Bioorganic & Medicinal Chemistry 19 (2011) 1866–1880

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Synthesis of new glycyrrhetinic acid derived ring A azepanone, 29-urea and 29-hydroxamic acid derivatives as selective 11β-hydroxysteroid dehydrogenase 2 inhibitors

Rawindra Gaware [a], Rupesh Khunt [a], Laszlo Czollner [a], Christian Stanetty [b], Thierry Da Cunha [c], Denise V. Kratschmar [c], Alex Odermatt [c], Paul Kosma [b], Ulrich Jordis [a], Dirk Claßen-Houben [d,*]

[a] Institute of Applied Synthetic Chemistry, University of Technology, Getreidemarkt 9, 1060 Vienna, Austria
[b] Department of Chemistry, University of Natural Resources and Applied Life Sciences, Muthgasse 18, 1190 Vienna, Austria
[c] Division of Molecular and Systems Toxicology, Pharmacenter, University of Basel, Klingelbergstrasse 50, 4056 Basel, Switzerland
[d] Onepharm Research and Development GmbH, Veterinärplatz 1, 1210 Vienna, Austria

ARTICLE INFO

Article history:
Received 14 December 2010
Revised 28 January 2011
Accepted 3 February 2011
Available online 2 March 2011

Keywords:
11β-Hydroxysteroid dehydrogenase
11β-HSD1
11β-HSD2
Selective inhibitor
Hydroxamic acid
Glycyrrhizin
Glycyrrhetinic acid
Carbenoxolone
Glucocorticoid metabolism
Cortisone metabolism
Inflammation
Chronic inflammatory disease
Cancer

ABSTRACT

Glycyrrhetinic acid, the metabolite of the natural product glycyrrhizin, is a well known nonselective inhibitor of 11β-hydroxysteroid dehydrogenase (11β-HSD) type 1 and type 2. Whereas inhibition of 11β-HSD1 is currently under consideration for treatment of metabolic diseases, such as obesity and diabetes, 11β-HSD2 inhibitors may find therapeutic applications in chronic inflammatory diseases and certain forms of cancer. Recently, we published a series of hydroxamic acid derivatives of glycyrrhetinic acid showing high selectivity for 11β-HSD2. The most potent and selective compound is active against human 11β-HSD2 in the low nanomolar range with a 350-fold selectivity over human 11β-HSD1. Starting from the lead compounds glycyrrhetinic acid and the hydroxamic acid derivatives, novel triterpene type derivatives were synthesized and analyzed for their biological activity against overexpressed human 11β-HSD1 and 11β-HSD2 in cell lysates. Here we describe novel 29-urea- and 29-hydroxamic acid derivatives of glycyrrhetinic acid as well as derivatives with the Beckman rearrangement of the 3-oxime to a seven-membered ring, and the rearrangement of the C-ring from 11-keto-12-ene to 12-keto-9(11)-ene. The combination of modifications on different positions led to compounds comprising further improved selective inhibition of 11β-HSD2 in the lower nanomolar range with up to 3600-fold selectivity.

1. Introduction

11β-Hydroxysteroid dehydrogenases (11β-HSDs) are microsomal enzymes belonging to the short-chain dehydrogenase/reductase (SDR) family. In humans and rodents, two isozymes, 11β-HSD1 and 11β-HSD2 have been identified which catalyze the interconversion of active 11β-hydroxyglucocorticoids and their inactive 11-keto counterparts (Fig. 1).[1–4]

11β-HSD1 NADPH-dependently activates the 11-ketosteroids cortisone (human) and 11-dehydrocorticosterone (rodents) to cortisol and corticosterone, respectively. 11β-HSD1 is highly expressed in many glucocorticoid target tissues, including liver, adipose tissue, skeletal muscle and macrophages. 11β-HSD2 is a NAD^+-dependent dehydrogenase and inactivates 11β-hydroxyglucocorticoids by oxidation in kidney, colon, placenta and inflamed tissue. In classical aldosterone target tissues, such as renal cortical collecting ducts and distal colon, 11β-HSD2 protects the mineralocorticoid receptor from activation by glucocorticoids.

Active glucocorticoids play a vital role in the regulation of carbohydrate, protein, lipid, and bone metabolism, the maturation and differentiation of cells, and the modulation of inflammatory responses and stress. These cortisol effects are mediated by the activation of glucocorticoid receptors. The local concentration of active cortisol in specific tissues is tuned by the pre-receptor metabolism performed by 11β-HSDs. Cortisone in plasma provides a pool of inactive precursor that can be converted to active glucocorticoids at sites where 11β-HSD1 reductase activity is

* Corresponding author. Present address: Savira Pharmaceuticals GmbH, Veterinärplatz 1, 1210 Vienna, Austria. Tel.: +43 1 250 77 5900; fax: +43 1 250 77 5999.
E-mail address: dirk.classen-houben@savira.at (D. Claßen-Houben).

0968-0896/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2011.02.005

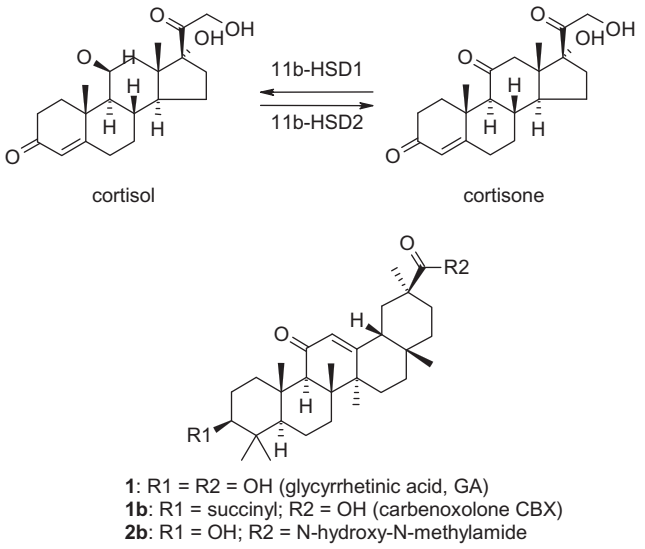


Figure 1. 11β-HSD enzymes catalyze the interconversion of cortisone and cortisol. Glycyrrhetinic acid (**1**) and carbenoxolone (**1b**) represent nonselective 11β-HSD inhibitors that together with the selective inhibitor (**2b**) serve as lead molecules in the present study.

predominant. Local excess or deficiency of glucocorticoids due to impaired function of 11β-HSDs has been associated with specific diseases, pointing to potential therapeutic indications for selective inhibitors of both 11β-HSD1 and 11β-HSD2.

Elevated 11β-HSD1-dependent glucocorticoid activation is associated with multiple features of the metabolic syndrome like insulin and leptin resistance, visceral obesity, dyslipidemia, and type 2 diabetes.[5–7] Over-expression of 11β-HSD1 within adipose tissue in transgenic mice results in insulin resistance, hyperlipidemia and visceral obesity, whereas 11β-HSD1 knockout mice show decreased triglyceride and cholesterol levels and resistance to stress-induced hyperglycemia.[8,9]

Glycyrrhetinic acid (**1**, GA), the metabolite of the natural product glycyrrhizin, inhibits both, 11β-HSD1 and 11β-HSD2.[10–12] Based on IC_{50} values in homogenates of rat liver and kidney, a 20-fold to 40-fold selectivity for 11β-HSD1 over 11β-HSD2 was reported for 11-deoxo-glycyrrhetinic acid, glycyrrhetinic acid 3-hemiphthalate and glycyrrhetol.[13] Other compounds that are not triterpenes have been identified as selective 11β-HSD1 inhibitors in high throughput screening campaigns and lead optimization programs by different pharmaceutical companies and are currently being developed for the treatment of metabolic diseases.[14–21]

Whereas beneficial effects have been reported for the inhibition of 11β-HSD1 in metabolic diseases such as obesity and diabetes, inhibition of 11β-HSD2 has been associated with enhanced renal sodium retention and increased blood pressure.[22] However, recent studies found an association of elevated 11β-HSD2 expression with (chronic) inflammatory diseases and cancer, and suggested that inhibition of this enzyme may have beneficial effects in these diseases.[23–28] Topical applications or targeted delivery to the tumor may be required for selective 11β-HSD2 inhibitors in order to avoid glucocorticoid-dependent activation of the mineralocorticoid receptor. Alternatively, 11β-HSD2 inhibitors may be applied in the treatment of patients on hemodialysis aiming to enhance potassium excretion by the colon.[29] Nevertheless, no selective 11β-HSD2 inhibitor has been developed so far and neither animal studies nor clinical trials have been executed based on the selective inhibition of 11β-HSD2. The main physiological function of 11β-HSD2 is to prevent the binding of active corticosteroids to the mineralocorticoid receptor in certain tissues including the kidneys. A selective inhibition of 11β-HSD2 can thus result in apparent mineralocorticoid excess syndrome due to excessive activation of the mineralocorticoid receptor by glucocorticoids. This might be the main reason that no selective inhibitor has been developed so far. Beside the hydroxamic acid derivatives of glycyrrhetinic acid reported previously by our group,[30] the only compound known to exhibit noteworthy selectivity for 11β-HSD2 is a hydroxyethylamide derivative of glycyrrhetinic acid reported by Vicker et al.[31] with only 36% inhibition of rodent 11β-HSD1 but 92% inhibition of rodent 11β-HSD2 at 10 μM. The reported IC_{50} value by Vicker et al. of 0.004 nM for 11β-HSD2 is very low[31], however, using human 11β-HSD2, an IC_{50} value in the lower micromolar range was obtained (own observations).[32] Recently, we were able to show, that hydroxamic acid derivatives of glycyrrhetinic acid are highly potent and selective inhibitors of human 11β-HSD2, whereof compound **2b** was the most potent (IC_{50} value of 2.9 nM) and selective (350-fold) inhibitor of human 11β-HSD2 reported to date.[30]

GA, as a non-selective inhibitor of both isozymes and the hydroxamic acid derivative **2b** are valuable starting points for the further development of selective 11β-HSD2 inhibitors. Earlier we reported different types of modifications, including the introduction of sulfur, halides, double bonds, additional hydroxy groups, the installation of an episulfide and ring-A expansions[32,33] as well as a combinatorial library of Ugi-type products[34], and most recently the installation of the C29 hydroxamic acid motif.[30]

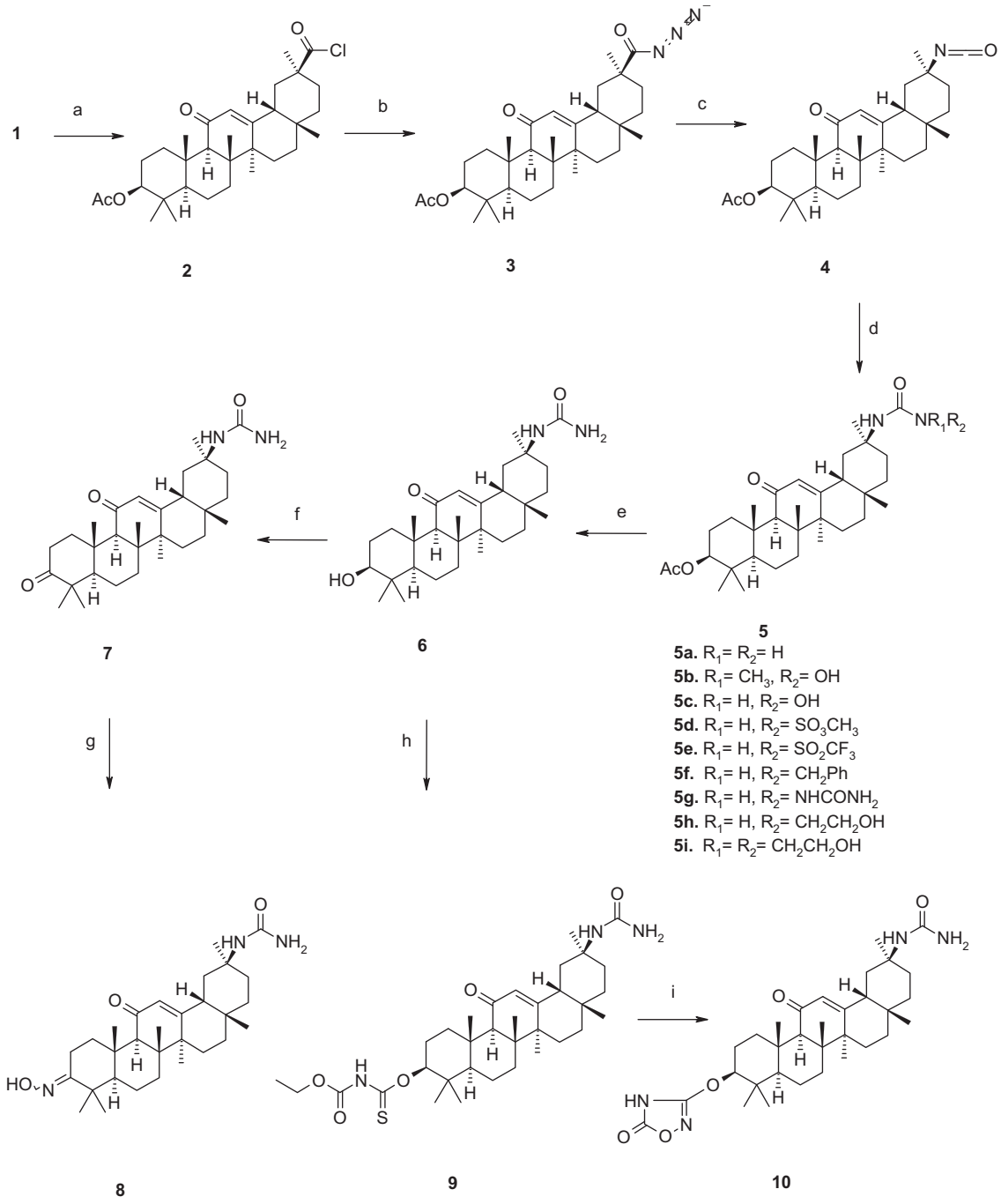
Here, we report the synthesis of a variety of 29-urea and 29-hydroxamic acid derivatives of glycyrrhetinic acid with inhibitory activity against human recombinant 11β-HSD1 and 11β-HSD2. Sophisticated derivatives with the Beckman rearrangement of the 3-oxime to a seven-membered Ring, and the rearrangement of the C-ring from 11-keto-12-ene to 12-keto-9(11)-ene are also included in the survey. Finally, the motif of the 29-urea and 20-hydroxamic acid was combined with modifications of ring A and at position 12 and activity of compounds against both isoenzymes of 11β-HSD were determined.

