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Original article

Discovery and structure–activity relationships of *ent*-Kaurene diterpenoids as potent and selective 11β-HSD1 inhibitors: Potential impact in diabetes

Xu Deng [a], Yu Shen [b], Jing Yang [a,b], Juan He [a], Yu Zhao [a], Li-Yan Peng [a], Ying Leng [b,*], Qin-Shi Zhao [a,*]

[a] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, China
[b] Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China

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ABSTRACT

The biological screening of a collection of nature occurring diterpenoids against 11β-HSD1 resulted in the discovery of the lead compound **1b**, which pointed to the therapeutic potential for type 2 diabetes. Subsequently, an optimization project was initiated. Starting from compound **1b** and its counterpart **2**, the hemi-synthesis was performed on kaurenic acid scaffolds yielding 36 derivatives. Further evaluations on both human and mouse 11β-HSD revealed that seven urea derivatives exhibited significant improved potency and selectivity. Especially, the urea **19a** has an IC_{50} (human 11β-HSD1) = 9.4 nM and selectivity index (human 11β-HSD) > 10,649. The 2D and 3D binding models of the complex **19a**/11β-HSD1 were generated using docking simulations. Based on the results, the structural–activity relationships (SARs) of compounds **1b** and **2** were also discussed.

1. Introduction

The prevalence of type 2 diabetes and obesity is one of the main public health threats in 21st century. It is estimated that more than 150 million people worldwide suffer from diabetes. And the number is expected to rise to 300 million by the year of 2025 [1]. However, the medicines for treatment of type 2 diabetes are far from sufficient. So the development of novel agents with good therapeutic index and low side effects is still in urgent demand [2].

Metabolic syndrome is a pre-diabetic state, which features with abdominal obesity, impaired glucose tolerance, dyslipidemia, low levels of high density lipoprotein (HDL), and hypertension [3]. When metabolic syndrome progresses to diabetes, complications associating with this disorder, including cardiovascular disease, kidney failure and diabetic retinopathy, become prominent. Recent investigations revealed that aberrant glucocorticoid receptor (GR) signaling was closely associated with metabolic syndrome. Glucocorticoid hormones, including cortisone and cortisol in human, are important regulators of glucose and lipid homeostasis [4]. Elevated level of glucocorticoids can lead to insulin resistance by decreasing insulin dependent glucose uptake, enhancing hepatic gluconeogenesis, and inhibiting insulin secretion from pancreatic cells. Patients with sustained glucocorticoid excess will develop dyslipidemia, visceral obesity, and other metabolic syndromes [5].

11β-hydroxysteroid dehydrogenase (11β-HSD) catalyzes the inter-conversion of the glucocorticoids, cortisone and cortisol, in human (Fig. 1) [6]. 11β-HSD has two isoforms, 11β-HSD1 and 11β-HSD2. 11β-HSD1, which is primarily found in liver, adipose and brain, converses the inactive cortisone to the active cortisol. Its counterpart 11β-HSD2, which is mainly expressed in kidney, catalyzes the reverse conversion. Both 11β-HSD1 and 11β-HSD2 are involved in maintenance of the balance of glucocorticoid hormones. Evidences from homozygous 11β-HSD1 knock-out mice model revealed that impaired function of 11β-HSD1 could result in reducing of gluconeogenesis and lipophilia in liver, and increasing of insulin sensitivity. However, inhibition of 11β-HSD2 would lead to sodium retention, hypokalemia and hypertension [7,8]. Therefore, selective inhibition of 11β-HSD1 will be a therapeutic strategy to combat type 2 diabetes and obesity [9–11].

* Corresponding authors.
E-mail address: qinshizhao@mail.kib.ac.cn (Q.-S. Zhao).

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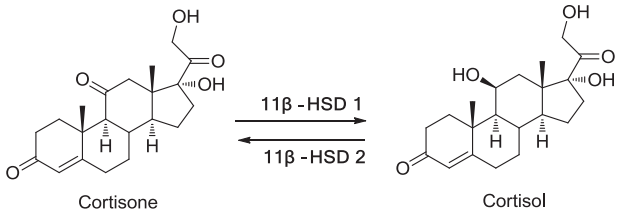


Fig. 1. Inter-conversion of cortisone and cortisol.

As a result, 11β-HSD1 has attracted considerable attention among medicinal chemistry community over the past few years [12–14]. A number of potent and selective 11β-HSD1 inhibitors was reported up to now, some of which are progressing to clinical trial (Fig. 2) [15]. However, most of the inhibitors are synthetic chemicals [12]. Nature-derived inhibitors were still scarce in literatures. So unearthing the selective 11β-HSD1 inhibitors from the inexhaustible natural product reservoir will be a promising project.

Herein, we presented the discovery of the *ent*-kaurene diterpenoids **1b** and **2** as selective 11β-HSD1 inhibitors along with their structure–activity relationship studies. Starting from the leads **1b** and **2**, thirty-six derivatives were designed and synthesized. The representatives were C-4 urea substituted analogs, which were accessible through Curtius rearrangement and subsequent nucleophilic addition. Enzymatic evaluations revealed that seven urea derivatives exhibited potent human 11β-HSD1 inhibitory activity and improved selectivity over human 11β-HSD2.

2. Discovery of the lead compounds

As a part of our efforts to identify novel drug candidates for type 2 diabetes, we discovered a novel selective 11β-HSD1 inhibitor, kaur-16-en-19-oic acid (**1b**), by screening a collection of natural products (Table 1). As shown in Table 1, compound **1b**, with IC_{50} (mouse) = 357 nM and selectivity index (mouse) = 1400, has superior activity and selectivity over the other counterparts.

However, several obstacles, including poor water solubility, lay ahead of compound **1b** on its way to a drug candidate. Therefore, it is important to initiate an optimization project to circumvent these problems. The limited amount of compound **1b** in plant had once bothered us a lot. Gratifyingly, the easily available 11β,15β-dihydroxy-kaur-16-en-19-oic acid (**2**) can serve as alternative. It is relatively abundant in *Nouelia insignis* Franch. Moreover, compound **2** has more functional groups, which facilitates structure modifications and structure–activity relationship studies. So both compounds **1b** and **2** were chosen as the starting materials for structural modifications (Fig. 3).

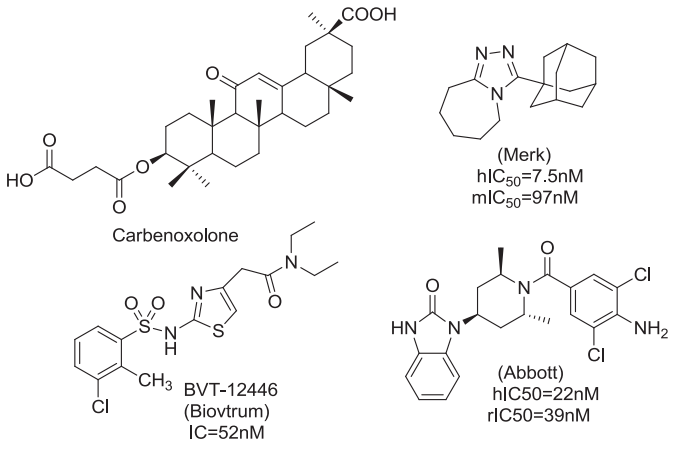


Fig. 2. Representative 11β-HSD1 inhibitors.

3. Chemistry

Starting from compounds **1b** and **2**, thirty-six structurally diverse derivatives varying on the positions C-11, C-15, C-16(17), and C-19 were designed and synthesized for the SARs studies.

Generally, the derivatives were designed on basis of bioisosteric replacement, functionality "knock-out" strategies combining with chemical accessibility. In medicinal chemistry, bioisosteric replacement is a frequently used strategy in lead optimization in order to gain the desired biological or physical properties of a compound. On the other hand, functionality "knock-out" is also a common protocol to clearly state the contributions of the structural motifs to the biological activities.

3.1. Modifications on C-11 position

The investigations on C-11 concerned modifications of the hydroxyl group into ester, ketone, alkene, and so on (Scheme 1). The C-11 hydroxyl group of compound **2** was selectively esterified by deprotonation with NaH and subsequent nucleophilic addition with CS_2 followed by trapping with MeI, affording compound **3** in moderate yield. Mitsunobu reaction of compound **2** with diethyl azodicarboxylate (DEAD) in THF led to the alkene **4** in good yield [16]. Treatment of compound **2** with the acyl isothiocyanate in the presence of base delivered a series of C-11 mono-esters, **5a**–**5d**. Moreover, two C-11 carbamic acid esters, **6a** and **6b**, were also prepared in good yield by reacting with triphosgene and amines (Scheme 1) [17,18].

3.2. Modifications on C-15, C-16,17 positions

To demonstrate the contributions of C-15 alcohol and C-16,17 alkene to the activity, a class of regio-selective reactions was also performed on these motifs (Scheme 2).

Using compound **1b** as the substrate, the C-16,17 alkene was oxidized to its corresponding bioisostere ketone **7**, under K_2OsO_4–$NaIO_4$ reaction system [19]. Reaction of compound **1b** with SeO_2 provided the allylic alcohol **8a** in moderate yield [20,21]. The structure of compound **8a** was assigned by comparing the spectra data with the literature [22]. The alcohol **8a** was further transformed to its fluoride derivative **8b** by treatment with diethylaminosulfur trifluoride (DAST). Based on the S_N2 mechanism of the reaction concerning DAST, the C-15 stereochemistry of compound **8b** was opposite to that of C-15 in compound **8a**.

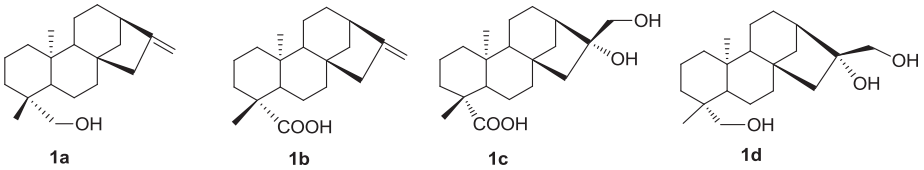
We also started our efforts toward the modifications of compound **2** on C-15 and C-16,17. The C-16,17 alkene group was hydrogenated enantioselectively with Pd/C under H_2 to afford the alkane **9** in excellent yield [23]. The epoxidation reaction of compound **2** with *meta*-chloroperoxybenzoic acid (*m*-CPBA) gave the target **10** as a single diastereoisomer. The high stereo-selectivity of compounds **9** and **10** is benefited from the directing effect of the hydroxyl group at position C-15 [23,24]. Treatment of compound **2** with the (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO) and phenyliodine diacetate (PIDA) in the presence of HOAc delivered the α,β unsaturated ketone **11** in good yield (Scheme 2) [25].

