, J. J.; Seckl, J. R.; Flier, J. S. *Science* **2001**, *294*, 2166–2170.
3. Masuzaki, H.; Yamamoto, H.; Kenyon, C. J.; Elmquist, J. K.; Morton, N. M.; Paterson, J. M.; Shinyama, H.; Sharp, M. G. F.; Fleming, S.; Mullins, J. J.; Seckl, J. R.; Flier, J. S. *J. Clin. Invest.* **2003**, *112*, 83–90.
4. Hadoke, P. W. F.; Iqbal, J.; Walker, B. R. *Br. J. Pharmacol.* **2009**, *156*, 689–712.
5. Boyle, C. D.; Kowalski, T. J. *Expert Opin. Ther. Pat.* **2009**, *19*, 801–825.
6. St. Jean, D. J., Jr.; Wang, M.; Fotsch, C. *Curr. Top. Med. Chem.* **2008**, *8*, 1508–1523.
7. Odermatt, A.; Gumy, C. *Biochem. Pharmacol.* **2008**, *76*, 1184–1193.
8. Gumy, C.; Thurnbichler, C.; Aubry, E. M.; Balazs, Z.; Pfisterer, P.; Baumgartner, L.; Stuppner, H.; Odermatt, A.; Rollinger, J. M. *Fitoterapia* **2009**, *80*, 200–205.
9. Li, W. L.; Zheng, H. C.; Bukuru, J.; De Kimpe, N. *J. Ethnopharmacol.* **2004**, *92*, 1–21.
10. Chen, J.; Li, W. L.; Wu, J. L.; Ren, B. R.; Zhang, H. Q. *Phytomedicine* **2008**, *15*, 98–102.
11. De Tommasi, N.; De Simone, F.; Cirino, G.; Cicala, C.; Pizza, C. *Planta Med.* **1991**, *57*, 414–416.
12. Li, W.-L.; Wu, J.-L.; Ren, B.-R.; Chen, J.; Lu, C.-G. *Am. J. Chin. Med.* **2007**, *35*, 705–711.
13. Lu, H.; Chen, J.; Li, W. L.; Ren, B. R.; Wu, J. L.; Kang, H. Y.; Zhang, H. Q.; Adams, A.; De Kimpe, N. *J. Ethnopharmacol.* **2009**, *122*, 486–491.
14. Lu, H.; Chen, J.; Li, W. L.; Ren, B. R.; Wu, J. L.; Zhang, H. Q. *Phytomedicine* **2009**, *16*, 967–971.
15. Noreen, W.; Wadood, A.; Hidayat, H. K.; Wahid, S. A. *Planta Med.* **1988**, *54*, 196–199.
16. Schuster, D.; Maurer, E. M.; Laggner, C.; Nashev, L. G.; Wilckens, T.; Langer, T.; Odermatt, A. *J. Med. Chem.* **2006**, *49*, 3454–3466.
17. Rollinger, J. M.; Steindl, T. M.; Schuster, D.; Kirchmair, J.; Anrain, K.; Ellmerer, E. P.; Langer, T.; Stuppner, H.; Wutzler, P.; Schmidtke, M. *J. Med. Chem.* **2008**, *51*, 842–851.
18. Amico, V.; Barresi, V.; Condorelli, D.; Spatafora, C.; Tringali, C. *J. Agric. Food Chem.* **2006**, *54*, 810–814.
19. Ganguly, S. N. *Phytochemistry* **1972**, *11*, 2893.
20. He, X.; Liu, R. H. *J. Agric. Food Chem.* **2007**, *55*, 4366–4370.
21. Li, E.-N.; Luo, J.-G.; Kong, L.-Y. *Phytochem. Anal.* **2009**, *20*, 338–343.
22. Liang, Z.; Aquino, R.; De Feo, V.; De Simone, F.; Pizza, C. *Planta Med.* **1990**, *56*, 330–332.
23. Banno, N.; Akihisa, T.; Tokuda, H.; Yasukawa, K.; Taguchi, Y.; Akazawa, H.; Ukiya, M.; Kimura, Y.; Suzuki, T.; Nishino, H. *Biol. Pharm. Bull.* **2005**, *28*, 1995–1999.
24. Ito, H.; Kobayashi, E.; Li, S.-H.; Hatano, T.; Sugita, D.; Kubo, N.; Shimura, S.; Itoh, Y.; Yoshida, T. *J. Nat. Prod.* **2001**, *64*, 737–740.
25. Biessels, H. W. A.; Van der Kerk-Van Hoof, A. C.; Kettenes-Van den Bosch, J. J.; Salemink, C. A. *Phytochemistry* **1974**, *13*, 203–207.
26. Fu, L.; Zhang, S.; Li, N.; Wang, J.; Zhao, M.; Sakai, J.; Hasegawa, T.; Mitsui, T.; Kataoka, T.; Oka, S.; Kiuchi, M.; Hirose, K.; Ando, M. *J. Nat. Prod.* **2005**, *68*, 198–206.

27. Tsichritzis, F.; Jakupovic, J. *Phytochemistry* **1990**, *29*, 3173–3187.
28. Seo, S.; Tomita, Y.; Tori, K. *Tetrahedron Lett.* **1975**, 7–10.
29. Lontsi, D.; Ngounou, N. F.; Tapondjou, A. L.; Sondengam, B. L.; Bodo, B.; Martin, M.-T. *Phytochemistry* **1998**, *49*, 2473–2476.
30. Shimizu, M.; Fukumura, H.; Tsuji, H.; Tanaami, S.; Hayashi, T.; Morita, N. *Chem. Pharm. Bull.* **1986**, *34*, 2614–2617.