2. Results and discussion

2.1. Chemistry

Commercial glycyrrhetinic acid (**1**) was refluxed in acetyl chloride to directly give acid chloride of 3-acetyl glycyrrhetinic acid **2**, which was further converted to the acid azide **3** with NaN_3 in acetone (Scheme 1). Curtius rearrangement in $CHCl_3$ gave isocyanate **4**, a central intermediate for a series of urea type compounds, in excellent overall yield. Treatment with ammonia gas gave parent urea **5a** and similarly, substituted urea compounds **5b–5i** were prepared by parallel reactions of **4** with amines (Method A) or amine hydrochlorides and $NaHCO_3$ (Method B) in good yields. Unsubstituted urea **5a** was structurally diversified by liberation of 3-hydroxy moiety (**6**), Jones oxidation to ketone **7** and formation of 3-oxime **8** (Scheme 1). Furthermore, alcohol **6** was converted to oxadiazolone **10** in two steps by first reacting with ethoxycarbonyl isothiocyanate in $CHCl_3$ (**9**) and subsequent ring closure using $NH_2OH{\cdot}HCl$ and LiOH as base.

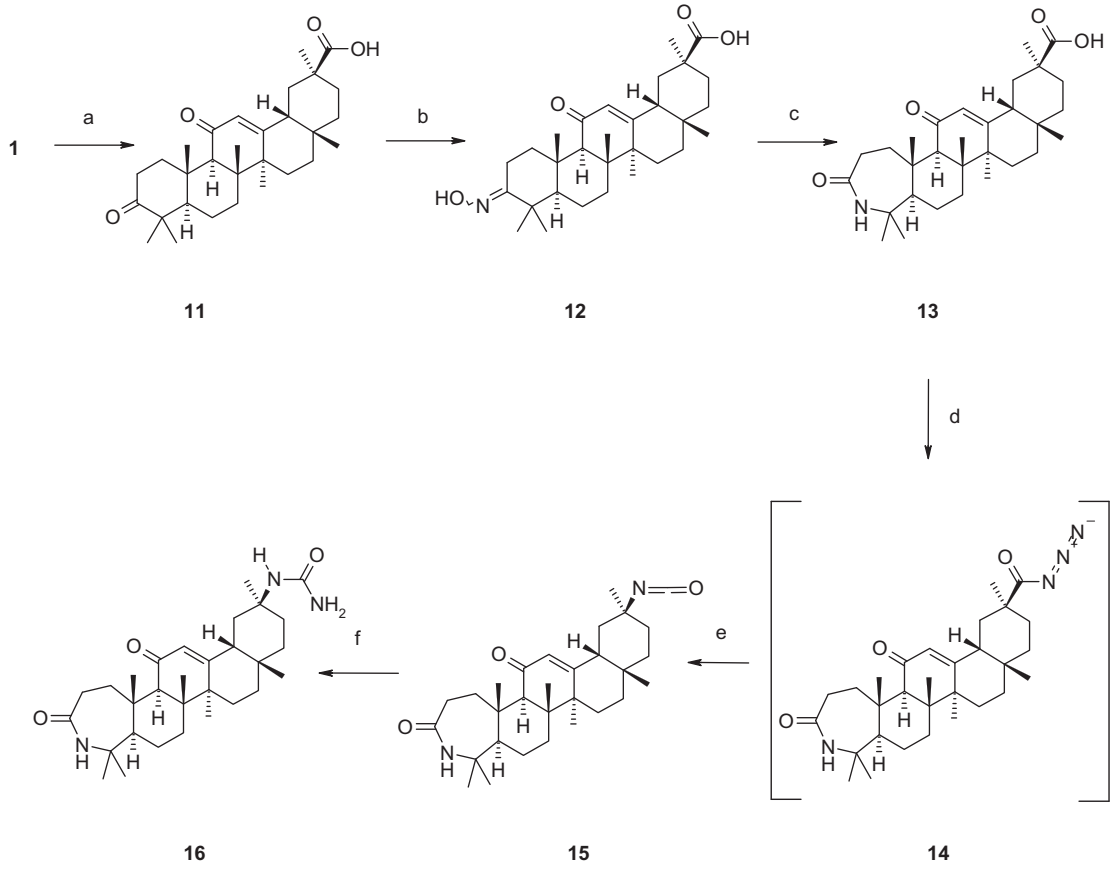
To further enrich the chemical diversity, two more sophisticated urea type glycyrrhetinic acid derivatives were prepared with modified A-ring (Scheme 2) and modified C-ring, respectively (Scheme 3). First, 3-keto-GA[32] **11** was converted to oxime **12** that was submitted to a Beckmann rearrangement leading to lactam **13**. Reaction conditions with PCl_5 in dry methylene chloride (DCM) gave the best results. Lactam **13** was converted to the corresponding urea **16** via acid azide **14** and isocyanate **15** in an analogous manner as described above. Furthermore, we prepared a urea derivative of **1** with inverted regiochemistry of the enone in ring C (Scheme 3). Initially the enone of **1** was reduced with



Scheme 1. C29 urea derivatives of glycyrrhetinic acid modified at C3 position. Reagents: (a) acetyl chloride, reflux (86%); (b) NaN_3 in acetone (90%); (c) $CHCl_3$, heat, (90%); (d) ammonia gas, amine hydrochlorides/$NaHCO_3$ in THF or amine in dry DCM (50% **5a**, 64% **5b**, 56% **5c**, 51% **5d**, 42% **5e**, 66% **5f**, 57% **5g**, 63% **5h**, 66% **5i**); (e) KOH in MeOH (72%); (f) Jones reagent in acetone (70%); (g) $NH_2OH.HCl$ in pyridine (58%); (h) ethoxycarbonyl isothiocyanate in $CHCl_3$ (26%); (i) $NH_2OH.HCl$, LiOH, in EtOH (6%).

$NaBH_4$ and NaOH in THF to give the 11-hydroxy derivative **17** as a mixture of both stereoisomers that was further converted to the diene **18** by acid catalyzed elimination. The oxidation of diene **18** using 3-chloro-perbenzoic acid resulted in the 12-oxo-9(11)-ene **19** that constitutes a regioisomer of **1**. Carboxylic acid **19** was converted to urea **23** via acid azide **21** and isocyanate **22** applying similar conditions as for compounds **5a** and **16** and was deprotected to give urea **24** with free 3-hydroxy moiety.

Both, the 3-oxadiazolone substitution in compound **10** as well as the lactam substructure in compound **16** are both structural elements that have not yet been included in our strive for selective 11β-HSD inhibitors. Therefore, we wanted to combine them with the *N*-methyl-hydroxamic acid moiety that was identified as most potent structural element at the C29 position earlier.[30] **1** was therefore converted to 3-oxadiazolone derivative **26** analogous to above by first reacting with ethoxycarbonyl isothiocyanate in $CHCl_3$ (**25**) and subsequent ring closure using $NH_2OH{\cdot}HCl$, and LiOH as base (Scheme 4). Subsequently, the acid moiety was activated as acid chloride and reacted with N-methyl hydroxylamine to deliver the corresponding hydroxamic acid **27**. In the same manner, the acid moiety of lactam 13 was converted to the *N*-methyl-hydroxamic acid **28** (Scheme 4).



Scheme 2. Ring A modified C29 urea derivative of glycyrrhetinic acid. Reagents: (a) Jones reagent in acetone (95%); (b) $NH_2OH.HCl$ in pyridine (85%) (c) PCl_5 in dry DCM (40%); (d) $SOCl_2$ in dry DCM, NaN_3 in acetone (90%); (e) in $CHCl_3$, heat (60%); (f) ammonia gas in $CHCl_3$ (73%).

2.2. Biology

Glycyrrhetinic acid (**1**) is a potent and nonselective inhibitor of both 11β-HSD isozymes. Shimoyama et al. reported a lower IC_{50} value using rat hepatic 11β-HSD1 homogenate (90 ± 2 nM) compared to rat renal 11β-HSD2 homogenate (360 ± 2 nM).[13] Potter et al. reported 85% inhibition of rat 11β-HSD1 and complete inhibition of rat 11β-HSD2 at a concentration of 10 μM of glycyrrhetinic acid.[31,35–37] We evaluated the inhibitory activity of glycyrrhetinic acid and its derivatives against recombinant human 11β-HSD1 and 11β-HSD2. Using a tenfold lower inhibitor concentration (1 μM) we found an inhibition of 83.9% of human 11β-HSD1 and 93.6% of human 11β-HSD2 for glycyrrhetinic acid. The licensed drug carbenoxolone (**1b**) had comparable potency with 87.7% inhibition of 11β-HSD1 and 98.2% inhibition of 11β-HSD2. As the lead compounds **1** and **1b** showed significant inhibition of both, 11β-HSD1 and 11β-HSD2 at 1 μM, novel derivatives were first screened at a concentration of 1 μM. As soon as first highly active compounds were found, further derivatives were screened at 0.2 μM. IC_{50} values were determined for selected compounds.

The crystal structure of 11β-HSD1 in complex with carbenoxolone (**1b**) and $NADP^+$ was analyzed for necessary and potential interactions between inhibitor and protein (PDB ID: 2BEL).[38,39] Besides multiple hydrophobic interactions of the triterpene core with surrounding amino acids (Thr124, Leu126, Leu171, Ala172, Tyr177, Val180, Tyr183, Leu217, Ala223, Ala226, Val227), specific interactions are formed with the carboxylic acid oxygen atom, the 11-keto function and the carbonyl oxygen of the ester between glycyrrhetinic acid and succinic acid. The carboxylic acid of glycyrrhetinic acid interacts with the hydrogen bond donor functions of Tyr183 and the 2′-hydroxy-group of the ribose of the nicotinamide nucleotide of $NADP^+$. The 11-keto function acts as a hydrogen bond acceptor for the hydroxy group of Ser170 and the backbone amide of Ala172. Finally, the carbonyl oxygen atom of the ester between glycyrrhetinic acid and succinic acid forms a hydrogen bond from the amide nitrogen of Leu217 as well as from a conserved water molecule. The free acid function of the succinate is not well resolved in the crystal structure that might be due to high conformational flexibility and lack of specific interactions.