3.3. Modifications on C-19 position

We then turned our hemi-synthesis explorations to the C-19 carboxyl acid, which was a top priority in our schedule.

Again, both compounds **1b** and **2** were used as the substrates. We first attempted to convert the C-19 carboxyl acid to the

Table 1
Discovery of 11β-HSD1 inhibitors..



1a 1b 1c 1d

Entry	Mouse (IC_{50})			Human (IC_{50})		
	11β-HSD1	11β-HSD2	HSD2/HSD1	11β-HSD1	11β-HSD2	HSD2/HSD1
1a	1.553 μM	>1 mM	>640	1.417 μM	10 μM	7.0
1b	357 nM	500 μM	1400	ND	ND	ND
1c	3.216 μM	>1 mM	>311	1.236 μM	>1 mM	>809
1d	2.805 μM	>1 mM	>357	0.91 μM	>1 mM	>1095
GA[a]	40.21 nM	ND	ND	29.1 nM	1.22 nM	0.42

[a] GA: glycyrrhetinic acid was used as a positive control; ND: not determined.

hydroxyl group. Refluxing of compounds **1b** and **2** with $LiAlH_4$ in THF furnished the desired alcohols **12** and **16** in excellent yields. Further treatment of the alcohol **12** with DAST afforded the fluoride **13** in moderate yield. Reaction of compound **2** with diazomethane in ether gave the methyl ester **17** quantitatively (Scheme 3).

Inspiring by the fact that the urea constitutes a family of potent selective 11β-HSD1 inhibitors [14,26,27], we envisaged to incorporate the urea scaffold into the core structure. The carboxyl group was converted to urea moiety in a two-step procedure. Upon heating compounds **1b** and **2** with diphenylphosphoryl azide (DPPA) in anisole, the Curtius rearrangement proceeded smoothly to afford intermediates **14** and **18** in excellent yields [28,29]. The C-4 stereochemistry of derivatives **14** and **18** is still *R* because Curtius reaction doesn't affect the stereochemistry of carbon adjacent to the carboxyl acid [28]. Subsequent nucleophilic addition of the isocyanates **14** and **18** with various reagents delivered a diverse set of urea derivatives **15a**–**15c** in moderate to excellent yields (Scheme 3).

4. Results and discussions

4.1. Protein based modeling

To gain insight into the interactions between the ligand and the receptor, the 2D and 3D binding models of the compound **19a** to mouse and human 11β-HSD1 were generated respectively based on the molecular docking (Fig. 4). It's found that the top ranking docking score for compound **19a** with mouse 11β-HSD1 is −6.98 kcal/mol, while with human 11β-HSD1 the score is −8.60 kcal/mol. These indicate that the binding affinity of compound **19a** with human 11β-HSD1 is stronger than that with mouse 11β-HSD1. The results are also in good agreement with the experimental results (IC_{50} (human 11β-HSD1) = 9.3 nM and IC_{50} (mouse 11β-HSD1) > 1 μM). Additionally, the hydrophobic interaction (indicated by dashed lines) between compound **19a** and human 11β-HSD1 is stronger than between compound **19a** and mouse 11β-HSD1, from the 2D interaction scheme (Fig. 4A and B). Besides, there are two hydrogen bonds between C-15 hydroxyl group and Tyr183 and Ser170 in the complex **19a**/mouse 11β-HSD1. And there is a strong hydrogen interaction with the O···O distance of 2.9 Å between C-15 hydroxyl group and the residue Tyr183 in complex **19a**/human 11β-HSD1 (Fig. 4).

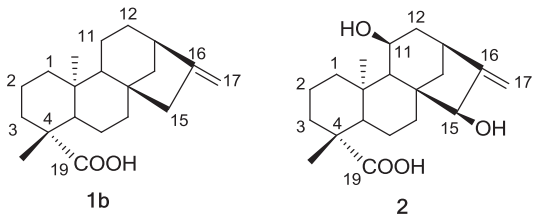


Fig. 3. Structure of compounds **1b** and **2**.

4.2. Biological effects of the derivatives

With derivatives secured, we then determined the enzymatic potency on inhibition of human and mouse 11β-HSD1 and 11β-HSD2 by scintillation proximity assay (SPA) using microsomes containing 11β-HSD1 or 11β-HSD2 [30]. Results are reported as the average of at least two independent experiments with at least two replicates at each concentration.

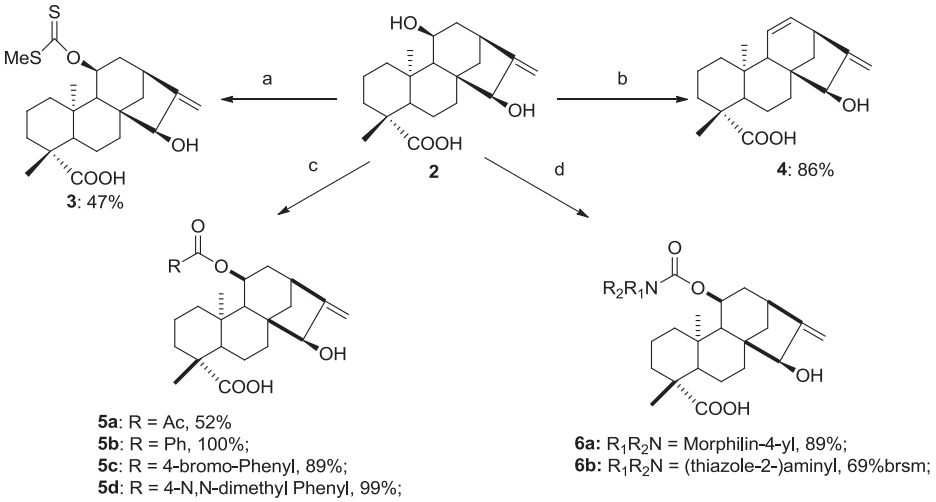
The preliminary evaluations of the derivatives were performed at the concentration of 1 μM. Glycyrrhetinic acid was used as the positive control [31] (Table 2).

The derivatives with inhibitory ratio more than 50% in preliminary tests were further evaluated to calculate the IC_{50} value and the selectivity ratio against 11β-HSD2 (Table 3).

At the discovery stage, several *ent*-kaurene diterpenoids were assessed for their 11β-HSD inhibitory activity and selectivity (Table 1). Structural comparisons between the active and the inactive demonstrated that the carboxyl group at C-18 (see compounds **1a** and **1b**) and the double bond at C-16,17 (see compounds **1c** and **1d**) were preferential to the hydroxyl group and the vicinal diol group respectively for the activity and selectivity.

On basis of the biological results illustrated in Tables 2 and 3, the structure–activity relationships of the leads **1b** and **2** were discussed herein.

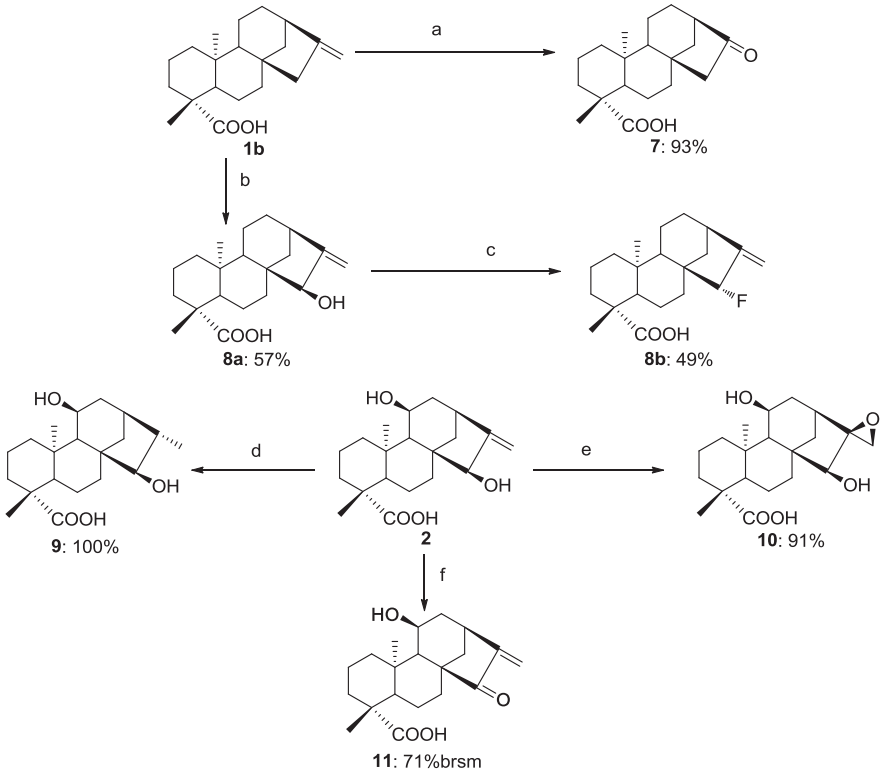
The effects of structural variations at C-11 on the activity and selectivity are first examined. Eliminating of the C-11 hydroxyl group to the alkene **4** exerted negligible effects on the inhibition of both mouse and human 11β-HSD1, indicating that the C-11 hydroxyl group is not necessary for the binding. The C-11 ester analogs **3** and **5a** showed an impressive improvement of the potency and selectivity on human 11β-HSD. These observations suggested that the proper non-polar functionality at C-11 was beneficial to the activity and selectivity on human 11β-HSD instead of mouse 11β-HSD. However, the potency on both mouse and human 11β-HSD1 was decreasing with increase in the steric bulk of C-11 (see esters **5b**–**5d**, **6a** and **6b**).