31. Taniguchi, S.; Imayoshi, Y.; Kobayashi, E.; Takamatsu, Y.; Ito, H.; Hatano, T.; Sakagami, H.; Tokuda, H.; Nishino, H.; Sugita, D.; Shimura, S.; Yoshida, T. *Phytochemistry* **2002**, *59*, 315–323.
32. Kubota, T.; Asaka, Y.; Miura, I.; Mori, H. *Helv. Chim. Acta* **1982**, *65*, 611–619.
33. Pardhy, M. R. S.; Bhattacharyya, S. C. *Indian J. Chem., Sect. B* **1978**, *16B*, 176–178.
34. Syamasundar, K. V.; Mallavarapu, G. R.; Krishna, E. M. *Phytochemistry* **1991**, *30*, 362–363.
35. Berman, H. M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T. N.; Weissig, H.; Shindyalov, I. N.; Bourne, P. E. *Nucleic Acids Res.* **2000**, *28*, 235–242.
36. Kim, K. W.; Wang, Z.; Busby, J.; Tsuruda, T.; Chen, M.; Hale, C.; Castro, V. M.; Svensson, S.; Nybo, R.; Xiong, F.; Wang, M. *Biochim. Biophys. Acta, Proteins Proteomics* **2006**, *1764*, 824–830.
37. Wolber, G.; Dornhofer, A. A.; Langer, T. *J. Comput. Aided Mol. Des.* **2006**, *20*, 773–788.
38. Hughes, K. A.; Webster, S. P.; Walker, B. R. *Expert Opin. Invest. Drugs* **2008**, *17*, 481–496.
39. Dzubak, P.; Hajduch, M.; Vydra, D.; Hustova, A.; Kvasnica, M.; Biedermann, D.; Markova, L.; Urban, M.; Sarek, J. *Nat. Prod. Rep.* **2006**, *23*, 394–411.
40. Miura, T.; Ueda, N.; Yamada, K.; Fukushima, M.; Ishida, T.; Kaneko, T.; Matsuyama, F.; Seino, Y. *Biol. Pharm. Bull.* **2006**, *29*, 585–587.
41. Fukushima, M.; Matsuyama, F.; Ueda, N.; Egawa, K.; Takemoto, J.; Kajimoto, Y.; Yonaha, N.; Miura, T.; Kaneko, T.; Nishi, Y.; Mitsui, R.; Fujita, Y.; Yamada, Y.; Seino, Y. *Diabetes Res. Clin. Pract.* **2006**, *73*, 174–177.
42. Yamaguchi, Y.; Yamada, K.; Yoshikawa, N.; Nakamura, K.; Haginaka, J.; Kunitomo, M. *Life Sci.* **2006**, *79*, 2474–2479.
43. Wen, X.; Sun, H.; Liu, J.; Wu, G.; Zhang, L.; Wu, X.; Ni, P. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4944–4948.
44. Hou, W.; Li, Y.; Zhang, Q.; Wei, X.; Peng, A.; Chen, L.; Wei, Y. *Phytother. Res.* **2009**, *23*, 614–618.
45. Shi, L.; Zhang, W.; Zhou, Y.-Y.; Zhang, Y.-N.; Li, J.-Y.; Hu, L.-H.; Li, J. *Eur. J. Pharmacol.* **2008**, *584*, 21–29.
46. Na, M.; Yang, S.; He, L.; Oh, H.; Kim, B. S.; Oh, W. K.; Kim, B. Y.; Ahn, J. S. *Planta Med.* **2006**, *72*, 261–263.
47. Takagi, S.; Miura, T.; Ishibashi, C.; Kawata, T.; Ishihara, E.; Gu, Y.; Ishida, T. *J. Nutr. Sci. Vitaminol.* **2008**, *54*, 266–268.
48. Jang, S.-M.; Yee, S.-T.; Choi, J.; Choi, M.-S.; Do, G.-M.; Jeon, S.-M.; Yeo, J.; Kim, M.-J.; Seo, K.-I.; Lee, M.-K. *Int. Immunopharmacol.* **2009**, *9*, 113–119.
49. Wen, X.; Sun, H.; Liu, J.; Cheng, K.; Zhang, P.; Zhang, L.; Hao, J.; Zhang, L.; Ni, P.; Zographos, S. E.; Leonidas, D. D.; Alexacou, K.-M.; Gimisis, T.; Hayes, J. M.; Oikonomakos, N. G. *J. Med. Chem* **2008**, *51*, 3540–3554.
50. Ali, H.; Houghton, P. J.; Soumyanath, A. *J. Ethnopharmacol.* **2006**, *107*, 449–455.
51. Wermuth, C. G.; Ganellin, C. R.; Lindberg, P.; Mitscher, L. A. *Pure Appl. Chem.* **1998**, *70*, 1129–1143.
52. Wolber, G.; Langer, T. *J. Chem. Inf. Comput. Sci.* **2005**, *45*, 160–169.
53. Collins, D. O.; Ruddock, P. L. D.; Chiverton de Grasse, J.; Reynolds, W. F.; Reese, P. B. *Phytochemistry* **2002**, *59*, 479–488.
54. Weng, J.-R.; Su, H.-J.; Yen, M.-H.; Won, S.-J.; Lin, C.-N. *Chin. Pharm. J.* **2003**, *55*, 267–272.
55. Gnoatto, S. C. B.; Dassonville-Klimpt, A.; Da Nascimento, S.; Galera, P.; Boumediene, K.; Gosmann, G.; Sonnet, P.; Moslemi, S. *Eur. J. Med. Chem.* **2008**, *43*, 1865–1877.
56. Ding, Y.; Kinjo, J.; Yang, C.; Nohara, T. *Phytochemistry* **1991**, *30*, 3703–3707.