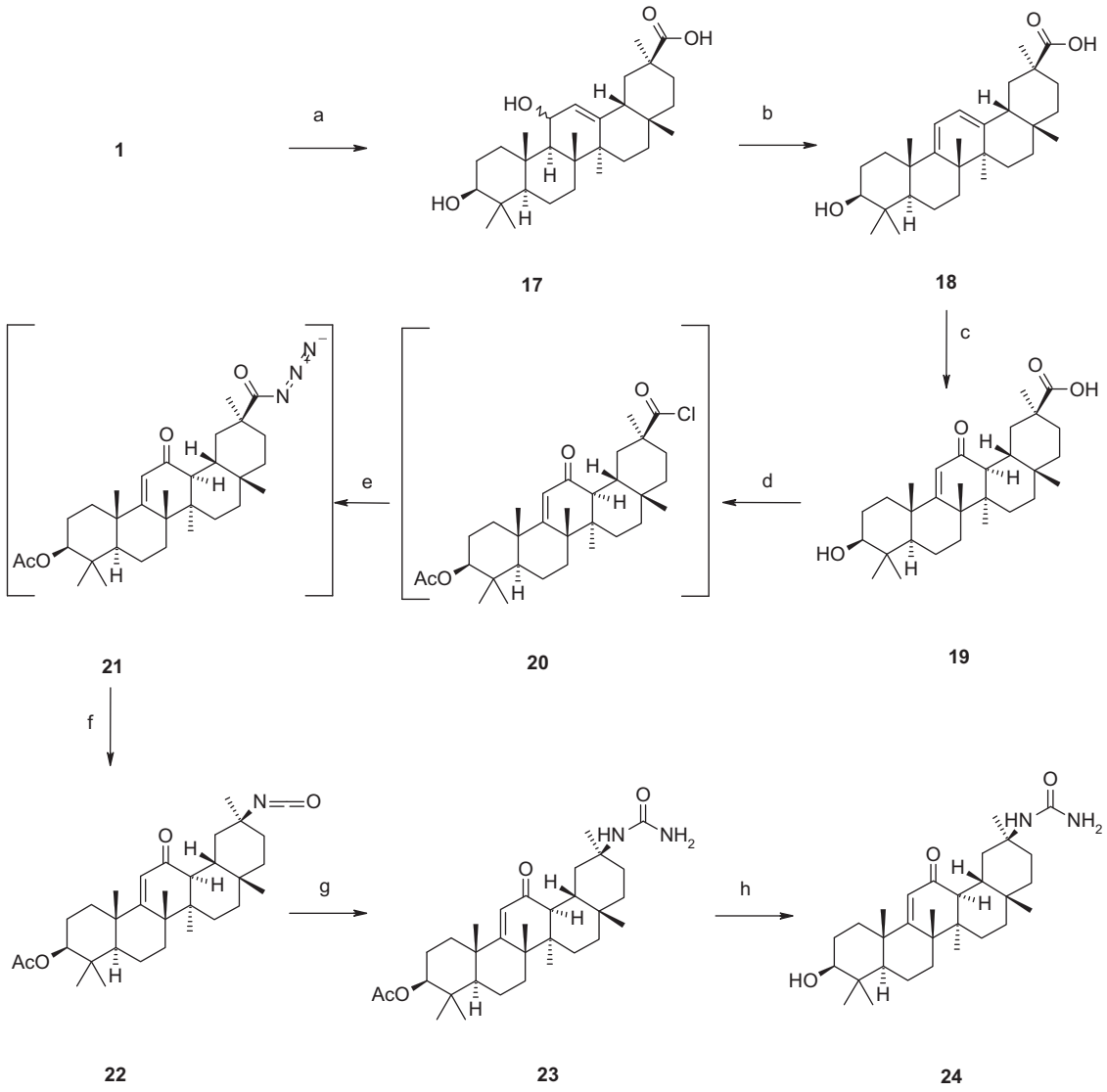
Recently, we reported that the substitution of position 3 in ring A is possible without loss of activity, as could be shown for the acetate **1c**, acetamide **1d**, ketone **11**, methoxyamine **12**, and certain sulfonamide derivatives[30] (Table 1). The 3-acetate **1c** has already been suggested as an inhibitor of 11β-HSD isozymes based on a pharmacophore model.[40]

Based on information derived from crystal structure of 11β-HSD1 with **1b**, we synthesized the azepanone derivative **13** and the oxadiazoline ether **26** that should retain the hydrogen bond with the backbone amide of Leu217. Both modifications had only a minor impact on selectivity and activity of glycyrrhetinic acid derivatives.

In addition, we could show, the favorable 11β-HSD2 inhibition of series of 3-acetyl glycyrrhetinic acid derivatives with the substitution of position 29 with a hydroxamic acid series, where especially 29-(*N*-methyl)-hydroxamic acid derivative **2b** comprised high activity and selectivity[30] (Table 2).

Based on the above structural information and the obtained results, an additional series of urea derivatives at position 29 with an acetate residue in position 3 were synthesized and evaluated for their biological activity.

The unsubstituted urea derivative **5a** inhibited 11β-HSD2 with similar activity as glycyrrhetinic acid **1** and showed a complete loss



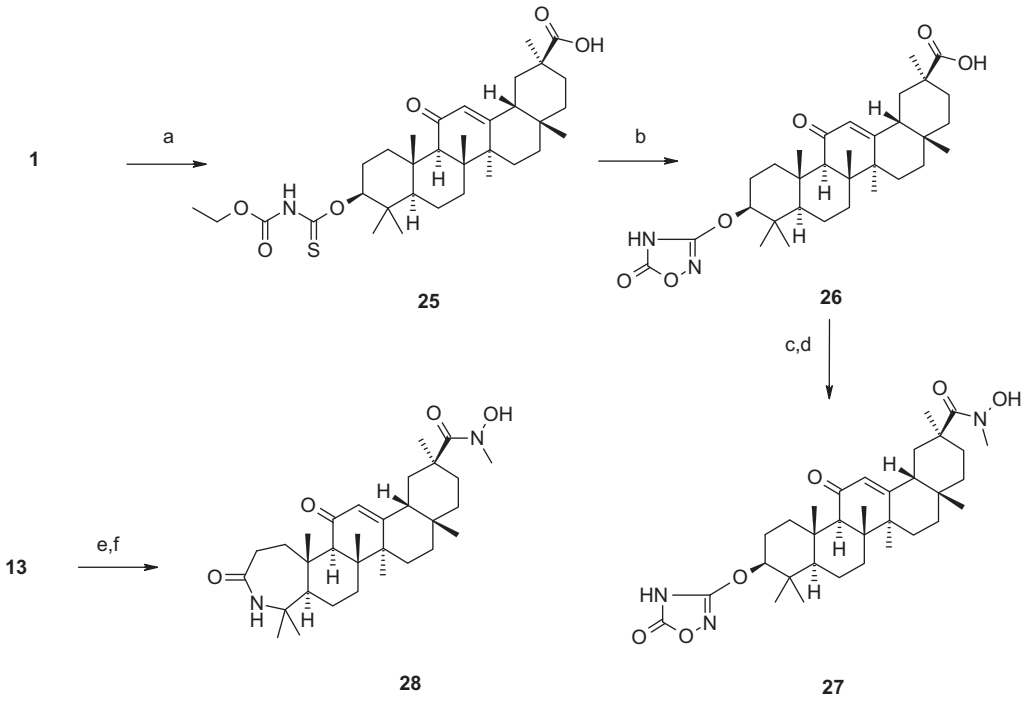
Scheme 3. C29 urea derivatives of glycyrrhetinic acid 12-oxo-9(11)ene derivative. Reagents: (a) $NaBH_4$, NaOH in 1:1 H_2O and THF (80%); (b) concd HCl in THF, reflux, (66%); (c) *m*-chloroperbenzoic acid, in $CHCl_3$ (24%); (d) acetyl chloride, reflux (96%); (e) NaN_3, acetone (60%); (f) in $CHCl_3$, heat (21%); (g) ammonia gas in $CHCl_3$ (73%); (h) KOH in MeOH (72%).

of activity against 11β-HSD1 (Table 2). The IC_{50} value of **5a** for 11β-HSD2 was sevenfold lower than the IC_{50} value of **1c**; however, the activity against 11β-HSD1 was in the similar range (Table 5). Therefore, we started to modify the distal nitrogen of the urea. The obtained *N*-hydroxy-*N*-methyl **5b** and bis-*N*-hydroxyethyl derivatives **5i** were almost inactive on both enzymes. Mono substitution as in the *N*-hydroxy **5c** or *N*-hydroxyethyl derivative **5h** retained at least some activity against 11β-HSD2 and showed a correlation between degree of substitution of the distal urea nitrogen and 11β-HSD2 inhibition (Table 2). With the methylsulfonamide **5d** and the trifluoromethylsulfonamide **5e** additional mono substituted glycyrrhetinic urea derivatives were synthesized, whereof **5e** had similar activity at 200 nM as the unsubstituted urea. For both compounds **5e** and **5a** determined IC_{50} values were in the same range for 11β-HSD2 with 194 and 104 nM, respectively. For 11β-HSD1 the IC_{50} values were calculated to 4.0 and 8.3 μM, resulting in 20-fold to 80-fold selectivity for 11β-HSD2 over 11β-HSD1 (Table 5). In summary, the introduction of a urea function at position 29 resulted in increased activity compared to the lead compound **1** with activity depending on the type of substitution of the urea nitrogen.

The inversion of the enone in ring C ring from 11-keto-12-ene to 12-keto-9(11)-ene as exemplified in compound **19** was predicted to retain the hydrogen bonds seen for the 11 carbonyl group at least partially with the 12 carbonyl group. However, this modification did not lead to an improvement of activity or selectivity. Therefore, this class was not further optimized and we focused on the combination of ring A and ring E modifications (Table 3).

Similar to 3-acetyl-GA **1c**, the 3-keto-GA **11** inhibited both enzymes with a preference for 11β-HSD2 (Table 1). In order to improve the activity of the compounds, we combined this modification with the unsubstituted urea on position 29 as in **5a** resulting in compound **7**. Both, compound **7** and the 3-hydroxy-derivative **6** were active against 11β-HSD2 but not 11β-HSD1. IC_{50} values were determined for both compounds. Compound **7** was comparably active as **5a** whereas compound **6** was even more active with an IC_{50} value of 17 nM and a selectivity of more than 300 (Table 5).

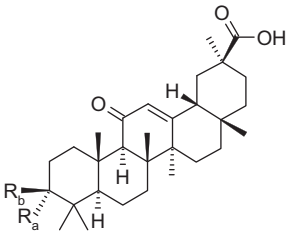
In parallel to these improved substances, the Beckmann rearrangement of ring A **13** and the 3-oxadiazolone derivative of glycyrrhetinic acid **26** were synthesized. Both compounds were active at 200 nM against 11β-HSD2 but there also remained some activity against 11β-HSD1 (Table 1). Therefore, the urea derivatives **10** and



Scheme 4. Ring A modified C29 hydroxamic acid and amine derivatives of glycyrrhetinic acid. Reagents: (a) ethoxycarbonyl isothiocyanate in $CHCl_3$ (80%); (b) $NH_2OH{\cdot}HCl$, LiOH, in EtOH (54%); (c) acetyl chloride; (d) *N*-methyl hydroxylamine hydrochloride, triethylamine in dry DCM (46%, two steps); (e) $SOCl_2$ in dry DCM; (f) *N*-methyl hydroxylamine hydrochloride, triethylamine in dry DCM (57%).

16 were synthesized. Both compounds, the Beckmann rearranged **16** and the 3-oxadiazolone **10** of the 29-urea derivative, were not only active at 200 μM against 11β-HSD2 but they also did not inhibit 11β-HSD1 at relevant concentrations (Table 4). Based on the

Table 1
Derivatives of the 3 hydroxy group of glycyrrhetinic acid and their inhibition of 11β-HSD1 and 11β-HSD2



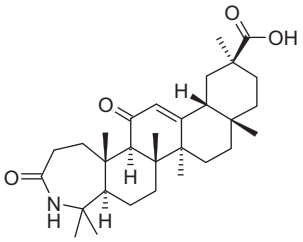
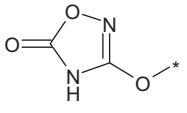
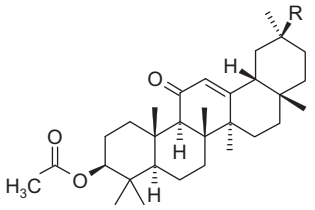
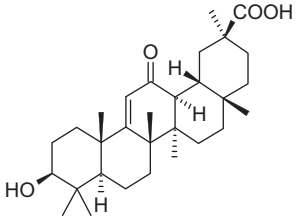
Compound	R_a	R_b	Concd [nM]	% Residual conversion [mean ± SD]	
				11β-HSD2	11β-HSD1
1	H	OH	1000	6 ± 2	16 ± 2
1b	H	$OCOCH_2CH_2COOH$	1000	2 ± 1	12 ± 4
1c[32]	H	$OCOCH_3$	1000	14 ± 1	12 ± 4
1d[30]	H	$NHCOCH_3$	1000	0.2 ± 0.4	36 ± 15
11	=O		1000	1 ± 1	36 ± 12
12	=NOH		1000	1 ± 1	n.d.
13			1000	21 ± 1	43 ± 8
26	H		1000	6 ± 1	23 ± 3

Table 2
Derivatives of the 29 carboxylic acid group of 3-acetyl-GA and their inhibition of 11β-HSD1 and 11β-HSD2



Compound	R	Concd [nM]	% Residual conversion [mean ± SD]	
			11β-HSD2	11β-HSD1
1c[32]	COOH	1000	14 ± 1	12 ± 4
2b[30]	$CONCH_3OH$	1000	2.6 ± 1.4	51 ± 11
		200	4.2 ± 5.0	97 ± 4
5a	$NHCONH_2$	1000	7.1 ± 2.3	95 ± 5
5c	NHCONHOH	200	34 ± 10	92 ± 4
5b	$NHCONCH_3OH$	200	110 ± 17	96 ± 7
5h	$NHCONHCH_2CH_2OH$	200	38 ± 1	97 ± 2
5i	$NHCON(CH_2CH_2OH)_2$	200	93 ± 25	102 ± 12
5d	$NHCONHSO_2CH_3$	200	20 ± 12	90 ± 5
5e	$NHCONHSO_2CF_3$	200	8 ± 8	91 ± 7

Table 3
Ring C modification of glycyrrhetinic acid and its inhibition of 11β-HSD1 and 11β-HSD2

Compound	Structure	Concd [nM]	% Residual conversion [mean ± SD]	
			11β-HSD2	11β-HSD1
19		200	85 ± 3	77 ± 7

determined IC_{50} values, the selectivity of the compounds was calculated to tenfold for **10** and more than thousand fold for **16**.