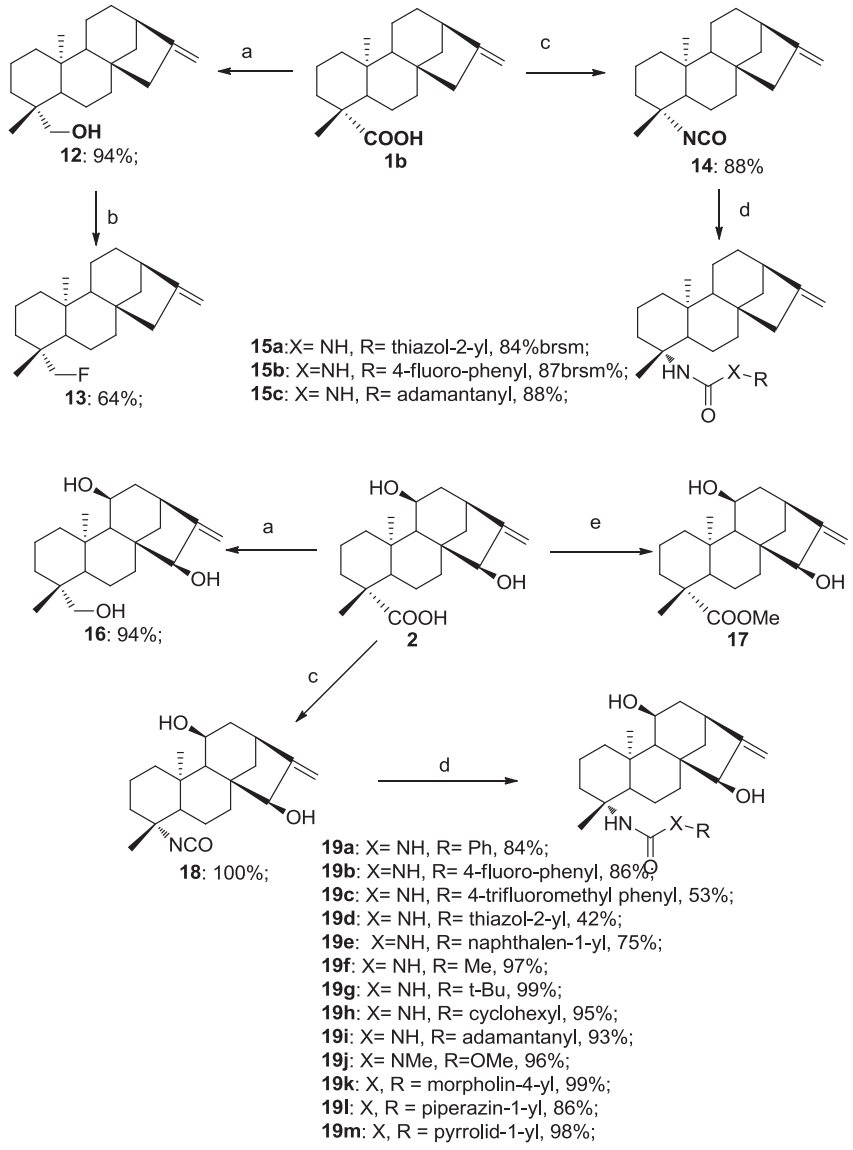
Scheme 1. Modifications on the hydroxyl group at position C-11. Reagents and Conditions: a) NaH, CS_2, then MeI, THF, RT; b) DEAD, PPh_3, THF, RT; c) RCOSCN, Et_3N, THF, 0 °C-RT; d) Triphosgene, Et_3N, R_1R_2NH, DCM, 0 °C-RT.

Next, the contributions of C-15 scaffold to the biological activities were discussed. The C-15 hydroxyl derivative **8a** was less active than compound **1b** on both mouse and human 11β-HSD1, but partially maintained the activity and selectivity on mouse 11β-HSD. Interestingly, the fluoride analog **8b** was also poorly active on both mouse and human 11β-HSD1. These observations revealed that the functionality combination of these groups was not tolerated by active site of both mouse and human 11β-HSD1. Oxidation of the C-15 hydroxyl group into ketone **11** produced negligible effects on the activity. These results demonstrated that C-15 hydroxyl group played a minor role for the binding, which was in agreement with the molecular docking predictions.

The ketone analog **7**, alkane analog **9**, the epoxide analog **10**, and the diol analog **1c** were designed to mimic the C-16,17 double bond and probe the binding characteristics in this region. However,



Scheme 2. Modifications at positions C-15, C-16 and C-17 Reagents and Conditions: a) K_2OsO_4, $NaIO_4$, 2,6-lutidine, *t*-BuOH/THF/H_2O, RT; b) SeO_2, 1,4-dioxane, RT; c) DAST, DCM, −78 °C; d) Pd/C, H_2, MeOH/EtOAc, RT; e) *m*-CPBA, $NaHCO_3$, DCM, RT; f) TEMPO, PIDA, HOAc, CH_3CN, RT.



Scheme 3. Modifications at positions C-18 Reagents and Conditions: a) $LiAlH_4$, THF, reflux; b) DAST, DCM, −78 °C; c) DPPA, Et_3N, anisole, 90 °C; d) R_1R_2NH, Et_3N, THF, RT-reflux; e) CH_2N_2, Et_2O, 0 °C.

replacements of the substitutes at the C-16,17 double bond by these bioisosteric manipulations resulted in a drop of potency on both mouse and human 11β-HSD1, which implied that the double bond was the optimum choice for this region. These results were also supported by the evidence that the C-16,17 alkene had hydrophobic interactions with residues Thr124, Thr222, Ile121, Ala223 and Tyr183, from the 2D binding schemes of the complex **19a**/human 11β-HSD1.

We then focused on the effects of the variations at the C-19 carboxyl group. The C-19 hydroxyl analogs **12** and **16**, the C-19 fluoride analog **13** were weakly active on both human and mouse 11β-HSD1, comparing to compound **1b** and **2**. The ester derivative **17** had comparable activity to compound **2** on both species 11β-HSD1. All these results highlighted the importance of electronic effects in this region. The urea modifications at C-19 gave a wide range of activities on human 11β-HSD1. Seven urea derivatives **15b**, **15c**, **19a**, **19b**, **19e**, **19h** and **19i** showed potent inhibitory activity on human 11β-HSD1, and were with modest to excellent selectivity over human 11β-HSD2 (Table 3). Especially, the most potent derivative **19a** was 100 folds more active than the parent compound **2** on human 11β-HSD1, and was with excellent selectivity over human 11β-HSD2 (Table 3), indicating that compound **19a** contained the optimal functional group combination for the binding. These superior features merit compound **19a** for further investigations.

We also noticed that mouse 11β-HSD1 and human 11β-HSD1 responded quite differently to the functionality changes. Human 11β-HSD1 was more sensitive to the even subtle structural variation, whereas, mouse 11β-HSD1 was rather obtuse. It was reasoned that there was significant structural variability between the active pockets of mouse 11β-HSD1 and human 11β-HSD1 [32].

5. Conclusions

In summary, we reported the discovery of an *ent*-kaurene diterpenoids, compound **1b**, as a novel selective 11β-HSD1 inhibitor. Using compound **1b** and its counterpart **2** as the starting materials, thirty-six structurally diverse derivatives were designed and synthesized.