Based on these findings and the previously identified 29-(*N*-methyl)-hydroxamic acid derivative **2b**, we decided to synthesize the respective combinations with Beckmann rearrangement of ring A **28** and the 3-oxadiazolone derivative **27**. Both 29-(*N*-methyl)-hydroxamic acid derivatives were more potent against 11β-HSD2 than the respective 29-urea derivatives with IC_{50} values of 15 and 11 nM for **27** and **28**, respectively. Whereas **27** also inhibited 11β-HSD1 with an IC_{50} value of 500 nM resulting in a selectivity of 30, **28** did not significantly inhibit the enzyme at concentrations up to 40 μM. The selectivity of **28** for 11β-HSD2 versus 11β-HSD1 was calculated be higher than 3600 (Table 5).

In summary, the combination of ring A and position 29 modifications led to increased activity and selectivity. The modification, especially, of the ring-A to the azepanone in combination with the previously reported 29-(*N*-methyl)hydroxamic acid derivative led to the most active and selective compound reported so far.

3. Conclusions

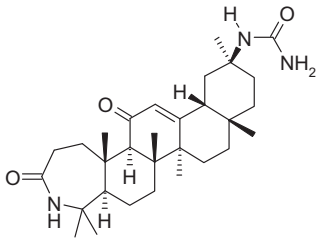
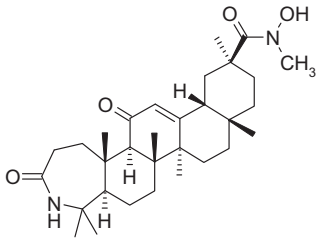
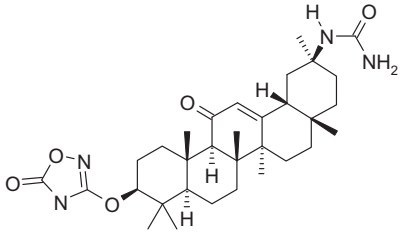
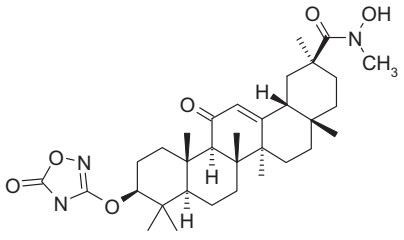
Glycyrrhetinic acid was used as a lead compound and starting point for the synthesis of novel, highly potent and selective inhibitors of human 11β-HSD2. In combination with previously published inhibitors,[30] this novel data reveals excellent structure activity relationships for glycyrrhetinic acid derivatives as inhibitors of human 11β-hydroxysteroid dehydrogenase 2. The selectivity for human 11β-HSD2 over 11β-HSD1 could be further improved for compounds synthesized. Compound **28** is the most selective compound reported so far with a selectivity of more than 3600 and should provide a useful mechanistic tool for further in vitro and in vivo studies in anti-inflammatory disease models.

4. Experimental

4.1. General

Compounds **1**, **2** and **13** have been prepared as reported before.[32] Whenever reasonable, solvents were purified and dried by standard procedures. Melting points were measured on a Büchi B-545 melting point apparatus or a Kofler-type Reichert Thermovar micro hot stage microscope and are uncorrected. Regarding NMR-assignment and nomenclature, the carboxylic acid of glycyrrhetinic acid was assigned as C29 and the adjacent methyl group as C30. NMR spectra were recorded at 297 K in the solvent indicated with a Bruker AC 200-, a Bruker DPX 300-, a Bruker AC 400-, and a Bruker DPX 400 spectrometer using standard Bruker NMR software. Spectra were referenced to tetramethylsilane via calibration with the residual solvent peaks.[41] Reactions were monitored by TLC on Silica Gel 60 F_{254} plates; spots were detected by UV light examination or visualized by spraying with anisaldehyde–sulfuric acid, molybdatophosphoric acid, mixture of molybdatophosphoric acid and Ce^{IV} ammonium nitrate or ninhydrine and heating. Normal phase column chromatography was performed on Silica Gel 60 (230–400 mesh, Merck). HPLC-HRMS analysis was carried out from CH_3CN solutions (concentration: 1–10 mg/L) using an HTC PAL

Table 4
Combination of 3-modifications and 29-modifications of glycyrrhetinic acid and their inhibition of 11β-HSD1 and 11β-HSD2

Compound	Structure	Concd [nM]	% Residual conversion [mean ± SD]	
			11β-HSD2	11β-HSD1
16		200	14 ± 9	78 ± 20
28		200	13 ± 8	73 ± 13
10		200	24 ± 1	81 ± 5
27		200	1.7 ± 2.8	20 ± 15

system auto sampler (CTC Analytics AG, Zwingen, Switzerland), an Agilent 1100/1200 HPLC with binary pumps, degasser and column thermostat (Agilent Technologies, Waldbronn, Germany) and Agilent 6210 ESI-TOF mass spectrometer (Agilent Technologies, Palo Alto, United States).

4.2. Synthesis of C29 urea (general procedure A)

To a solution of **4** (1.0 mmol) in DCM (25 mL), the amine (1.5 mmol) was added and the reaction mixture was stirred for 3 h at ambient temperature. Water (30 mL) was added to the reaction mixture and the layers were separated. The aqueous layer was extracted with DCM (2 × 25 mL), the combined DCM layer were washed with water (30 mL), dried over Na_2SO_4 and the solvent was removed under vacuum to get the crude product that was purified by flash chromatography (SiO_2, MeOH–DCM, gradient elution) to give the urea product.

4.3. Synthesis of C29 urea (general procedure B)

A mixture of $NaHCO_3$ (1.5 mmol) and the amine hydrochloride (1.5 mmol) in THF (25.0 mL) was stirred for 15 min at room temperature. To this suspension, isocyanate **4** (1.0 mmol) was added and the reaction mixture was stirred for 2 h at ambient temperature. Water (25 mL) was added and the mixture extracted with DCM (2 × 25 mL). The combined DCM layers were washed with water (25 mL), dried over Na_2SO_4 and evaporated to get crude product that was purified by flash chromatography (SiO_2, MeOH–DCM, gradient elution) to give the urea product.

4.4. Cleavage of C3 *O*-acetyl group (general procedure C)

To the solution of C3 *O*-acetyl compound (1.0 mmol) in methanol (25.0 mL) KOH (12.0 mmol) was added and the reaction mixture was stirred at ambient temperature. After 16 h the solvent was evaporated, and the residue was diluted with water (50 mL). The pH was adjusted to 2–3 using 2 N HCl. The reaction mixture was extracted with DCM (2 × 50 mL), the combined organic layers were washed with water (50 mL), brine (50 mL), dried over Na_2SO_4 and evaporated. The crude product was purified on SiO_2 using 0–10% gradient of MeOH and DCM.

4.5. (3β,18β,20β)-3-(Acetoxy)-11-oxo-olean-12-en-29-oyl chloride (2)

A stirred suspension of **1** (9.4 g, 20 mmol) in acetyl chloride was heated up to 50 °C for 1 h resulting in a clear solution. Excess acetyl chloride was removed under vacuum and the residue triturated

Table 5
IC_{50} values and selectivities of glycyrrhetinic acid and novel selective 11β-HSD2 inhibitors

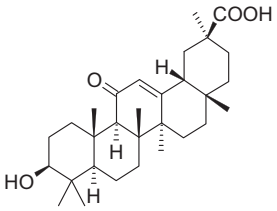
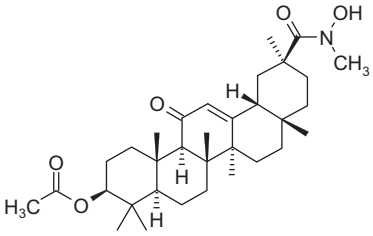
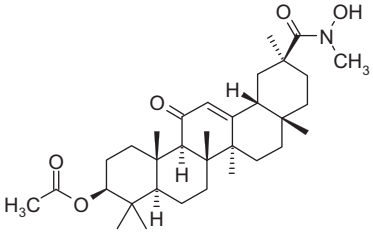
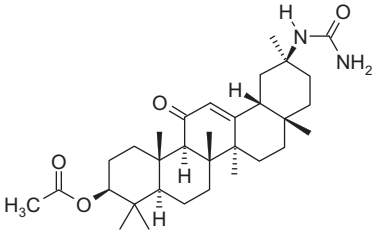
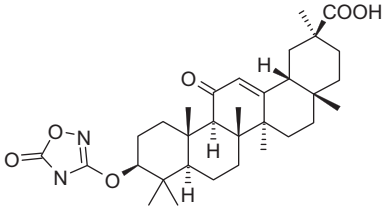
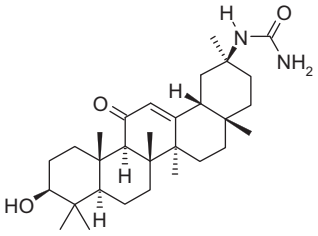
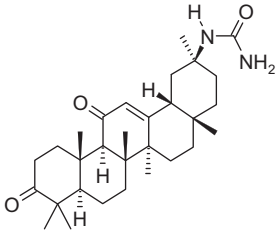
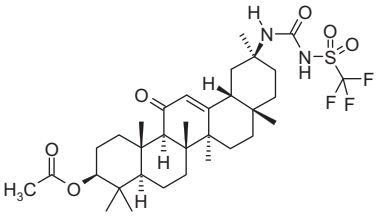
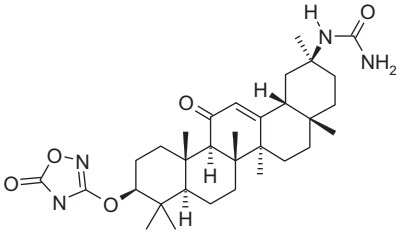
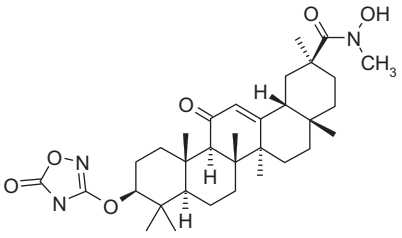
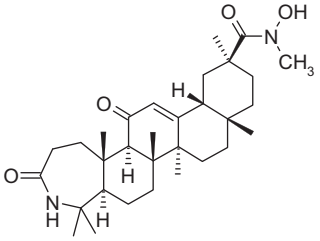
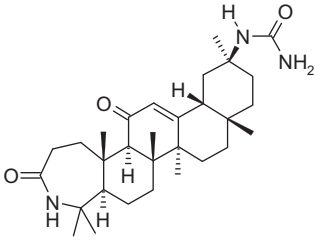
Compound	Structure	11β-HSD2		11β-HSD1		Selectivity HSD2/HSD1
		IC_{50} [nM]	95% CI	IC_{50} [nM]	95% CI	
1		257	186–354	778	631–960	3
1c		750	416–1350	7100	2200–23000	10
2b[30]		2.9	0.65–13	1010	747–1370	350
5a		104	91–120	8300	5500–12600	80
26		11	8.5–13	140	95–206	13
6		17	8.7–32	5400	3800–7800	324
7		45	33–60	>10000		>224

Table 5 (*continued*)

Compound	Structure	11β-HSD2		11β-HSD1		Selectivity HSD2/HSD1
		IC_{50} [nM]	95% CI	IC_{50} [nM]	95% CI	
5e		194	146–258	4000	3100–5100	20
10		>4000		>40000		
27		15	12–18	504	318–796	34
28		11	8.0–15	>40000		>3600
16		33	23–48	45000	29000–72000	1360

using diethyl ether (50 mL).The residue was filtered and dried in vacuo to yield **2** as white powder (9.1 g, 86%). mp 289–292 °C (lit.[42]: 297–303 °C).

4.6. (3β,18β,20β)-3-(Acetoxy)-20-isocyanato-30-norolean-12-en-11-one (4)

A suspension of **2** (5.00 g, 9.41 mmol) and sodium azide (1.5 g, 23 mmol) in acetone (300 mL) was stirred with for 30 min. Ice water (500 mL) was added and the readily formed precipitate was filtered and dried in air. It was re-dissolved in $CHCl_3$ (500 mL) and refluxed for 12 h. The solvent was evaporated to obtain the crude product that was purified on SiO_2 with a gradient of 0–10% diethyl ether and methylene chloride to give **4** as white powder (4.3 g, 89.6%). mp 295–297 °C (Lit.[43]: 299–300 °C). ^1H NMR (200 MHz, $CDCl_3$): δ 5.63 (s, 1H), 4.55–2.42 (m, 1H), 2.84–2.69 (m, 1H), 2.36–2.18 (m, 2H), 2.03 (s, 3H), 1.42- 1.95 (m, 19H), 1.35 (s, 3H), 1.30 (s, 3H), 1.14 (s, 3H), 1.12 (s, 3H), 0.88 (s, 3H), 0.86 (s, 6H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 199.76, 170.94, 168.17, 128.56, 122.11, 80.53, 61.70, 58.59 54.97, 47.21, 45.34, 44.44, 43.17, 38.75, 38.00, 36.90, 36.17, 34.77, 32.64, 31.84, 31.70, 28.14, 28.00, 26.28, 26.16, 23.51, 23.29, 21.27, 18.65, 17.33, 16.65, 16.37.

4.7. (3β,18β,20β)-*N*-[-3-(Acetoxy)-11-oxo-30-norolean-12-en-20-yl] urea (5a)

Ammonia gas was introduced to a solution of **4** (2.0 g, 3.9 mmol) in $CHCl_3$ (200 mL) for 15 min at 0–5 °C. Excess of ammonia was removed by purging air with. The solvent was removed under vacuum and the residue purified by flash chromatography on silica using a 0–2% gradient of diethyl ether and methylene chloride to give **5a** as white powder (1.5 g, 73%, HPLC >99%). ^1H NMR (200 MHz, $CDCl_3$): δ 5.68 (s, 1H), 4.50–2.40 (m, 1H),

2.80–2.64 (m, 1H), 2.15- 2.31 (m, 4H), 2.03 (s, 3H), 1.40- 1.97 (m, 19H), 1.32 (s, 3H), 1.30 (s, 3H), 1.13 (s, 3H), 1.10 (s, 3H), 0.89 (s, 6H), 0.79 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 200.41, 171.03, 170.10, 158.86, 128.06, 80.58, 61.71, 54.95, 52.28, 52.18, 46.59, 45.45, 43.33, 43.09, 38.89, 38.01, 36.87, 35.78, 32.67, 31.88, 31.28, 28.54, 28.39, 28.01, 26.20, 23.53, 23.16, 21.30, 18.64, 17.32, 16.66, 16.37. HRMS: calcd $[M+H]^+$: 527.3843, found $[M+H]^+$: 527.3842.

4.8. *N*-Hydroxy-*N*-methyl-*N′*-[(3β,18β,20β)-3-(acetoxy)-11-oxo-30-norolean-12-en-20-yl] urea (5b)

Prepared according to general procedure (B) from **4** (100.0 mg, 0.20 mmol) and *N*-methyl-hydroxylamine hydrochloride (25.0 mg, 0.30 mmol) to yield **5b** as a white powder (70.0 mg, 64%, HPLC >99%). ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.61 (s, 1H), 4.59–2.42 (m, 1H), 3.09 (s, 3H), 2.85–2.67 (m, 1H), 2.41–2.75 (m, 22H), 2.04 (s, 3H), 1.34 (s, 3H), 1.33 (s, 3H), 1.14 (s, 3H), 1.10 (s, 3H), 0.86 (s, 6H), 0.81 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 200.87, 171.03, 170.36, 160.98, 128.12, 80.51, 61.71, 54.96, 52.33, 47.08, 45.54, 43.41, 42.85, 38.77, 38.73, 38.02, 36.93, 35.69, 32.68, 31.87, 31,38, 28.44, 28.27, 28.00, 26.34, 26.20, 23.52, 23.10, 21.28, 18.66, 17.30, 16.66, 16.39. CI-MS, m/z ($I_{rel.}$ (%)): 556.94 $[M]^+$ (18.94), 556.02 (8.10), 552.95 (10.79), 552.25 (5.70), 543.04 (17.38), 541.86 (21.09), 540.77 (100.00), 539.79 (46.27), 538.45 (11.70), 468.09 (15.48). Anal. Calcd for $C_{33}H_{52}N_2O_5$: C, 71.19; H, 9.41; N, 5.03. Found: C, 67.15; H, 8.91; N, 4.67.

4.9. *N*-Hydroxy-*N′*-[(3β,18β,20β)-3-(acetoxy)-11-oxo-30-norolean-12-en-20-yl] urea (5c)

Prepared according to general procedure (B) from **4** (200.0 mg, 0.39 mmol) and hydroxylamine hydrochloride (41.0 mg, 0.59 mmol) to give **5c** as a white powder (120.0 mg, 56%, HPLC >97%). ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.81 (s, 1H), 5.70 (s, 1H), 4.60–2.42 (m, 1H), 2.84–2.69 (m, 1H), 2.35- 0.75 (m, 22H), 2.04 (s, 3H), 1.33 (m, 6H), 1.13, (s, 3H), 1.09 (s, 3H), 0.86 (m, 6H), 0.81 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 201.04, 171.01, 170.61, 161.23, 128.12, 80.52, 61.67, 54.94, 52.58, 47.04, 45.46, 43.34, 41.27, 38.74, 38.02, 36.96, 35.54, 32.89, 32.69, 31.82, 28.47, 28.30, 28.01, 26.41, 26.31,23.52, 23.02, 21.29, 18.68, 17.29, 16.66, 16.40. CI-MS, m/z ($I_{rel.}$ (%)): 560.02 $[M+NH_4]^+$ (1.17), 559.12 (0.57), 526.58 (18.41), 525.53, (27, 45), 524.49 (17.07), 510.97 (38.73), 509.96 (100.00), 299.87 (19.35), 259.02 (19.22), 134.99 (15.95). Anal. Calcd for $C_{32}H_{50}N_2O_5$: C, 70.81; H, 9.29; N, 5.16. Found: C, 68.52; H, 9.51; N, 4.81.

4.10. *N*-Methylsulfonyl-*N′*-[(3β,18β,20β)-3-(acetoxy)-11-oxo-30-norolean-12-en-20-yl] urea (5d)

Prepared according to general procedure (A) from **4** (200.0 mg, 0.39 mmol) and methane sulfonamide (56.0 mg, 0.59 mmol) to yield **5d** as a white powder (120.0 mg, 50.6%, HPLC >96%). ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.62 (s, 1H), 4.60–2.42 (m, 1H), 3.24 (s, 3H), 2.80–2.65 (m, 1H), 2.33 (s, 1H), 2.17–0.70 (m, 21H), 2.05 (s, 3H), 1.36 (s, 3H), 1.33 (s, 3H), 1.14 (s, 3H), 1.11 (s, 3H), 0.87 (m, 6H), 0.84 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 200.38, 171.13, 169.26, 150.74, 128.26, 80.58, 61.73, 54.93, 53.71, 46.73, 45.48, 43.30, 42.10, 42.02, 38.75, 38.01, 36.88, 35.69, 32.65, 31.86, 31.32, 28.40, 28.01, 27.86, 26.31, 26.10, 23.51, 23.20, 21.31, 18.63, 17.30, 16.66, 16.37. CI-MS, m/z ($I_{rel.}$ (%)): 621.54 $[M+NH_4]^+$ (3.76), 578.54 (10.81), 526.43 (18.45), 525.49 (15.89), 524.43 (12.16), 511.01 (31.94), 509.98, (100.00), 508.96 (6.39), 485.80 (2.90), 484.91 (13.99). Anal. Calcd for $C_{33}H_{52}N_2O_6S$: C, 65.53; H, 8.67; N, 4.63; S, 5.30. Found: C, 62.84; H, 9.04; N, 4.19; S, 4.89.

4.11. *N*-[(3β,18β,20β)-3-(Acetoxy)-11-oxo-30-norolean-12-en-20-yl]-*N′*-[(trifluoromethyl)sulfonyl] urea (5e)

Prepared according to general procedure (A) from **4** (200.0 mg, 0.39 mmol) and trifluoro methane sulfonamide (87.0 mg, 0.58 mmol) to give **5e** as a white powder (110.0 mg, 42%, HPLC >99%). ^{1}H NMR (200 MHz, pyridine-d_5): δ 5.76 (s, 1H), 4.79–2.64 (m, 1H), 3.18–2.90 (m, 1H), 2.44–2.70 (m, 22H), 2.03 (s, 3H), 1.43 (s, 3H), 1.36 (s, 3H), 1.23 (s, 3H), 1.05 (s, 3H), 0.90 (s, 3H), 0.89 (s, 3H), 0.77 (s, 3H). ^{13}C NMR (50 MHz, pyridine-d_5): δ 199.96, 171.08, 170.15, 162.28, 128.84, 80.93, 62.31, 55.50, 53.57, 47.48, 45.92, 44.02, 42.03, 39.61, 38.71, 37,82, 36.43, 33.23, 33.03, 32.43, 28.96, 28.60, 28.55, 27.12, 27.05, 24.44, 23.79, 21.58, 19.18, 18.08, 17.44, 17.13. CI-MS, m/z ($I_{rel.}$ (%)): 675.89 $[M+NH_4]^+$ (1.0); 526.86 (45.03); 525.90 (100.00); 525.01 (38.22); 524.19 (45.03); 523.33 (24.61); 510.98 (28.27); 510.98 (28.27); 509.92 (58.90); 466.92 (31.67); 190.97 (45.80); 95.04 (24.07). Anal. Calcd for $C_{33}H_{49}F_3N_2O_6S$: C, 60.13; H, 7.50; N, 4.25; F, 8.65; S, 4.87. Found: C, 58.15; H, 7.95; N, 3.88; F, 7.88; S, 4.43.

4.12. *N*-[(3β,18β,20β)-3-(Acetoxy)-11-oxo-30-norolean-12-en-20-yl]-*N′*-(phenylmethyl) urea (5f)

Prepared according to general procedure (A) from **4** (200.0 mg, 0.39 mmol) and benzyl amine (63.0 mg, 0.59 mmol) to yield **5f** as a white powder (160.0 mg, 66%, HPLC >99%). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.32–2.20 (m, 5H), 5.56 (s, 1H), 4.57–2.42 (m, 1H), 4.41–2.12 (m, 2H), 2.84–2.60 (m, 1H), 2.29–2.70 (m, 22H), 2.03 (s, 3H),1.29 (m, 6H), 1.13 (s, 3H), 1.09 (s, 3H), 0.86 (m, 6H), 0.71 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 200.08, 171.07, 169.62, 157.95, 139.55, 128.60, 128,45, 128.13, 127.14, 80.60, 61.68, 54.95, 52.40, 46.54, 45.41, 44.24, 43.41, 43.28, 38.80, 38.01, 36.83, 35.74, 32.66, 31.79, 31.12, 28.71, 28.28, 28.02, 26.32, 26.14, 23.50, 23.20, 21.30, 18.62, 17.34, 16.67, 16.37. HRMS: calcd $[M+H]^+$: 617.4313, found $[M+H]^+$: 617.4326.

4.13. *N*-[(3β,18β,20β)-3-(Acetoxy)-11-oxo-30-norolean-12-en-20-yl]-1,2-hydrazinedicarboxamide (5g)

Prepared according to general procedure (B) from **4** (200.0 mg, 0.39 mmol) and semicarbazide hydrochloride (66.0 mg, 0.59 mmol) to give **5g** as a white powder (130 mg, 57%, HPLC >99%). ^{1}H NMR (200 MHz, pyridine-d_5): δ 5.78 (s, 1H), 4.80–2.64 (m, 1H), 3.15–2.90 (m, 1H), 2.44–2.70 (m, 25H), 2.03 (s, 3H), 1.52 (s, 3H), 1.25 (s, 3H), 1.22 (s, 3H), 1.00 (s, 3H), 0.89 (s, 3H), 0.88 (s, 3H), 0.77 (s, 3H). ^{13}C NMR (50 MHz, pyridine-d_5): δ 199.74, 171.03, 169.98, 162.08, 159.43, 128.92, 80.89, 62.21, 55.43, 52.99, 47.42, 45.89, 43.93, 42.17, 39.48, 38.70, 37.78, 36.57, 33.36, 33.16, 32.44, 29.16, 28.91, 28.55, 27.10, 26.92, 24.42, 23.76, 21.57, 19.15, 18.05, 17.45, 17.13. CI-MS, m/z ($I_{rel.}$ (%)): 584.83 $[M]^+$ (6.72), 583.91 (5.47), 559.02 (6.06), 523.51 (10.68), 522.58 (13.67), 510.96 (40.53), 509.96 (100.00), 508.81 (6.52), 484.89 (16.29), 483.98 (49.73). HRMS: calcd $[M+H]^+$: 585.4010, found $[M+H]^+$: 585.4017.

4.14. *N′*-(2-Hydroxyethyl)-*N*-[(3β,18β,20β)-3-(acetoxy)-11-oxo-30-norolean-12-en-20-yl] urea (5h)

Prepared according to general procedure (A) from **4** (100.0 mg, 0.1961 mmol) and ethanol amine (21.4 mg, 0.35 mmol) to yield **5h** as a white powder (70.0 mg, 63%, HPLC >99%). ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.65 (s, 1H), 4.47–2.39 (m, 1H), 3.60 (s, 2H), 3.24 (s, 2H), 2.67–2.61 (m, 1H), 2.28 (s, 1H), 2.11-0.5 (m, 22H), 1.98 (s, 3H), 1.27 (s, 3H), 1.22 (s, 3H), 1.06 (s, 3H), 1.04 (s, 3H), 0.80 (m, 6H), 0.73 (s, 3H). ^{13}C NMR13C NMR (50 MHz, $CDCl_3$): δ 201.28. 171.32, 170.94, 159.63, 127.88, 80.45, 63.08, 61.71, 54.91, 52.31, 46.60, 45.57,

43.39, 42.97, 42.86, 38.76, 38.01, 36.88, 35.90, 32.63, 31.88, 31.88, 28.68, 28.42, 28.00, 26.41, 26.20, 23.49, 23.16, 21.27, 18.62, 17.29, 16.66, 16.40.