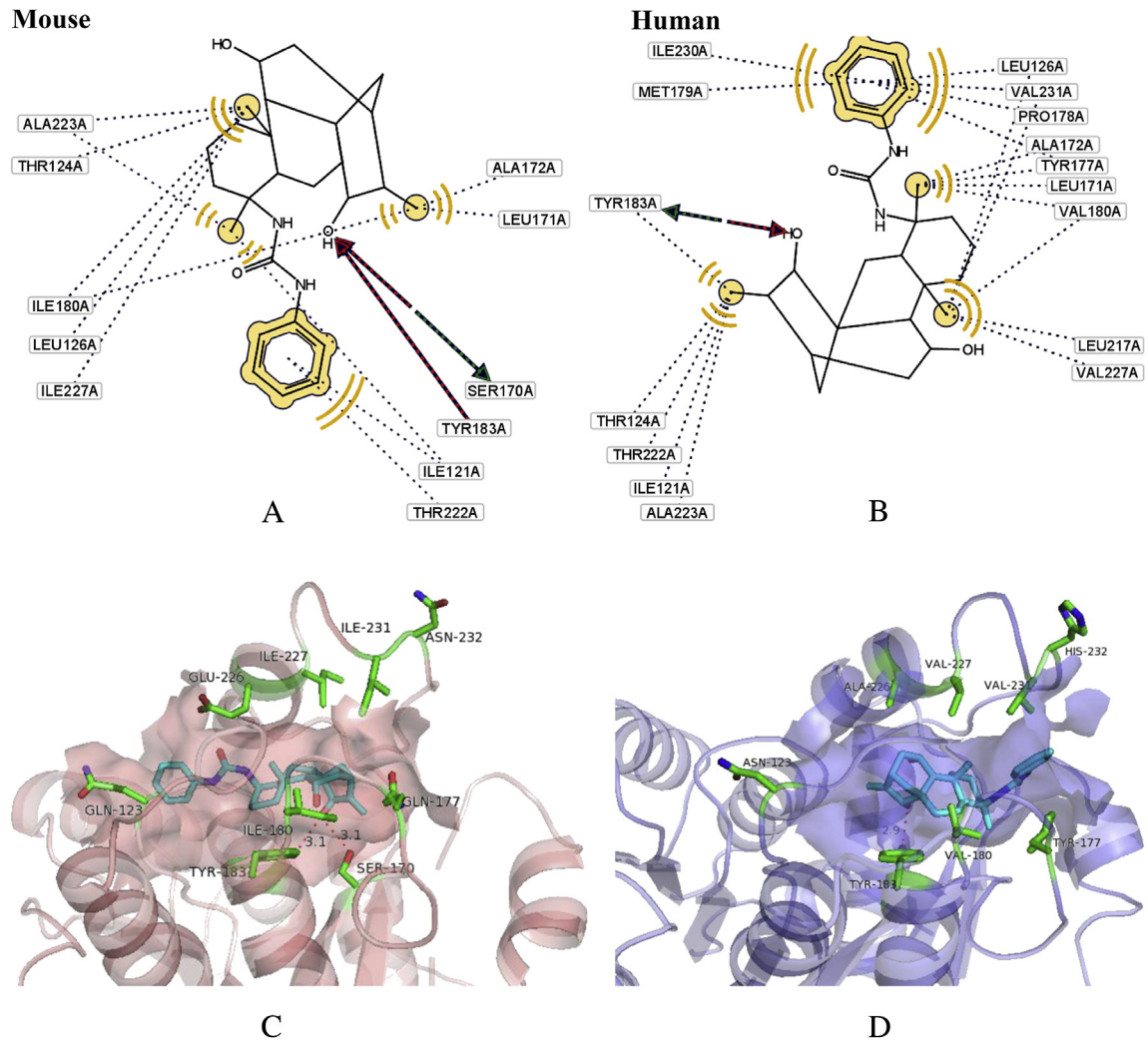


Fig. 4. Two-dimensional (2D) and three-dimensional (3D) interaction schemes between compound **19a** and mouse/human 11β-HSD1. The figures were prepared using LigandScout (http://www.inteligand.com/ligandscout/) and PyMOL (http://pymol.sourceforge.net/). The ligands and the residues near the pocket which are different in mouse and human 11β-HSD1 are shown as sticks (receptor carbon in green and ligand carbon in cyan). The left two figures belong to mouse 11β-HSD1 and the right two figures belong to human 11β-HSD1. Critical different residues of the binding pocket are labeled in the 3D interaction views. Hydrogen bonds are shown as dotted lines in both 2D and 3D views. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The derivatives were further evaluated on SPA for their 11β-HSD inhibitory potency and selectivity. It's found that the acetyl ester **5a** and several urea derivatives, **19a**, **19b**, **19e**, **19h**, exhibited significant improved potency and selectivity on human 11β-HSD1. Moreover, the 2D and 3D interaction schemes of compound **19a** and human/mouse 11β-HSD1 were generated by docking simulation. The present work enriched the structural diversity of selective 11β-HSD1 inhibitors.

6. Experimental sections

6.1. General information

All reactions were performed under argon atmosphere using flame-dried glassware unless otherwise noted. CH_2Cl_2, CH_3CN and 1,4-dioxane were distilled over CaH_2. THF was distilled over sodium/benzophenone ketyl. All reagents were commercially available and used without further purification unless indicated otherwise. Thin layer chromatographies were carried out on Merck silica plates (0.25 mm layer thickness). Flash chromatography was performed with 300–400 mesh silica gels. Yields reported were for isolated, spectroscopically pure compounds. ^{1}H- and ^{13}C-NMR experiments were performed on a Bruker AM-400 and DRX-500 NMR spectrometer at ambient temperature. The residual solvent protons (^{1}H) or the solvent carbons (^{13}C) were used as internal standards. ^{1}H-NMR data are presented as follows: chemical shift in ppm downfield from tetramethylsilane (multiplicity, coupling constant, integration). The following abbreviations are used in reporting NMR data: s, singlet; d, doublet; t, triplet; dd, doublet of doublets; dt, doublet of triplets; m, multiplet. EI-MS and HR-EI-MS were taken on a VG Auto Spec-3000 or on a Finnigan MAT 90 instrument.

6.2. Chemistry

6.2.1. Isolation of ent-kaur-16-en-19-ol (**1a**), ent-kaur-16-en-19-oic acid (**1b**), ent-16β, 17-dihydroxy-19-kauranoic acid (**1c**), ent-16β,17,19-kaurenetriol (**1d**)

Dried samples of aerial part of *Diplopterygium gigantea (Wall.) Nakai* (5.4 kg) were ground and extracted with 95% ethanol. The extraction was subjected to macroporous resin eluting with 95% methanol. The crude product was then purified by silica gel chromatography with petrol ether/acetone mixture to give five fractions. The fraction of the general chromatography eluted with petrol ether/acetone (20:80) was further chromatographed over silica gel columns, Sephadex LH column and RP-18 reverse column

Table 2
Enzymatic assay.[a]

Entry	Inhibitory ratio	
	Mouse 11β-HSD1 (%)	Human 11β-HSD1 (%)
1b	82.39	81.79
2	16.54	39.72
3	11.06	59.99
4	19.58	43.57
5a	12.40	74.91
5b	21.28	29.33
5c	32.67	24.82
5d	33.98	32.31
6a	35.23	30.07
6b	11.60	15.49
7	19.58	56.31
8a	55.54	35.17
8b	55.97	47.13
9	11.60	15.49
10	16.78	27.32
11	25.27	19.59
12	20.43	28.58
13	37.67	31.20
14	55.52	28.35
15a	18.24	19.74
15b	25.89	63.98
15c	18.17	55.66
16	27.26	17.42
17	25.06	42.26
18	16.83	6.14
19a	24.40	94.96
19b	7.92	64.54
19c	3.69	44.71
19d	5.39	9.70
19e	52.23	68.81
19f	19.44	2.12
19g	11.32	11.81
19h	16.34	75.95
19i	20.77	65.69
19j	16.07	0.24
19k	21.39	13.89
19l	12.71	23.15
19m	9.21	44.60
GA[b]	96.68	94.53

[a] The value was determined at the concentration of 1 μM. And the values are averages of two determinations and deviation from the average is 10% of the average value.
[b] GA: glycyrrhetinic acid, which was used as a positive control.

to give compound **1a** (450 mg), compound **1b** (3.0 g), compound **1c** (8 mg), and compound **1d** (8 mg). Compounds **1a** [33], **1b** [33], **1c** [34], and **1d** [34] were characterized by comparing the spectral data with the references indicated.

Table 3
Enzymatic assay.[a]

Entry	Mouse (IC_{50})			Human (IC_{50})		
	11β-HSD1	11β-HSD2	HSD2/HSD1	11β-HSD1	11β-HSD2	HSD2/HSD1
1b	64.2 nM			131.4 nM	19.0 μM	144.8
3	>1 μM	ND	ND	770.6 nM	>100 μM	>129.7
5a	>1 μM	ND	ND	116.4 nM	228.7 μM	1965.4
8a	258.6 nM	>100 μM	>386.7	>1 μM	ND	ND
15b	>1 μM	ND	ND	330.8 nM	>100 μM	>302.3
15c	>1 μM	ND	ND	407.3 nM	>100 μM	>245.7
19a	>1 μM	ND	ND	9.4 nM	>1 mM	>10649.0
19b	>1 μM	ND	ND	249.0 nM	>100 μM	>401.6
19e	>1 μM	ND	ND	266.1 nM	1.6 μM	6.2
19h	>1 μM	ND	ND	165.4 nM	>100 μM	> 604.6
19i	>1 μM	ND	ND	403.7 nM	0.7 μM	1.7
GA[b]	2.0 nM	ND	ND	0.83 nM	1.22 nM	0.4012

[a] Values are averages of two determinations and deviation from the average is 10% of the average value.
[b] GA: glycyrrhetinic acid, which was used as a positive control; ND: not determined.

*6.2.2. Isolation of 11β,15β-dihydroxy-kaur-16-en-19-oic acid (**2**)*

The pulverized branch and leaf of *N. insignis* Franch (22 kg) was extracted with 95% ethanol and concentrated to give a residue (750 g). The residue was subjected to silica gel chromatography eluting with petrol ether/acetone (from 90:10 to 30:70) to separate into fractions. The polar fraction eluted with petrol ether/acetone (30:70) was further purified over silica gel columns, Sephadex LH column and RP-18 reverse column to give compound **2** (65 g). Compound **2** was characterized by comparing the spectral data with the references indicated [35].

*6.2.3. 11β-((Methylthio)-thioxomethoxy)-15β-hydroxy-kaur-16-en-19-oic acid (**3**)*

To a suspension of NaH (32 mg, 60%, 1 mmol) in THF (1.0 mL) at 0 °C under N_2 was added a solution of compound **2** (33 mg, 0.1 mmol) in THF (0.5 mL) dropwise. The mixture was stirred at 0 °C for 0.5 h. Then CS_2 (39 mg, 0.5 mmol) was added dropwise. The mixture was warmed to the room temperature and continued stirring for 2 h. Then MeI (35 mg, 0.25 mmol) was added dropwise and stirred for another 4 h until no starting material was detected by TLC judgment. A saturated aqueous NH_4Cl solution was added to quench the reaction. The mixture was extracted with dichloromethane (10 mL × 3). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The crude product was subjected to flash chromatography on silica gel (ethyl acetate/petrol ether = 1:3) to afford compound **3** as white foams (20 mg, 47%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 6.30 (s, 1H), 5.18 (s, 1H), 5.18 (s, 1H), 3.86 (d, *J* = 4 Hz, 1H), 2.76 (s, 1H), 2.63 (s, 3H), 2.18 (d, *J* = 8 Hz, 1H), 2.04–2.09 (m, 3H), 2.01 (s, 1H), 1.82–1.89 (m, 2H), 1.79 (d, *J* = 16 Hz, 1H), 1.69 (s, 1H), 1.63 (d, *J* = 12 Hz, 1H), 1.53 (dd, *J* = 20 Hz, 8 Hz, 1H), 1.51 (d, *J* = 20 Hz, 1H), 1.34 (dd, *J* = 12 Hz, 8 Hz, 1H), 1.25 (s, 1H), 1.24 (s, 3H), 1.19 (dd, *J* = 20 Hz, 8 Hz, 1H), 1.09 (d, *J* = 4 Hz, 1H), 1.07 (s, 1H), 0.85 (s, 3H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 216.7, 183.8, 151.4, 109.3, 89.9, 66.5, 57.0, 56.1, 45.4, 43.7, 42.6, 40.2, 38.6, 38.6, 38.4, 37.4, 35.9, 29.6, 28.8, 20.9, 19.2, 18.9, 15.0; HR-EI-MS (*m*/*z*): calcd. for $C_{22}H_{32}O_4S_2$ $[M]^+$, 424.1742, found 424.1744.

*6.2.4. 15β-Hydroxy-kaur-11(12),16-dien-19-oic acid (**4**) [16]*

To a solution of DEAD (87 mg, 0.5 mmol) in THF (1.5 mL) at 0 °C were added compound **2** (33 mg, 0.1 mmol) and PPh_3 (65 mg, 0.25 mmol) successively. The mixture was warmed to the room temperature and was stirred for 8 h until no starting material was detected. Then the reaction was quenched with water. The aqueous phase was extracted with EtOAc (8 mL × 3). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The crude product was subjected to flash chromatography on silica gel (ethyl acetate/petrol ether = 1:4) to afford compound **4** as white foams (28 mg, 89%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 6.02 (t, *J* = 8 Hz, 8 Hz, 1H), 5.48 (dd, *J* = 8 Hz, 8 Hz, 1H), 4.86 (s, 1H), 4.81 (d, *J* = 4 Hz, 1H), 3.88 (s, 1H), 2.83 (dd, *J* = 8 Hz, 4 Hz, 1H), 2.13 (m, 2H), 1.97 (s, 1H), 1.84 (m, 3H), 1.76 (m, 1H), 1.54 (d, *J* = 12 Hz, 1H), 1.45 (m, 1H), 1.25 (s, 3H), 1.18 (d, *J* = 12 Hz, 2H), 1.07 (m, 3H), 0.86 (s, 3H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 183.8, 157.6, 134.2, 125.4, 103.1, 83.4, 55.1, 48.8, 44.9, 43.7, 42.3, 39.7, 38.8, 38.4, 37.5, 34.1, 28.8, 21.3, 18.9, 15.4; HR-EI-MS (*m*/*z*): calcd. for $C_{20}H_{28}O_3$ $[M]^+$, 316.2042, found 316.2038.

*6.2.5. Typical procedures for compounds **5a**–**5d** (preparation of compound **5b**)*

To a solution of compound **2** (17 mg, 0.05 mmol) in THF (1.0 mL) at 0 °C were added Et_3N (15 mg, 0.15 mmol) and benzoyl isothiocyanate (16 mg, 0.1 mmol) successively. The mixture was warmed to the room temperature and was stirred for 1.5 h until no starting material was detected. Then the reaction was quenched with water. The aqueous phase was extracted with EtOAc (8 mL × 3). The

combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The crude product was subjected to flash chromatography on silica gel (ethyl acetate/petrol ether = 1:3) to afford compound **5b** as white foams (22 mg, 100%).

*6.2.5.1. 11β-Acetyloxy-15β-hydroxy-kaur-16-en-19-oic acid (**5a**).* Compound **5a** (10 mg, 53%) was prepared from compound **2** (17 mg, 0.05 mmol) as white foam according to the synthetic procedure described for **5b**. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 5.21 (s, 1H), 5.14 (s, 1H), 4.93 (s, 1H), 4.83 (s, 1H), 2.68 (s, 1H), 2.17 (s, 3H), 1.99 (d, J = 10 Hz, 1H), 1.95 (d, J = 15 Hz, 2H), 1.90 (s, 3H), 1.83–1.85 (m, 3H), 1.58 (s, 1H), 1.43–1.52 (m, 2H), 1.24 (s, 3H), 1.03–1.04 (m, 3H), 0.90 (s, 3H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 183.6, 170.4, 152.6, 108.8, 81.9, 66.4, 56.4, 56.2, 44.4, 43.7, 42.5, 40.1, 38.6, 38.3, 37.4, 36.0, 28.8, 21.2, 20.9, 18.9, 15.0; HR-EI-MS (m/z): calcd. for $C_{22}H_{32}O_5$ $[M]^+$, 376.2250, found 376.2247.

*6.2.5.2. 11β-Benzoyloxy-15β-hydroxy-kaur-16-en-19-oic acid (**5b**).* Compound **5b** (22 mg, 100%) was prepared from compound **2** (17 mg, 0.05 mmol) as white foam according to the synthetic procedure described above. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.05 (d, J = 8 Hz, 2H), 7.66 (t, J = 8 Hz, 1H), 7.51 (t, J = 8 Hz, 2H), 5.11 (s, 1H), 5.02 (s, 1H), 4.04 (d, J = 4 Hz, 1H), 3.76 (s, 2H), 2.63 (s, 1H), 2.33 (d, J = 16 Hz, 1H), 1.92–2.05 (m, 5H), 1.69–1.80 (m, 4H), 1.60 (s, 1H), 1.55 (d, J = 16 Hz, 1H), 1.47 (dd, J = 8 Hz, 4 Hz, 1H), 1.43 (s, 3H), 1.31 (d, J = 12 Hz, 1H), 1.25 (d, J = 12 Hz, 1H), 1.18 (dd, J = 12 Hz, 4 Hz, 1H), 1.09 (dd, J = 12 Hz, 4 Hz, 1H), 0.99 (s, 3H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 172.8, 162.2, 158.2, 134.3, 130.3, 129.1, 128.8, 106.0, 82.3, 66.7, 56.3, 53.4, 45.5, 44.4, 42.9, 40.0, 38.8, 38.7, 37.8, 37.7, 35.6, 28.2, 21.3, 18.8, 16.1; HR-EI-MS (m/z): calcd. for $C_{27}H_{34}O_5$ $[M]^+$, 438.2406, found 438.2414.

*6.2.5.3. 11β-(4-Bromo-benzoyloxy)-15β-hydroxy-kaur-16-en-19-oic acid (**5c**).* Compound **5c** (23 mg, 89%) was prepared from compound **2** (17 mg, 0.05 mmol) as white solid according to the synthetic procedure described for **5b**. ^{1}H-NMR (CD_3OD, 400 MHz) δ 7.83 (d, J = 4 Hz, 2H), 7.51 (d, J = 4 Hz, 2H), 4.96 (s, 1H), 4.93 (s, 1H), 3.84 (d, J = 4 Hz, 1H), 3.67 (s, 1H), 2.50 (s, 1H), 2.09 (d, J = 12 Hz, 1H), 1.94 (d, J = 12 Hz, 1H), 1.91 (d, J = 8 Hz, 1H), 1.79 (t, J = 8 Hz, 4H), 1.73 (d, J = 12 Hz, 1H), 1.57 (dt, J = 8 Hz, 4 Hz, 2H), 1.45 (s, 1H), 1.34–1.38 (m, 3H), 1.13 (s, 3H), 1.09 (d, J = 4 Hz, 1H), 1.03–1.06 (m, 3H), 0.99 (d, J = 4 Hz, 1H), 0.97 (d, J = 4 Hz, 1H), 0.81 (s, 3H), 0.79 (d, J = 4 Hz, 2H); ^{13}C-NMR (125 MHz, MeOD) δ 180.9, 168.5, 158.0, 132.0, 131.8, 130.1, 129.7, 128.2, 106.5, 82.6, 66.3, 56.8, 54.9, 45.3, 44.0, 42.2, 40.7, 39.7, 39.6, 38.3, 38.3, 36.4, 30.0, 29.2, 21.8, 19.5, 15.7; HR-EI-MS (m/z): calcd. for $C_{27}H_{33}O_5Br$ $[M]^+$, 516.1511, found 516.1533.

*6.2.5.4. 11β-(4-Dimethylamimo-benzoyloxy)-15β-hydroxy-kaur-16-en-19-oic acid (**5d**).* Compound **5d** (24 mg, 99%) was prepared from compound **2** (17 mg, 0.05 mmol) as white solid according to the synthetic procedure described for **5b**. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.73 (d, J = 10 Hz, 2H), 6.55 (d, J = 10 Hz, 2H), 4.92 (s, 1H), 4.88 (s, 1H), 3.91 (s, 3H), 3.82 (dd, J = 15 Hz, 5 Hz, 1H), 3.62 (s, 1H), 2.91 (s, 6H), 2.46 (s, 1H), 2.17 (d, J = 15 Hz, 1H), 1.91 (s, 1H), 1.82–1.89 (m, 4H), 1.79 (d, J = 15 Hz, 2H), 1.70 (d, J = 15 Hz, 2H), 1.54 (t, J = 15 Hz, 1H), 1.43 (d, J = 20 Hz, 2H), 1.34 (d, J = 15 Hz, 1H), 1.27 (s, 3H), 1.12 (s, 3H), 1.08 (s, 2H), 1.04 (d, J = 5 Hz, 1H), 1.00 (s, 1H), 0.90–0.96 (m, 2H), 0.86 (s, 3H); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 173.6, 162.6, 157.3, 154.1, 132.2, 114.6, 110.6, 105.8, 81.9, 65.7, 56.2, 53.9, 45.1, 43.2, 41.6, 39.9, 39.6, 38.8, 38.7, 37.5, 35.6, 29.3, 28.6, 28.0, 21.1, 18.8, 15.8; HR-EI-MS (m/z): calcd. for $C_{29}H_{39}NO_5$ $[M]^+$, 481.2828, found 481.2821.

*6.2.6. Typical procedure for compounds **6a**–**6b** (preparation of compound **6a**) [17,18]*

To a solution of compound **2** (17 mg, 0.05 mmol) and triphosgene (15 mg, 0.05 mmol) in DCM (1.0 mL) was added Et_3N (15 mg, 0.15 mmol) dropwise. The mixture was stirred at 0 °C for 0.5 h, and then morpholine (9 mg, 0.1 mmol) was added dropwise. The mixture was stirred for another 2.5 h until no starting material was detected by TLC. Then the reaction was quenched with water. The aqueous phase was extracted with EtOAc (8 mL × 3). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The crude product was subjected to flash chromatography on silica gel (ethyl acetate/petrol ether = 1:1.5) to afford compound **6a** as white foam (20 mg, 89%).

*6.2.6.1. 11β-((Morphilin-4-yl)-carbonyl-oxy)-15β-hydroxy-kaur-16-en-18-oic acid (**6a**).* ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 5.19 (d, J = 4 Hz, 1H), 5.07 (s, 1H), 4.99 (s, 1H), 3.78 (s, 1H), 3.61–3.69 (m, 6H), 3.50–3.56 (m, 2H), 2.64 (d, J = 8 Hz, 1H), 2.30 (d, J = 16 Hz, 1H), 2.16 (s, 1H), 2.09 (d, J = 12 Hz, 1H), 1.87–1.93 (m, 2H), 1.78 (t, J = 12 Hz, 12 Hz, 2H), 1.64 (t, J = 12 Hz, 12 Hz, 1H), 1.53–1.55 (m, 1H), 1.50 (s, 1H), 1.46 (d, J = 12 Hz, 2H), 1.27 (s, 3H), 1.24 (d, J = 4 Hz, 2H), 1.06–1.16 (m, 4H), 0.97 (s, 3H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 176.7, 157.1, 154.5, 106.8, 83.2, 77.6, 71.4, 67.3, 61.1, 54.0, 47.0, 46.6, 45.2, 44.6, 41.6, 40.1, 40.0, 39.6, 39.4, 39.0, 38.0, 36.0, 28.3, 22.4, 20.3, 18.9, 15.8; HR-EI-MS (m/z): calcd. for $C_{25}H_{37}NO_6$ $[M]^+$, 447.2621, found 447.2640.