4.15. *N*′,*N*′-Bis(2-hydroxyethyl)-*N*-[(3β,18β,20β)-3-(acetoxy)-11-oxo-30-norolean-12-en-20-yl] urea (5i)

Prepared according to general procedure (A) from **4** (150 mg, 0.294 mmol) and diethanol amine (93.0 mg, 0.883 mmol) to give **5i** as a white solid (120.0 mg, 66%, HPLC >99%). ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.60 (s, 1H), 4.54–2.46 (m, 1H), 3.91 (br s, 2H), 3.81–2.64 (m, 4H), 3.48–2.29 (m, 4H), 2.81–2.63 (s, 1H), 2.39–2.68 (m, 21H) 2.04 (s, 3H), 1.34 (s, 3H), 1.32 (s, 3H), 1.13 (s, 3H), 1.10 (s, 3H), 0.87 (m, 6H), 0.83 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 200.55, 171.01, 170.28, 160.73, 128.05, 80.55, 61.79, 61.70, 54.94, 52.61, 50.91, 46.75, 45.47, 43.36, 43.23, 38.72, 38.01, 36.89, 35.82, 32.66, 31.86, 31.25, 28.59, 28.37, 28.01, 26.34, 26.26, 23.52, 23.20, 21.29, 18.64, 17.33, 16.66, 16.38.

4.16. *N*-[(3β,18β,20β)-3-Hydroxy-11-oxo-30-norolean-12-en-20-yl] urea (6)

Urea **5a** (3.0 g, 5.7 mmol) was hydrolyzed according to general procedure (C) to get **6** as a white powder (2.0 g, 72%, HPLC >99%). ^{1}H NMR (200 MHz, pyridine-d_5): δ 5.89 (s, 1H), 3.52–2.40 (m, 1H), 3.18–2.90 (m, 1H), 2.56- 0.75 (m, 27H), 1.28 (s, 6H), 1.23 (s, 3H), 1.06 (s, 3H), 1.04 (s, 3H), 0.80 (s, 3H). ^{13}C NMR (50 MHz, pyridine-d_5): δ 200.35, 170.49, 160.51, 128.90, 78.35, 62.58, 55.75, 52.83, 47.21, 46.00, 43.98, 43.56, 40.24, 38.05, 36.76, 33.43, 33.53 32.65, 32.54, 29.43, 29.20, 29.05, 28.55, 27.17, 27.01, 23.80, 19.27, 18.37, 17.27, 17.06. HRMS: calcd $[M+H]^+$: 485.3738, found $[M+H]^+$: 485.3743.

4.17. *N*-[(3β,18β,20β)-3,11-Dioxo-30-norolean-12-en-20-yl] urea (7)

Jones reagent (5 mL) was added over 20 min to a suspension of **6** (100.0 mg, 0.21 mmol) in acetone (30.0 mL) until the solution turned brown and stirred for 30 min at 0–2 °C. Isopropanol was added to the reaction mixture until the brown color disappeared. The reaction mixture was filtered and washed with DCM (2 × 50 mL). The combined filtrates were distilled under vacuum to get the crude product that was purified on SiO_2 using 0–2% MeOH and DCM to give **7** (70.0 mg, 70%, HPLC >98%). ^{13}C NMR (50 MHz, pyridine-d_5): δ 216.32, 199.28, 170.55, 159.98, 128.65, 61.62, 55.52, 52.79, 48.21, 47.49, 45.73, 44.08, 43.42, 40.40, 37.45, 36.69, 34.89, 32.73, 32.57, 32.52, 29.40, 29.08, 27.16, 27.00, 26.91, 23.65, 21.99, 19.44, 18.95, 16.33. HRMS: calcd $[M+H]^+$: 483.3581, found $[M+H]^+$: 483.3593.

4.18. *N*-[(3β,18β,20β)-3-(Hydroxyimino)-11-oxo-30-norolean-12-en-20-yl] urea (8)

To a solution of **7** (200 mg, 0.414 mmol) in pyridine (2.5 mL) $NH_2OH.HCl$ (150.0 mg, 2.16 mmol) was added. After stirring the solution for 2 h at 50 °C, the solvent was removed in vacuo. The residue was treated with water (5 mL), the resulting solid was filtered, washed with excess of water and dried for 6 h at 60 °C under vacuum to get **8** (120.0 mg, 58.3%, HPLC >99%). ^{1}H NMR (200 MHz, pyridine-d_5): δ 5.61 (s, 1H), 3.56–2.35 (m, 1H), 3.27–2.08 (m, 1H), 2.63–2.63 (m, 23H), 1.58 (s,3H), 1.36 (s, 3H), 1.34 (s, 3H), 1.25 (s, 3H), 1.14 (s, 3H), 1.06 (s, 3H), 0.76 (s, 3H). ^{13}C NMR (50 MHz, pyridine-d_6): δ 199.64, 170.25, 164.41, 160.15, 128.76, 62.00, 56.15, 52.82, 47.37, 45.90, 44.02, 43.52, 40.82, 39.86, 37.94, 36.72, 32.96, 32.58, 32.52, 29.40, 29.07, 28.35, 27.11, 26.95, 24.28, 23.70, 19.14, 18.96, 17.95, 16.36. Anal. Calcd for $C_{30}H_{47}N_3O_3$: C, 72.40; H, 9.52; N, 8.44. Found: C, 69.56; H, 9.17; N, 8.03. HRMS: calcd $[M+H]^+$: 498.3690, found $[M+H]^+$: 498.3691.

4.19. (3β,18β,20β)-3-[(Ethoxycarbonylamino)thioxo-methoxy]-11-oxo-olean-12-en-29-oic acid (9)

To a solution of **6** (500.0 mg, 1.1 mmol) in $CHCl_3$ (30 mL), ethoxycarbonyl isocyanate (271.0 mg, 2.1 mmol) was added and the reaction mixture was stirred at 60 °C under argon atmosphere. After 75 h the reaction mixture was evaporated, and the residue purified by flash chromatography on silica with a gradient 0–20% methanol and methylene chloride to yield **9** as a white powder (150 mg, 23.6%). ^{1}H NMR (200 MHz, pyridine-d_5): δ 5.91 (s, 1H), 4.15 (q, J = 7.12 Hz, 2H), 3.52–2.40 (m, 1H), 3.22–2.10 (m, 1H), 2.59–2.70 (m, 24H), 1.55 (s, 3H), 1.31 (s, 3H), 1.30 (s, 3H), 1.23 (s, 3H), 1.12 (s, 3H), 1.11 (s, 3H), 1.09 (s, 3H), 0.90 (s, 3H). ^{13}C NMR (50 MHz, pyridine-d_5): δ 199.97, 180. 97, 169.35, 154.48, 151.68, 129.19, 78.31, 63.56, 62.66, 55.74, 54.94, 47.68, 46.00, 43.99, 42.50, 40.25, 38.09, 36.65, 33.42, 32.61, 32.21, 30.40, 29.18, 29.02, 28.10, 27.12, 27.01, 23.79, 23.38, 19.28, 18.37, 17.28, 17.04, 14.56. Anal. Calcd for $C_{34}H_{53}N_3O_5S$: C, 66.31; H, 8.67; N, 6.82. Found: C, 66.28; H, 8.52; N, 6.75.

4.20. (3β,18β,20β)-[3-(1,2,4-Oxadiazole-5(2*H*)-one)]-11-oxo-olean-12-en-29-oic acid (10)

A suspension of **9** (150 mg, 0.25 mmol) and hydroxyl amine hydrochloride (119.0 mg, 1.71 mmol) in EtOH (30 mL) was heated to 35–20 °C until a clear solution was obtained. LiOH (41.0 mg, 0.97 mmol) was added over 20 min and the reaction mixture was stirred at room temperature for 1 h and refluxed for 15 h. The solvent was evaporated and the resulting residue treated with water (50 mL) and extracted with DCM (2 × 25 mL). The combined DCM layers were washed with water, dried over Na_2SO_4 and concentrated under vacuum to yield the crude product that was purified on SiO_2 using 0–20% gradient of MeOH and DCM to give **10** (10 mg, 6%, HPLC >99%). ^{1}H NMR (200 MHz, pyridine-d_5): δ 5.65 (s, 1H), 4.70 (m, 1H), 3.44–2.22 (m, 1H), 2.66–2.70 (m, 24H), 1.30 (s, 3H), 1.21 (s, 6H), 1.07 (s, 3H), 0.93 (s, 3H), 0.83 (s, 6H). ^{13}C NMR (50 MHz, pyridine-d_5): δ 202.85, 173.56, 172.12, 162.58, 156.05, 128.24, 77.91, 62.54, 55.51, 54.65, 54.55, 48.11, 46.06, 44.29, 40.19, 37.96, 32.64, 32.56, 30.40, 30.36, 30.05, 29.21, 28.89, 28.49, 28.30, 27.10, 27.01, 23.66, 19.34, 18.35, 17.16, 17.00. HRMS: calcd $[M+Na]^+$: 567.2246, found $[M+Na]^+$: 567.2237.

4.21. (18β,20β)-3-(Hydroxyimino)-11-oxo-olean-12-en-29-oic acid (12)

The product was prepared using the procedure as described for **8** from **11** (950.0 mg, 2.026) to give **12** (830.0 mg, 85%). ^{1}H NMR (200 MHz, pyridine-d_5): δ 5.97 (s, 1H), 3.48 (m, 1H), 3.21 (m, 1H), 2.50–2.70 (m, 21H), 1.38 (s, 3H), 1.37 (s, 3H), 1.34 (s, 3H), 1.33 (s, 3H), 1.15 (s, 3H), 1.10 (s, 3H), 0.77 (s, 3H). ^{13}C NMR (50 MHz, pyridine-d_5): δ 199.75, 179.61, 170.36, 164.42, 129.07, 62.12, 56.22, 49.16, 45.97, 44.55, 43.98, 42.10, 40.86, 39.88, 38.87, 37.99, 32.98, 32.60, 32.00, 29.19, 29.15, 28.36, 27.24, 27.05, 24.28, 23.89, 19.16, 18.98, 17.94, 16.40.

4.22. (5a*R*,7a*R*,7b*S*,9a*S*,12*S*,13a*R*,15a*R*,15b*S*)-2,3,4,5,5a,6,7,7a,7b,8,9,9a,10,11,12,13,13a,15,15a,15b-Eicosahydro-5,5,7a,7b,9a,12,15b-heptamethyl-3,15-dioxo-1*H*-chryseno[2,1-*c*]azepine-12-carboxylic acid (13)

To a solution of **12** (5.0 g, 10.33 mmol) in dry DCM (100 mL), PCl_5 (12.9 g, 61.95 mmol) was added and the reaction mixture was stirred at room temperature. After 30 min, ice-water (50 mL)

was added to the reaction mixture and the DCM layer was washed with water and dried over Na_2SO_4. Evaporation gave the crude product that was purified on SiO_2 with a gradient of 0–5% methanol and methylene chloride to give **13** as a pale yellow powder (2.0 g, 40%, HPLC >98%). ^{1}H NMR (200 MHz, pyridine-d_5): δ 5.98 (s, 1H), 3.20–2.86 (m, 1H), 2.68–0.70 (m, 22H), 1.48 (s, 3H), 1.37 (s, 3H), 1.32, (s, 6H), 1.25 (s, 3H), 1.09 (s, 3H), 0.76 (s, 3H). ^{13}C NMR (50 MHz, pyridine-d_5): δ 199.38, 179.63, 177.27, 170.00, 129.30, 62.11, 56.54, 54.58, 49.11, 46.08, 44.54, 44.17, 42.08, 41.15, 40.10, 38.84, 34.00, 34.24, 33.33, 32.80, 32.66, 32.00, 29.16, 27.47, 27.14, 27.04, 23.67, 21.90, 18.80, 18.54.

4.23. (5a*R*,7a*R*,7b*S*,9a*S*,12*S*,13a*R*,15a*R*,15b*S*)-2,3,4,5,5a,6,7,7a,7b,8,9,9a,10,11,12,13,13a,15,15a,15b-Eicosahydro-5,5,7a,7b,9a,12,15b-heptamethyl-12-isocyanato-3,15-dioxo-1*H*-chryseno-[2,1-*c*]azepine (15)

To a solution of **13** (200.0 mg, 0.42 mmol) in dry DCM, $SOCl_2$ (123 mg, 1.1 mmol) was added and the reaction mixture was stirred at room temperature. After 1 h, volatiles were removed under vacuum and the resulting treated with acetone (15 mL) followed by a solution of NaN_3 (55.0 mg, 0.85 mmol) in water (2 mL). After 1 h, volatiles were removed and the resulting residue distributed between water and DCM (2 × 20 mL). The combined DCM layers were washed with water and dried over Na_2SO_4 and evaporated. The resulting crude product **14** was refluxed in $CHCl_3$ 4 h followed by evaporation and flash chromatography using a gradient of 0–5% methanol in methylene chloride to give **15** as white powder (120.0 mg, 60%). ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.59 (s, 1H), 2.69–2.17 (m, 6H), 2.00–0.70 (m, 16H), 1.31 (s, 6H), 1.28 (s, 3H), 1.25 (s, 3H), 1.22 (s, 3H), 1.10 (s, 3H), 0.83 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 199.06, 177.58, 167.96, 128.68, 122.06, 61.06, 58.56, 56.26, 54.55, 47.18, 45.44, 44.33, 43.41, 40.39, 38.20, 36.10, 34.70, 34.22, 32.33, 31.86 (2C), 31.66, 28.13, 26.75, 26.15, 26.07, 23.04, 20.98, 18.38, 17.23. Anal. Calcd for $C_{30}H_{44}N_2O_3$: C, 75.00; H, 9.23; N, 5.83. Found: C, 74.64; H, 8.95; N, 5.72.