*6.2.6.2. 11β-((N-2-thiazolyl)-carbonyl-oxy)-15β-hydroxy-kaur-16-en-18-oic acid (**6b**).* Compound **6b** (11 mg, 69% base on the recovery starting material (brsm)) was prepared from compound **2** (17 mg, 0.05 mmol) as white solid according to the synthetic procedure described for **6a**, with 5 mg starting material recovered. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.27 (d, J = 4 Hz, 1H), 6.83 (d, J = 4 Hz, 1H), 5.10 (s, 1H), 4.91 (s, 1H), 3.79 (s, 1H), 2.58 (s, 1H), 2.24 (d, J = 12 Hz, 1H), 1.98 (dd, J = 12 Hz, 8 Hz, 1H), 1.79–1.97 (m, 5H), 1.72 (d, J = 16 Hz, 1H), 1.64 (s, 1H), 1.45 (d, J = 12 Hz, 1H), 1.42 (d, J = 12 Hz, 1H), 1.22 (s, 3H), 1.18 (s, 3H), 1.13 (s, 1H), 1.08 (d, J = 16 Hz, 2H), 1.04 (d, J = 8 Hz, 2H), 0.89 (s, 3H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 183.7, 162.4, 157.2, 152.6, 136.5, 112.9, 106.6, 83.2, 72.7, 56.3, 51.8, 44.9, 44.3, 40.5, 40.3, 38.9, 38.7, 38.6, 38.1, 36.0, 30.1, 29.5, 21.7, 19.5, 15.9; HR-EI-MS (m/z): calcd. for $C_{24}H_{30}N_2O_5S$ $[M]^+$, 458.1875, found 458.1883.

*6.2.7. 17-Norkaur-16-oxo-19-oic acid (**7**) [19]*

To a solution of compound **1b** (30 mg, 0.1 mmol) in *t*-BuOH/THF/H_2O (0.75 mL/0.5 mL/0.25 mL) were added 2,6-lutidine (20 mg, 0.2 mmol) and K_2OsO_4 (2 mg, 0.004 mmol) successively. The mixture was stirred at room temperature for 10 min, then $NaIO_4$ (87 mg, 0.4 mmol) was added in one portion. The mixture was stirred for another 3 h until no starting material was detected by TLC. Then the reaction was quenched with a saturated Na_2SO_3 aqueous solution. The aqueous phase was extracted with EtOAc (8 mL × 3). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The crude product was subjected to flash chromatography on silica gel (ethyl acetate/petrol ether = 1:4) to afford compound **7** as white foam (28 mg, 93%). The structure of compound **7** was assigned by comparing the spectra data with that reported in the literature [36].

*6.2.8. 15α-Hydroxy-kaur-16-en-19-oic acid (**8a**) [20,21]*

To a solution of compound **1b** (30 mg, 0.1 mmol) in 1,4-dioxane (1.0 mL) was added SeO_2 (17 mg, 0.15 mmol) in one portion. The mixture was stirred at room temperature under N_2 for 2 h until no starting material was detected by TLC. Then the solvent was removed under vacuo. The crude product was subjected to flash chromatography on silica gel (ethyl acetate/petrol ether = 1:4) to afford compound **8a** (18 mg, 57%). The structure of compound **8a** was characterized by comparing with the spectra data in the literature [22].

*6.2.9. 11β,15β-dihydroxy-kaur-19-oic acid (**9**)*

To a solution of compound **2** (17 mg, 0.05 mmol) in MeOH (4.0 mL) was added Pd/C (3.0 mg, 17%wt) in one portion. The mixture was stirred at room temperature under H_2 for 1 h until no starting material was detected by TLC. Then the insoluble substance was removed. The filtrate was concentrated under vacuo, and the crude product was subjected to flash chromatography on silica gel (ethyl acetate/petrol ether = 1:2) to afford compound **9** as white foams (16 mg, 94%). ^{1}H-NMR ($CDCl_3$:CD_3OD = 1:1, 500 MHz) δ 3.8 (d, J = 5 Hz, 1H), 3.28 (s, 2H), 3.24 (s, 1H), 2.33 (d, J = 10 Hz, 2H), 2.17 (t, J = 10 Hz, 1H), 2.07 (d, J = 15 Hz, 1H), 1.73–1.86 (m, 8H), 1.64 (d, J = 10 Hz, 1H), 1.35 (d, J = 10 Hz, 1H), 1.29 (d, J = 10 Hz, 2H), 1.17 (s, 1H), 1.15 (d, J = 5 Hz, 3H), 1.10 (s, 3H), 1.07 (d, J = 15 Hz, 1H), 0.96 (t, J = 10 Hz, 1H), 0.87 (s, 3H); ^{13}C-NMR ($CDCl_3$:CD_3OD = 1:1, 125 MHz) δ 182.4, 65.2, 61.8, 57.1, 52.1, 50.3, 44.6, 40.2, 39.3, 39.0, 37.9, 35.6, 35.4, 34.0, 29.5, 20.9, 19.7, 15.9, 11.3; HR-EI-MS (m/z): calcd. for $C_{20}H_{32}O_4$ $[M]^+$, 336.2144, found 336.2155.

*6.2.10. 11β,15β-dihydroxy-16β(17)-epoxy-kaur-19-oic acid (**10**)*

To a suspension of compound **2** (33 mg, 0.1 mmol) and $NaHCO_3$ (17 mg, 0.2 mmol) in DCM (1.0 mL) was added *m*-CPBA (26 mg, 70%, 0.1 mmol) in one portion. The mixture was stirred at room temperature for 1.5 h until no starting material was detected by TLC. Then the reaction was quenched with a saturated Na_2SO_3 aqueous solution. The aqueous phase was extracted with EtOAc (10 mL × 3). The combined organic layers were washed with H_2O and brine successively, dried over anhydrous Na_2SO_4 and concentrated. The crude product was subjected to flash chromatography on silica gel (ethyl acetate/petrol ether = 1:1) to afford compound **10** as white foams (32 mg, 91%). ^{1}H-NMR (CD_3OD, 400 MHz) δ 4.37 (s, 1H), 3.67 (d, J = 12 Hz, 1H), 3.63 (d, J = 12 Hz, 1H), 3.08 (s, 1H), 2.48 (t, J = 8 Hz, 1H), 2.20 (d, J = 16 Hz, 1H), 2.01 (t, J = 12 Hz, 2H), 1.79–1.89 (m, 4H), 1.73 (d, J = 12 Hz, 1H), 1.64 (s, 1H), 1.56 (dt, J = 12 Hz, 4 Hz, 1H), 1.27 (s, 1H), 1.21 (s, 3H), 1.12–1.16 (m, 2H), 1.05 (d, J = 12 Hz, 2H), 1.02 (s, 3H); ^{13}C-NMR (CD_3OD, 100 MHz) δ 180.4, 86.9, 78.0, 76.6, 62.3, 56.5, 51.3, 45.4, 43.1, 41.2, 38.4, 38.3, 37.7, 37.6, 36.6, 34.3, 28.7, 20.9, 18.8, 17.4; HR-EI-MS (m/z): calcd. for $C_{20}H_{30}O_5$ $[M]^+$, 350.2093, found 350.2100.

*6.2.11. 11β-Hydroxy-kaur-15-oxo-16-en-19-oic acid (**11**) [25]*

To a suspension of compound **2** (17 mg, 0.05 mmol) in CH_3CN (1.0 mL) was added TEMPO (2 mg, 0.013 mmol), iodobenzene diacetate (25 mg, 0.077 mmol) and HOAc (10 mg, 0.17 mmol) successively. The mixture was stirred at room temperature for 6 h until most starting material was conversed by TLC. Then the reaction was quenched with a saturated Na_2SO_3 aqueous solution. The aqueous phase was extracted with EtOAc (10 mL × 3). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The crude product was subjected to flash chromatography on silica gel (ethyl acetate/petrol ether = 1:2) to afford compound **11** as white foams (5 mg, 72% brsm). ^{1}H-NMR (CD_3OD, 400 MHz) δ 6.30 (s, 1H), 5.76 (s, 1H), 4.54 (s, 1H), 3.87 (s, 1H), 3.56 (s, 1H), 2.93 (d, J = 12 Hz, 1H), 2.69 (d, J = 12 Hz, 1H), 2.56 (m, 1H), 2.46–2.50 (m, 3H), 2.32–2.34 (m, 3H), 1.85–2.00 (m, 5H), 1.75 (s, 3H), 1.69–1.77 (m, 4H), 1.53–1.62 (m, 3H), 1.47 (s, 3H); ^{13}C-NMR (CD_3OD, 100 MHz) δ 210.9, 180.0, 150.1, 112.1, 65.2, 62.3, 55.7, 50.4, 43.1, 40.0, 39.2, 38.5, 37.4, 36.6, 36.2, 33.5, 28.5, 19.5, 18.5, 15.1; HR-EI-MS (m/z): calcd. for $C_{20}H_{28}O_4$ $[M]^+$, 332.1988, found 332.1992.

*6.2.12. Typical procedure for compounds **12** and **16** (preparation of compound **12**)*

To a suspension of $LiAlH_4$ (14 mg, 0.37 mmol) in THF (1.0 mL) at 0 °C under N_2 was added a solution of compound **1b** (30 mg, 0.1 mmol) in THF (0.5 mL) dropwise. The mixture was warmed to the room temperature and stirred for 2 h until no starting material was detected by TLC judgment. A saturated aqueous NH_4Cl solution was added to quench the reaction and the mixture was stirred for 0.5 h. The aqueous phase was extracted with EtOAc (10 mL × 3). The combined organic layers were washed with H_2O and brine successively, dried over anhydrous Na_2SO_4 and concentrated. The crude product was subjected to flash chromatography on silica gel (ethyl acetate/petrol ether = 1:10) to afford compound **12** as white foams (24 mg, 84%).

*6.2.12.1. 19-Hydroxy-kaur-16-ene (**12**).* The structure of compound **12** was assigned by comparing the spectra data with the literature [37].

*6.2.12.2. 11β,15β,19-Trihydroxy-kaur-16-ene (**16**).* Compound **16** (30 mg, 94%) was prepared from compound **2** (33 mg, 0.1 mmol) as white solid according to the synthetic procedure described for **12**. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 4.76 (s, 1H), 4.74 (s, 1H), 3.65 (d, J = 4 Hz, 1H), 3.45 (s, 1H), 3.44 (d, J = 12 Hz, 1H), 3.06 (s, 5H), 2.31 (s, 1H), 1.70 (d, J = 12 Hz, 2H), 1.64 (m, 1H), 1.55 (d, J = 16 Hz, 1H), 1.35–1.43 (m, 1H), 1.27 (s, 1H), 1.17–1.20 (m, 2H), 1.02–1.06 (m, 2H), 0.98 (s, 3H), 0.85 (t, J = 12 Hz, 1H), 0.73–0.78 (m, 2H), 0.68 (s, 3H), 0.67 (s, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 155.8, 102.9, 65.4, 56.7, 56.1, 49.0, 44.1, 43.9, 41.5, 40.4, 39.6, 39.1, 38.6, 35.5, 33.1, 27.0, 20.4, 18.2, 18.1, 18.0; HR-EI-MS (m/z): calcd. for $C_{20}H_{32}O_3$ $[M]^+$, 320.2351, found 320.2353.

*6.2.13. Typical procedure for compounds **8b** and **13** (preparation of compound **13**)*

To a solution of compound **12** (15 mg, 0.05 mmol) in DCM (1.0 mL) at −78 °C under N_2 was added a solution of DAST (16 mg, 0.1 mmol) in DCM (0.5 mL) dropwise. The mixture was stirred for 0.5 h until no starting material was detected by TLC judgment. A saturated aqueous NH_4Cl solution was added to quench the reaction. The aqueous phase was extracted with EtOAc (10 mL × 3). The combined organic layers were washed with H_2O and brine successively, dried over anhydrous Na_2SO_4 and concentrated. The crude product was subjected to flash chromatography on silica gel (chloroform/petrol ether = 1:2) to afford compound **13** as white foams (7 mg, 48%).

*6.2.13.1. 19-Fluoro-kaur-16-ene (**13**).* ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 4.80 (s, 1H), 4.74 (s, 1H), 4.15 (dd, J = 60 Hz, 12 Hz, 1H), 3.70 (dd, J = 48 Hz, 12 Hz, 1H), 3.64 (s, 2H), 2.65 (s, 1H), 2.06 (m, 2H), 1.94 (d, J = 12 Hz, 1H), 1.88 (d, J = 12 Hz, 1H), 1.76 (m, 1H), 1.65 (m, 1H), 1.57 (s, 3H), 1.48 (m, 4H), 1.35 (m, 1H), 1.26 (s, 1H), 1.09 (d, J = 4 Hz, 1H), 1.03 (d, J = 4 Hz, 3H), 0.99 (s, 4H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 155.6, 103.0, 65.1, 64.9, 56.7, 56.0, 48.9, 47.9, 43.8, 41.3, 40.1, 39.5, 39.0, 37.4, 35.8, 35.7, 33.1, 30.9, 29.6, 27.5, 27.4, 20.3, 18.1, 18.0; HR-EI-MS (m/z): calcd. for $C_{20}H_{31}F$ $[M]^+$, 290.2410, found 290.2447.

*6.2.13.2. 15β-Fluoro-kaur-16-en-19-oic acid (**8b**).* Compound **8b** (6 mg, 49%) was prepared from compound **8a** (12 mg, 0.038 mmol) as white solid according to the synthetic procedure described for **13**. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 5.51 (d, J = 4 Hz, 1H), 5.33 (d, J = 8 Hz, 1H), 5.23 (d, J = 4 Hz, 1H), 4.97 (s, 1H), 4.86 (s, 1H), 4.62 (d, J = 56 Hz, 1H), 2.83 (s, 1H), 2.65 (d, J = 4 Hz, 2H), 2.18 (s, 1H), 2.14 (dd, J = 8 Hz, 4 Hz, 1H), 2.10 (d, J = 8 Hz, 1H), 1.92 (d, J = 16 Hz, 4H), 1.82 (dd, J = 12 Hz, 4 Hz, 4H), 1.65 (d, J = 8 Hz, 2H), 1.62 (d, J = 4 Hz, 4H), 1.55 (s, 2H), 1.45–1.52 (m, 6H), 1.37 (dd, J = 16 Hz, 8 Hz, 1H), 1.32 (s, 3H), 1.09–1.18 (m, 5H), 1.03 (d, J = 8 Hz, 2H), 0.80–0.88 (m, 5H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 169.8, 139.3, 139.2, 111.7, 111.7, 102.6, 100.8, 81.7, 80.1, 56.3, 56.2, 51.7, 48.9, 47.0, 43.5, 41.9, 40.8, 40.1, 40.0, 38.5, 37.7, 37.7, 36.2, 32.4, 29.6, 27.2, 25.2, 20.3, 18.7, 18.5, 15.2; HR-EI-MS (m/z): calcd. for $C_{20}H_{29}FO_2$ $[M]^+$, 320.2152, found 320.2144.

*6.2.14. Typical procedure for compounds **14** and **18** (preparation of compound **18**) [28,29]*

To a solution of compound **2** (33 mg, 0.3 mmol) in anisole (1.0 mL) at room temperature was added DPPA (28 mg, 0.1 mmol) and Et_3N (15 mg, 0.15 mmol), successively. The mixture was warmed to 90 °C and stirred for 1.5 h until no starting material was detected by TLC. The mixture was cooled to the room temperature, and then was subjected to flash chromatography on silica gel (ethyl acetate/petrol ether = 1:10) directly to afford compound **18** as white foams (34 mg, 100%).

*6.2.14.1. 4α-Isocyanato-11β,15β-dihydroxy-kaur-16-ene (**18**).* ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 5.09 (s, 1H), 5.01 (s, 1H), 4.01 (d, *J* = 4 Hz, 1H), 3.77 (dd, *J* = 16 Hz, 12 Hz, 2H), 2.63 (s, 1H), 2.03 (d, *J* = 16 Hz, 1H), 1.95 (d, *J* = 16 Hz, 2H), 1.76 (t, *J* = 12 Hz, 12 Hz, 5H), 1.53 (s, 2H), 1.44 (m, 2H), 1.39 (d, *J* = 4 Hz, 1H), 1.38 (s, 3H), 1.12 (m, 2H), 1.06 (s, 3H), 1.03 (d, *J* = 12 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 158.0, 121.6, 106.1, 82.2, 66.2, 59.1, 54.2, 53.7, 44.3, 43.0, 41.3, 38.9, 38.8, 37.8, 37.2, 36.0, 31.8, 19.4, 18.0, 16.5; HR-EI-MS (*m*/*z*): calcd. for $C_{20}H_{29}NO_3$ [M]$^+$, 331.2147, found 331.2144.

*6.2.14.2. 4α-Isocyanato -kaur-16-ene (**14**).* Compound **14** (26 mg, 88%) was prepared from compound **1b** (30 mg, 0.1 mmol) as white solid according to the synthetic procedure described for **18**. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 4.81 (s, 1H), 4.75 (s, 1H), 2.66 (s, 1H), 2.07 (s, 2H), 2.04 (s, 1H), 1.87 (d, *J* = 12 Hz, 1H), 1.68–1.78 (m, 3H), 1.43–1.59 (m, 9H), 1.36 (s, 3H), 1.26 (s, 2H), 1.15 (s, 3H), 1.06 (d, *J* = 4 Hz, 1H), 0.95 (d, *J* = 8 Hz, 1H), 0.77 (td, *J* = 12 Hz, 4 Hz, 4 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 155.5, 103.1, 59.1, 55.2, 55.1, 49.1, 43.9, 43.8, 41.5, 40.1, 39.9, 39.4, 39.0, 33.1, 31.8, 29.6, 19.9, 18.2, 18.0, 16.6; HR-EI-MS (*m*/*z*): calcd. for $C_{20}H_{29}NO$ [M]$^+$, 299.2249, found 299.2242.

*6.2.15. Typical procedure for compounds **15a**–**15c** and **19a**–**19m** (preparation of compound **19h**)*

To a solution of compound **18** (33 mg, 0.1 mmol) in THF (1.0 mL) at room temperature was added Et_3N (30 mg, 0.3 mmol) and cyclohexyl amino (30 mg, 0.3 mmol) successively. The mixture was stirred at room temperature for 2.5 h until no starting material was detected by TLC. The solvent was removed under vacuo. The crude product was subjected to flash chromatography on silica gel (ethyl acetate/petrol ether = 1:1.5) directly to afford compound **19h** as white foams (41 mg, 95%).

*6.2.15.1. N-(2-thiazolyl)-N-(kaur-16-ene-4α-yl) urea (**15a**).* Compound **15a** (12 mg, 84%brsm) was prepared from compound **14** (15 mg, 0.05 mmol) as white solid according to the synthetic procedure described for **19h**. Another 6 mg starting material was recovered. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.37 (d, *J* = 4 Hz, 1H), 6.79 (d, *J* = 4 Hz, 1H), 4.82 (s, 1H), 4.76 (s, 1H), 2.95 (d, *J* = 16 Hz, 1H), 2.67 (s, 1H), 2.09 (s, 2H), 1.86 (dd, *J* = 16 Hz, 8 Hz, 1H), 1.70 (d, *J* = 16 Hz, 1H), 1.61 (d, *J* = 8 Hz, 1H), 1.47 (s, 3H), 1.21 (s, 2H), 1.13–1.18 (d, *J* = 4 Hz, 3H), 1.01 (d, *J* = 8 Hz, 1H), 0.83–0.87 (m, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 162.8, 155.6, 153.7, 136.7, 110.3, 103.1, 56.5, 55.6, 48.9, 44.0, 43.8, 40.8, 40.1, 40.0, 39.0, 36.5, 33.2, 27.6, 19.7, 17.9, 17.9; HR-EI-MS (*m*/*z*): calcd. for $C_{23}H_{33}N_3OS$ [M]$^+$, 399.2344, found 399.2341.