4.24. *N*-[(5a*R*,7a*R*,7b*S*,9a*S*,12*S*,13a*R*,15a*R*,15b*S*)-2,3,4,5,5a,6,7,7a,7b,8,9,9a,10,11,12,13,13a,15,15a,15b-Eicosahydro-5,5,7a,7b,9a,12,15b-heptamethyl-3,15-dioxo-1*H*-chryseno[2,1-*c*]azepine-12-yl] urea (16)

Compound **16** was prepared using the procedure for the preparation of **5a** from **15** (120.0 mg, 0.25 mmol) as a white powder (90.0 mg, 72.4%). ^{1}H NMR (200 MHz, methanol-d_4): δ 5.66 (s, 1H), 3.36–2.28 (m, 2H), 2.72–2.70 (m, 23H), 1.43 (s, 3H), 1.39 (s, 3H), 1.36 (s, 3H), 1.30 (s, 3H), 1.29 (s, 3H), 1.19 (s, 3H), 0.90 (s, 3H). ^{13}C NMR (50 MHz, methanol-d_4): δ 201.42, 179.76, 172.64, 161.51, 129.10, 62.84, 57.69, 54.00, 53.23, 48.12, 46.73, 44.97, 43.75, 41.34, 40.78, 36.90, 33.47, 33.06, 33.00, 32.93, 32.77, 29.15, 28.78, 27.45, 27.31, 27.15, 23.55, 22.51, 18.90, 18.75. CI-MS, m/z ($I_{rel.}$ (%)): 499.17 (11.95); 498.12 $[M]^+$ (36.85); 482.13 (7.52); 481.15 (19.94); 456.19 (15.86); 455.24 (42.26); 355.09 (14.06); 354.24 (13.17); 339.19 (23.84); 338.18 (100.00). HRMS: calcd $[M+H]^+$: 498.3690, found $[M+H]^+$: 498.3692.

4.25. (3β,18β,20β)-3-Hydroxy-olean-9(11),12-dien-29-oic acid (18)

Acid **1** (4.0 g, 8.50 mmol), sodium borohydride (14.0 g, 370 mmol) and sodium hydroxide (2.0 g, 50.0 mmol) were dissolved in a mixture of THF (100 mL) and of water (100 mL). The reaction mixture was refluxed for 4 h and quenched using a 5% solution of NaH_2PO_4 and extracted in EtOAc (2 × 100 mL). The combined EtOAc layers were washed with water, brine, dried over Na_2SO_4 and solvent removed under vacuum to get **17** as a white solid (3.2 g, 80% yield). Mixture of isomers that determined by TLC were unstable in air and used without purification. An aliquot of 1.3 g, (2.8 mmol) was dissolved in THF (25 mL). A drop of concd HCl was added and the reaction mixture was refluxed for 6 h. The solvent was removed under vacuum and water (50 mL) was added to the residue. The product was extracted into diethyl ether and the crude product obtained after evaporation was purified on silica a using 0–20% gradient of diethyl ether and methylene chloride to get **18** as a white solids (1.0 g, 66%, HPLC >99%). ^{1}H NMR (200 MHz, pyridine-d_5): δ 5.85 (d, J = 5.78 Hz, 1H), 5.71 (d, J = 5.77 Hz, 1H), 3.54–2.33 (m, 1H), 2.69–2.50 (m, 1H), 2.15–2.80 (m, 21H), 1.33 (s, 3H), 1.26 (s, 3H), 1.22 (s, 3H), 1.21 (s, 3H), 1.17 (s, 3H), 1.06 (s, 3H), 0.91 (s, 3H). ^{13}C NMR (50 MHz, pyridine-d_5): δ 179.97, 155.70, 147.22, 122.21, 116.46, 78.21, 52.21, 47.39, 44.69, 43.98, 43.51, 41.25, 40.01, 39.61, 39.42, 38.22, 32.95, 32.47, 32.13, 29.39(2C), 29.26, 29.22, 28.00, 26.49, 26.02, 21.62, 20.78, 19.20, 17.07.

4.26. (3β,18β,20β)-3-Hydroxy-12-oxo-olean-9(11)-en-29-oic acid (19)

A mixture of **18** (4.8 g, 10.55 mmol) and m-chloroperbenzoic acid in $CHCl_3$ (120 mL) was stirred for 24 h at ambient temperature. The reaction mixture washed with saturated sodium bicarbonate solution (50 mL), brine (50 mL), water (2 × 50 mL) and dried over sodium sulfate. The solvent was distilled under vacuum and the crude product was purified by flash chromatography with a 0–20 gradient of acetone and methylene chloride to give **19** as a white powder (940.0 mg, 24%, HPLC >94%). ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.80 (s, 1H), 3.33–2.10 (m, 1H), 2.18–2.66 (m, 23H), 1.41 (s, 3H), 1.37 (s, 3H), 1.23 (s, 3H), 1.03 (s, 3H), 1.01 (m, 6H), 0.82 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 202.28, 184.95, 178.54, 123.20, 78.06, 54.07, 50.83, 43.73, 42.68, 41.20, 39.93, 39.56, 39.21, 36.48, 35.15, 33.98, 32.86, 31.78, 29.86, 29.74, 29.65, 28.05, 27.46, 26.71, 26.52, 25.78, 24.77, 24.65, 17.52, 15.66. HRMS: calcd $[M+H]^+$: 471.3469, found $[M+H]^+$: 471.3493.

4.27. (3β,18β,20β)-3-(Acetoxy)-12-one-30-norolean-9(11)-en-20-isocyanato (22)

A suspension of **19** (1.0 g, 2.12 mmol) in acetyl chloride (10 mL) was refluxed for 40 min when a clear solution was obtained. Acetyl chloride was distilled under vacuum and the crude solid re-crystallized from acetone to get **20** as white solid (1.0 g, 96% yield) that was used without purification to prepare **22** as described for **4** (1.0 g, 1.88 mmol) as white powder (200 mg, 21% yield). ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.75 (s, 1H), 4.53–2.41 (m, 1H), 3.09–2.01 (m, 1H), 2.36–2.80 (m, 20H), 2.05 (s, 3H), 1.40 (s, 3H), 1.32 (s, 3H), 1.21 (s, 3H), 0.99 (s, 3H), 0.90 (s, 9H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 201.12, 177.99, 170.86, 123.01, 121.90, 79.63, 58.91, 50.26, 47.35, 45.48, 41.89, 39.81, 38.14, 37.09, 36.44, 36.42, 36.09, 35.24, 32.83, 32.03, 31.99, 30.90, 27.94, 26.84, 26.05, 25.89, 23.96, 23.83, 21.83, 21.24, 17.83, 16.65. Anal. Calcd for $C_{31}H_{47}NO_2S$: C, 80.00; H, 10.20; N, 3.01. Found: C, 79.64; H, 9.73; N, 3.21.

4.28. *N*-[(3β,18β,20β)-3-(Acetoxy)-12-oxo-30-norolean-9(11)-en-20-yl] urea (23)

Compound **23** was prepared as described for **5a** from **22** (2.0 g, 3.9 mmol) as white powder (1.5 g, 73% yield, HPLC >99%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.78 (s, 1H), 4.55–2.45 (m, 1H), 3.08–2.01 (m, 1H), 2.01–2.70 (m, 23H), 2.07 (s, 3H), 1.43 (s, 3H), 1.31 (s, 3H), 1.24 (s, 3H), 0.98 (s, 3H), 0.96 (s, 3H), 0.93 (s, 3H), 0.92 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 202.50, 179.56, 170.90, 159.24, 122.78, 79.60, 77.24, 53.08, 50.25, 47.56, 45.62, 42.10, 39.99, 38.20, 36.60, 36.28,

36.12, 35.63, 32.82, 32.12, 28.71, 27.97, 26.91, 26.13, 26.07, 24.00, 23.94, 23.86, 21.71, 21.26, 17.84, 16.70. HRMS: calcd $[M+H]^+$: 527.3843, found $[M+H]^+$: 527.3852.

4.29. *N*-[(3β,18β,20β)-3-Hydroxy-12-oxo-30-norolean-9(11)-en-20-yl] urea (24)

Urea **23** (3.0 g, 5.7 mmol) was hydrolyzed according to general procedure (C) to give **24** as a white powder (2.0 g, 72%). ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.78 (s, 1H), 3.29–2.15 (m, 1H), 3.07–2.91 (m, 1H), 2.36–2.70 (m, 24H), 1.40 (s, 3H), 1.28 (s, 3H), 1.21 (s, 3H), 1.04 (s, 3H), 0.96 (s, 3H), 0.95 (s, 3H), 0.84 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 202.82, 180.38, 159.27, 122.64, 77.87, 52.87, 50.11, 47.58, 45.64, 42.07, 40.14, 39.29, 36.61, 36.51, 36.43, 35.62, 32.87, 32.10 (2C), 28.72, 28.09, 27.40, 26.91, 26.13, 26.00, 23.95, 23.89, 21.83, 17.96, 15.64. HRMS: calcd $[M+H]^+$: 485.3738, found $[M+H]^+$: 485.3740.

4.30. (3β,18β,20β)-3-[(Ethoxycarbonylamino)thioxomethoxy]-11-oxo-olean-12-en-29-oic acid (25)

Prepared as per procedure described for **9** from **1** (1.40 g, 3.0 mmol) to give **25** as a white powder (1.43 g, 80%). ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.72 (s, 1H), 5.19–2.04 (m, 1H), 4.20 (q, J = 7.12 Hz, 2H), 2.97–2.77 (m, 1H), 2.39 (s, 1H), 2.05–2.70 (m, 21H), 1.35 (s, 3H), 1.27 (s, 3H), 1.20 (s, 3H), 1.17 (s, 3H), 1.11 (s, 3H), 1.00 (s, 3H), 0.94 (s, 3H), 0.82 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 200.27, 188.99, 181.87, 169.79, 148.80, 128.31, 91.08, 62.34, 61.61, 54.91, 48.20, 45.47, 43.79, 43.20, 40.80, 38.68, 38.52, 37.70, 36.93, 32.64, 31.83, 30.86, 28.53, 28.44, 27.79, 26.44, 26.35, 23.40, 22.35, 18.64, 17.30, 17.09, 16.47, 14.23. Anal. Calcd for $C_{34}H_{51}NO_6S$: C, 67.85; H, 8.54; N, 2.33. Found: C, 67.66; H, 8.52; N, 2.18.

4.31. (3β,18β,20β)-[3-(1,2,4-Oxadiazole-5(2*H*)-one)]-11-oxo-olean-12-en-29-oic acid (26)

Prepared according to procedure described for **10** from **25** (1.3 g, 2.16 mmol) to yield **26** (700 mg, 54%, HPLC >99%). ^{1}H NMR (200 MHz, pyridine-d_5): δ 5.95 (s, 1H), 4.69–2.51 (m, 1H), 3.33–2.95 (m, 1H), 2.54–2.70 (m, 22H), 1.40 (s, 3H), 1.33 (s, 3H), 1.23 (s, 3H), 1.07 (s, 3H), 1.02 (s, 3H), 0.91 (s, 3H), 0.77 (s, 3H). ^{13}C NMR (50 MHz, pyridine-d_5): δ 199.74, 179.61, 170.51, 163.39, 160.31, 128.98, 88.35, 62.18, 55.23, 49.16, 45.94, 44.56, 43.98, 42.09, 39.26, 39.13, 38.86, 37.71, 33.12, 32.60, 32.01, 29.16, 28.54, 27.25, 27.04, 24.02, 23.57, 22.26, 19.19, 17.98, 17.05, 16.87. Anal. Calcd for $C_{32}H_{46}N_2O_6$: C, 69.30; H, 8.36; N, 5.05. Found: C, 68.80; H, 8.15; N, 4.82.

4.32. (3β,18β,20β)-*N*-Hydroxy-*N*-methyl-[3-(1,2,4-oxadiazole-5(2*H*)-one)]-11-oxo-olean-12-en-29-amide (27)

A suspension of **26** (300 mg, 0.55 mmol) in acetylchloride (5 mL) heated to 40- 45 °C for 3 h and excess of acetylchloride was removed under vacuum. The residue was dissolved in dry DCM (20 mL) and to this stirred solution *N*-methyl hydroxylamine hydrochloride (67.8 mg, 0.82 mmol) and triethylamine (500 mg, 4.95 mmol) were added at room temperature. After 15 h reaction mixture was quenched with water (50 mL), layers were separated; the aqueous layer was extracted with DCM (2 × 25 mL). The combined DCM layers were washed with 1 N HCl, water and dried over Na_2SO_4 and evaporated in vacuo to get a crude product that was purified by flash chromatography on silica with a gradient of 0–10% methanol and methylene chloride to give **27** as white powder (145 mg, 46%). ^{1}H NMR (200 MHz, pyridine-d_5): δ 6.01, (s, 1H), 4.71–2.52, (m, 1H), 3.46, (s, 3H), 3.24–2.04, (m, 1H), 2.77–2.44 (m, 4H), 2.25–2.70 (m, 18H), 1.46 (s, 3H), 1.39 (s,3H), 1.22 (s, 3H), 1.07 (s, 3H), 1.02 (s, 3H), 0.91 (s, 3H), 0.79 (s, 3H). ^{13}C NMR (50 MHz, pyridine-d_5): δ 199.72, 176.45, 170.94, 163.56, 160.51, 129.00, 88.31, 62.11, 55.26, 49.11, 45.86, 45.07, 44.05, 43.74, 39.26, 39.15, 39.07, 39.02, 37.71, 33.19, 32.58, 32.29, 29.23, 28.55, 27.39, 27.23, 26.75, 23.76, 23.59, 19.21, 17.98, 17.06, 16.89. CI- MS, m/z ($I_{rel.}$ (%)): 601.45 $[M+NH_4]^+$ (13.71), 600.40 (35.84), 599.49 (25.52), 598.61 (32.53), 597.65 (21.03), 498.37 (19.25), 484.10 (24.54), 483.11 (56.03), 482.26 (100.00), 481.24 (33.76) 480.32 (21.11). Anal. Calcd for $C_{33}H_{49}N_3O_6$: C, 67.90; H, 8.46; N, 7.20. Found: C, 67.68; H, 8.27; N, 6.95.

4.33. (5a*R*,7a*R*,7b*S*,9a*S*,12*S*,13a*R*,15a*R*,15b*S*)-2,3,4,5,5a,6,7,7a,7b,8,9,9a,10,11,12,13,13a,15,15a,15b-Eicosahydro-5,5,7a,7b,9a,12,15b-heptamethyl-3,15-dioxo-*N*-hydroxy-*N*-methyl-1*H*-chryseno[2,1-*c*]azepine-12-carboxylic acid amid (28)

To the stirred suspension of 13 (200.0 mg, 0.42 mmol) in dry DCM (25 mL) was added $SOCl_2$ (125 mg, 1.1 mmol) at room temperature. After 1 h, DCM and thionylchloride was removed in vacuo to get residue that was diluted with dry DCM (25 mL) and to this stirred suspension triethylamine (168.0 mg, 1.7 mmol) and *N*-methyl hydroxyl amine hydrochloride (52.0 mg, 0.62 mmol) were added at room temperature. After 24 h water (20 mL) was added to the reaction mixture, the layers were separated, the aqueous layer extracted with DCM (2 × 25 mL) and the combined DCM layers were washed with 1 N HCl solution, water and finally dried over Na_2SO_4. The solvent was removed in vacuo and the crude product was purified by flash chromatography on SiO_2 with a mixture of 0–10% methanol and methylene chloride to give **28** as white powder (120.0 mg, 56.6%, HPLC >98%). ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.93, (s, 1H), 5.84 (s, 1H), 3.46 (s, 3H), 2.75–2.70 (m, 22H), 1.36 (s, 3H), 1.35 (s, 3H), 1.33 (s, 3H), 1.28 (s, 3H), 1.20 (s, 3H), 1.14 (s, 3H), 0.80 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 199.87, 177.96, 174.44, 170.27, 128.57, 61.06, 56.61, 54.42, 50.67, 45.46, 43.71, 43.56, 42.22, 40.44, 38.30, 38.26, 37.71, 34.21, 32.61, 32.41, 31.87, 31.64, 28.55, 26.82, 26.70, 26.39, 26.10, 22.86, 21.09, 18.41, 17.42. CI- MS, m/z ($I_{rel.}$ (%)): 513.16 $[M]^+$ (100.00); 514.20 (36.12); 484.24 (23.92); 338.24 (17.12); 515.21 (9.10); 485.25 (8.86); 526.98 (8.12); 528.91 (8.02); 511.46 (7.38); 497.28 (7.15). Anal. Calcd for $C_{31}H_{48}N_2O_4$: C, 72.62; H, 9.44; N, 5.46. Found: C, 72.28; H, 9.17; N, 5.08.

4.34. Biology

The activity of 11β-HSD1 and 11β-HSD2 was measured as described previously.[44] Briefly, HEK-293 cells were transfected with pcDNA3 plasmids containing either human 11β-HSD1 or 11β-HSD2 with a C-terminal FLAG epitope. 11β-HSD1 dependent reduction of [1,2-^{3}H]-labeled cortisone (American Radiolabeled Chemicals, St. Louis, MO) to cortisol was measured in cell lysates for 10 min at 37 °C in a volume of 22 μL containing a final concentration of 200 nM cortisone and 500 μM NADPH. 11β-HSD2 dependent oxidation of cortisol to cortisone was measured similarly using [1,2,6,7-^{3}H]-cortisol (Amersham Pharmacia, Piscataway, NJ, USA) at a final concentration of 50 nM and NAD^+ (500 μM). Inhibitors at final concentrations between 1 nM and 40 μM were diluted from stock solutions in dimethylsulfoxide and immediately used for activity assays. 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 and 95% confidence intervals (CI)) were obtained from three independent experiments.

Acknowledgments

The work was supported by the ZIT Zentrum für Innovation und Technologie GmbH (Vienna Spot of Excellence: 182081). A.O. has a Chair in Molecular and Systems Toxicology by the Novartis Research Foundation and was supported by the Swiss National Science Foundation, Grant 31003A-124912.

References and notes

1. White, P. C.; Mune, T.; Agarwal, A. K. *Endocr. Rev.* **1997**, *18*, 135.
2. Stewart, P. M.; Krozowski, Z. S. *Vitam. Horm.* **1999**, *57*, 249.
3. Walker, E. A.; Stewart, P. M. *Trends Endocrinol. Metab.* **2003**, *14*, 334.
4. Seckl, J. R.; Walker, B. R. *Endocrinology* **2001**, *142*, 1371.
5. Atanasov, A. G.; Odermatt, A. *Endocr. Metab. Immune Disord. Drug Targets* **2007**, *7*, 125.
6. Tomlinson, J. W.; Walker, E. A.; Bujalska, I. J.; Draper, N.; Lavery, G. G.; Cooper, M. S.; Hewison, M.; Stewart, P. M. *Endocr. Rev.* **2004**, *25*, 831.
7. Morton, N.; Seckl, J. *Front. Hormone Res.* **2007**, *36*, 146.
8. Kotelevtsev, Y.; Holmes, M. C.; Burchell, A.; Houston, P. M.; Schmoll, D.; Jamieson, P.; Best, R.; Brown, R.; Edwards, C. R.; Seckl, J. R.; Mullins, J. J. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 14924.
9. Masuzaki, H.; Paterson, J.; Shinyama, H.; Morton, N. M.; Mullins, J. J.; Seckl, J. R.; Flier, J. S. *Science* **2001**, *294*, 2166.
10. Buhler, H.; Perschel, F. H.; Hierholzer, K. *Biochim. Biophys. Acta* **1991**, *1075*, 206.
11. Monder, C.; Stewart, P. M.; Lakshmi, V.; Valentino, R.; Burt, D.; Edwards, C. R. *Endocrinology* **1989**, *125*, 1046.
12. Stewart, P. M.; Wallace, A. M.; Valentino, R.; Burt, D.; Shackleton, C. H.; Edwards, C. R. *Lancet* **1987**, *2*, 821.
13. Shimoyama, Y.; Hirabayashi, K.; Matsumoto, H.; Sato, T.; Shibata, S.; Inoue, H. *J. Pharm. Pharmacol.* **2003**, *55*, 811.
14. Boyle, C. D.; Kowalski, T. J. *Expert Opin. Ther. Pat.* **2009**, *19*, 801.
15. Hughes, K. A.; Webster, S. P.; Walker, B. R. *Expert Opin. Invest. Drugs* **2008**, *17*, 481.
16. Su, X.; Vicker, N.; Potter, B. V. *Prog. Med. Chem.* **2008**, *46*, 29.
17. Fotsch, C.; Wang, M. *J. Med. Chem.* **2008**, *51*, 4851.
18. Wang, M. *Curr. Opin. Investig. Drugs* **2006**, *7*, 319.
19. Fotsch, C.; Askew, B. C.; Chen, J. J. *Expert Opin. Ther. Pat.* **2005**, *15*, 289.
20. Rask, E.; Walker, B. R.; Soderberg, S.; Livingstone, D. E.; Eliasson, M.; Johnson, O.; Andrew, R.; Olsson, T. *J. Clin. Endocrinol. Metab.* **2002**, *87*, 3330.
21. Tomlinson, J. W.; Stewart, P. M. *Horm. Metab. Res.* **2002**, *34*, 746.
22. Ferrari, P. *Biochim. Biophys. Acta* **2010**, *1802*, 1178.
23. Oppermann, U.; Filling, C.; Hult, M.; Shafqat, N.; Wu, X.; Lindh, M.; Shafqat, J.; Nordling, E.; Kallberg, Y.; Persson, B.; Jornvall, H. *Chem. Biol. Interact.* **2003**, *143–144*, 247.
24. Rabbitt, E. H.; Gittoes, N. J. L.; Stewart, P. M.; Hewison, M. *J. Steroid Biochem. Mol. Biol.* **2003**, *85*, 415.
25. Hundertmark, S.; Buhler, H.; Rudolf, M.; Weitzel, H. K.; Ragosch, V. *J. Endocrinol.* **1997**, *155*, 171.
26. Haas, C. S.; Creighton, C. J.; Pi, X.; Maine, I.; Koch, A. E.; Haines, G. K., III; Ling, S.; Chinnaiyan, A. M.; Holoshitz, J. *Arthritis Rheum.* **2006**, *54*, 2047.
27. Schmidt, M.; Weidler, C.; Naumann, H.; Anders, S.; Schoelmerich, J.; Straub, R. H. *Arthritis Rheum.* **2005**, *52*, 1711.
28. Yakirevich, E.; Morris, D. J.; Tavares, R.; Meitner, P. A.; Lechpammer, M.; Noble, L.; de, R. A. F.; Gomez-Sanchez, C. E.; Wang, L. J.; Sabo, E.; Delellis, R. A.; Resnick, M. B. *Am. J. Surg. Pathol.* **2008**, *32*, 874.
29. Farese, S.; Kruse, A.; Pasch, A.; Dick, B.; Frey, B. M.; Uehlinger, D. E.; Frey, F. J. *Kid. Int.* **2009**, *76*, 877.
30. Stanetty, C.; Czollner, L.; Koller, I.; Shah, P.; Gaware, R.; Cunha, T. D.; Odermatt, A.; Jordis, U.; Kosma, P.; Claßen-Houben, D. *Biorg. Med. Chem.* **2010**, *18*, 7522.
31. Vicker, N.; Su, X.; Lawrence, H.; Cruttenden, A.; Purohit, A.; Reed, M. J.; Potter, B. V. L. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3263.
32. Beseda, I.; Czollner, L.; Shah, P. S.; Khunt, R.; Gaware, R.; Kosma, P.; Stanetty, C.; del Ruiz-Ruiz, M. C.; Amer, H.; Mereiter, K.; Da Cunha, T.; Odermatt, A.; Classen-Houben, D.; Jordis, U. *Biorg. Med. Chem.* **2010**, *18*, 433.
33. Amer, H.; Mereiter, K.; Stanetty, C.; Hofinger, A.; Czollner, L.; Beseda, I.; Jordis, U.; Kueenburg, B.; Classen-Houben, D.; Kosma, P. *Tetrahedron* **2010**, *66*, 4390.
34. Czollner, L.; Beseda, I.; Jordis, U.; Stanetty, C.; Amer, H.; Del Ruiz-Ruiz, M. C.; Kosma, P.; Classen-Houben, D. In *13th International Electronic Conference on Synthetic Organic Chemistry*, 2009, czoll1/1.
35. Su, X. D.; Lawrence, H.; Ganeshapillai, D.; Cruttenden, A.; Purohit, A.; Reed, M. J.; Vicker, N.; Potter, B. V. L. *Biorg. Med. Chem.* **2004**, *12*, 4439.
36. Su, X.; Vicker, N.; Lawrence, H.; Smith, A.; Purohit, A.; Reed, M. J.; Potter, B. V. *J. Steroid Biochem. Mol. Biol.* **2007**, *104*, 312.
37. Potter, B. V. L.; Purohit, A.; Reed, M. J.; Vicker, N. WO Patent 2002072084, 2002, 96 pp.
38. 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.
39. Wu, X.; Kavanagh, K.; Svensson, S.; Elleby, B.; Hult, M.; Von Delft, F.; Marsden, B.; Jornvall, H.; Abrahmsen, L.; Oppermann, U. Doi:10.2210/pdb2bel/pdb, 2004.
40. Schuster, D.; Maurer, E. M.; Laggner, C.; Nashev, L. G.; Wilckens, T.; Langer, T.; Odermatt, A. *J. Med. Chem.* **2006**, *49*, 3454.
41. Gottlieb, H. E.; Kotlyar, V.; Nudelman, A. *J. Org. Chem.* **1997**, *62*, 7512.
42. Petrenko, N. I.; Petukhova, V. Z.; Shakirov, M. M.; Shul'ts, E. E.; Tolstikov, G. A. *Russ. J. Org. Chem.* **2000**, *36*, 982.
43. Brieskorn, C. H.; Sax, H. *Arch. Pharm.* **1970**, *303*, 905.
44. Classen-Houben, D.; Schuster, D.; Da Cunha, T.; Odermatt, A.; Wolber, G.; Jordis, U.; Kueenburg, B. *J. Steroid Biochem. Mol. Biol.* **2009**, *113*, 248.