*6.2.15.2. N-(4-Fluoro-phenyl)-N-(kaur-16-ene-4α-yl) urea (**15b**).* Compound **15b** (11 mg, 87%brsm) was prepared from compound **14** (15 mg, 0.05 mmol) as white solid according to the synthetic procedure described for **19h**. Another 5 mg starting material was recovered. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.17–7.21 (m, 2H), 6.88 (t, *J* = 8 Hz, 1H), 4.73 (s, 1H), 4.67 (s, 1H), 2.71 (d, *J* = 12 Hz, 1H), 2.56 (s, 1H), 1.99 (s, 2H), 1.88 (d, *J* = 12 Hz, 1H), 1.77 (d, *J* = 12 Hz, 1H), 1.67 (d, *J* = 12 Hz, 1H), 1.40–1.57 (m, 7H), 1.31 (s, 3H), 1.01–1.04 (m, 2H), 0.97 (s, 3H), 0.88 (d, *J* = 12 Hz, 1H), 0.73 (t, *J* = 12 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 159.7, 157.3, 155.6, 155.4, 135.3, 121.7, 121.6, 115.4, 115.2, 103.0, 56.4, 55.4, 55.0, 48.5, 43.8, 43.7, 40.7, 39.9, 39.7, 38.9, 36.4, 33.0, 27.5, 19.2, 17.8, 17.1; HR-EI-MS (*m*/*z*): calcd. for $C_{26}H_{35}N_2OF$ [M]$^+$, 410.2733, found 410.2717.

*6.2.15.3. N-(tricyclo(3.3.1.1^{3,7})-dec-1-yl)-N-(kaur-16-ene-4α-yl) urea (**15c**).* Compound **15b** (21 mg, 88%) was prepared from compound **14** (15 mg, 0.05 mmol) as white solid according to the synthetic procedure described for **19h**. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 4.80 (s, 1H), 4.75 (s, 1H), 3.91 (s, 1H), 2.74 (d, *J* = 16 Hz, 1H), 2.65 (s, 1H), 2.06 (d, *J* = 4 Hz, 5H), 1.94 (s, 3H), 1.93 (s, 3H), 1.86 (d, *J* = 12 Hz, 1H), 1.78 (d, *J* = 12 Hz, 1H), 1.65 (s, 7H), 1.60 (s, 4H), 1.56 (d, *J* = 8 Hz, 3H), 1.38–1.42 (m, 2H), 1.40 (s, 1H), 1.38 (s, 3H), 1.26 (s, 1H), 1.15 (s, 3H), 1.09 (m, 1H), 0.92 (d, *J* = 8 Hz, 1H), 0.80 (dt, *J* = 12 Hz, 4 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 156.4, 155.4, 103.1, 56.6, 55.6, 55.0, 50.8, 48.9, 44.0, 43.8, 42.5, 40.8, 40.2, 39.9, 39.1, 36.7, 36.4, 33.2, 29.5, 27.9, 19.6, 18.0, 17.9; HR-EI-MS (*m*/*z*): calcd. for $C_{30}H_{46}N_2O$ [M]$^+$, 450.3610, found 450.3613.

*6.2.15.4. N-(phenyl)-N-(11β,15β-dihydroxy-kaur-16-ene-4α-yl) urea (**19a**).* Compound **19a** (32 mg, 84%) was prepared from compound **18** (33 mg, 0.1 mmol) as white solid according to the synthetic procedure described for **19h**. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.77 (d, *J* = 8 Hz, 1H), 7.70 (t, *J* = 8 Hz, 1H), 7.43 (t, *J* = 8 Hz, 1H), 5.52 (s, 1H), 5.49 (s, 1H), 4.39 (s, 1H), 4.21 (s, 1H), 3.25 (t, *J* = 16 Hz, 1H), 3.06 (s, 1H), 2.43 (m, 2H), 2.31 (s, 2H), 2.25 (d, *J* = 16 Hz, 1H), 2.19 (m, 2H), 2.01 (s, 1H), 1.92 (d, *J* = 16 Hz, 1H), 1.86 (s, 3H), 1.61 (m, 3H), 1.47 (s, 3H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 156.6, 155.1, 138.9, 128.2, 121.8, 118.9, 105.6, 81.3, 64.7, 55.2, 54.4, 54.2, 44.0, 41.2, 38.9, 38.5, 38.0, 36.6, 36.1, 35.4, 28.2, 28.2, 26.9, 18.3, 17.2, 16.4; HR-EI-MS (*m*/*z*): calcd. for $C_{26}H_{36}N_2O_3$ [M]$^+$, 424.2726, found 424.2733.

*6.2.15.5. N-(4-Fluoro-phenyl)-N-(11β,15β-dihydroxy-kaur-16-ene-4α-yl) urea (**19b**).* Compound **19b** (19 mg, 86%) was prepared from compound **18** (17 mg, 0.05 mmol) as white solid according to the synthetic procedure described for **19h**. ^{1}H-NMR (CD_3OD, 400 MHz) δ 7.17 (dd, *J* = 8 Hz, 4 Hz, 2H), 6.87 (t, *J* = 8 Hz, 2H), 4.98 (s, 1H), 4.94 (s, 1H), 3.85 (d, *J* = 4 Hz, 1H), 3.32 (s, 1H), 2.67 (d, *J* = 12 Hz, 1H), 2.52 (d, *J* = 4 Hz, 1H), 1.85 (t, *J* = 12 Hz, 2H), 1.76 (dd, *J* = 12 Hz, 4 Hz, 2H), 1.69 (s, 1H), 1.64 (dd, *J* = 16 Hz, 4 Hz, 1H), 1.55 (d, *J* = 12 Hz, 1H), 1.46 (s, 1H), 1.35 (d, *J* = 12 Hz, 4 Hz, 2H), 1.31 (s, 3H), 1.27 (d, *J* = 4 Hz, 1H), 1.22 (m, 1H), 1.17 (s, 2H), 1.05 (m, 4H), 0.93 (d, *J* = 12 Hz, 1H), 0.89 (s, 3H); ^{13}C-NMR (MeOD, 100 MHz) δ 159.7, 157.2, 155.6, 135.3, 121.7, 115.4, 115.2, 106.2, 81.9, 65.4, 55.6, 55.1, 54.6, 44.4, 42.0, 39.4, 39.0, 38.5, 37.1, 36.6, 36.0, 29.5, 27.5, 18.8, 17.7, 17.0; HR-EI-MS (*m*/*z*): calcd. for $C_{26}H_{35}N_2O_3F$ [M]$^+$, 442.2632, found 442.2629.

*6.2.15.6. N-(4-Trifluoronmethyl-phenyl)-N-(11β,15β-dihydroxy-kaur-16-ene-4α-yl) urea (**19c**).* Compound **19c** (13 mg, 53%) was prepared from compound **18** (17 mg, 0.05 mmol) as white solid according to the synthetic procedure described for **19h**. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.40 (s, 4H), 5.02 (s, 1H), 4.98 (s, 1H), 3.91 (d, *J* = 4 Hz, 1H), 3.71 (s, 2H), 2.73 (d, *J* = 12 Hz, 1H), 2.58 (d, *J* = 8 Hz, 1H), 2.13 (s, 1H), 1.91 (m, 3H), 1.67 (m, 5H), 1.51 (s, 1H), 1.42 (d, *J* = 16 Hz, 2H), 1.35 (s, 3H), 1.20 (s, 3H), 1.02 (s, 3H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 157.5, 154.5, 143.2, 125.9, 125.9, 117.8, 106.2, 81.9, 65.4, 55.6, 55.1, 54.6, 45.0, 41.1, 40.7, 38.3, 37.9, 36.6, 36.2, 36.0, 29.6, 27.6, 18.6, 18.5, 17.6; HR-EI-MS (*m*/*z*): calcd. for $C_{27}H_{35}N_2O_3F_3$ [M]$^+$, 492.2600, found 492.2599.

*6.2.15.7. N-(Thiazol-2-yl)-N-(11β,15β-dihydroxy-kaur-16-ene-4α-yl) urea (**19d**).* Compound **19d** (11 mg, 42%) was prepared from compound **18** (17 mg, 0.05 mmol) as white solid according to the synthetic procedure described for **19h**. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.2

(d, J = 4 Hz, 1H), 6.79 (d, J = 4 Hz, 1H), 4.99 (s, 1H), 4.96 (s, 1H), 3.89 (d, J = 4 Hz, 1H), 3.70 (s, 1H), 3.28 (s, 1H), 2.72 (d, J = 12 Hz, 1H), 2.56 (d, J = 8 Hz, 1H), 2.12 (s, 1H), 1.97 (d, J = 12 Hz, 1H), 1.90 (d, J = 12 Hz, 1H), 1.78 (m, 2H), 1.69 (d, J = 12 Hz, 1H), 1.62 (d, J = 16 Hz, 1H), 1.50 (s, 2H), 1.43 (d, J = 12 Hz, 2H), 1.36 (s, 3H), 1.18 (s, 3H), 1.03 (s, 3H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 157.5, 152.8, 134.1, 111.8, 106.4, 82.1, 65.6, 55.9, 54.8, 44.8, 42.1, 39.6, 39.3, 38.7, 37.4, 36.7, 36.2, 29.0, 27.5, 19.2, 17.9, 17.47; HR-EI-MS (m/z): calcd. for $C_{23}H_{33}N_3O_3S$ $[M]^+$, 431.2243, found 431.2223.

*6.2.15.8. N-(Naphthalen-1-yl)-N-(11β,15β-dihydroxy-kaur-16-ene-4α-yl) urea (**19e**).* Compound **19e** (9 mg, 75%brsm) was prepared from compound **18** (17 mg, 0.05 mmol) as white solid according to the synthetic procedure described for **19h**. Another 8 mg starting material was recovered. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.14 (d, J = 8 Hz, 1H), 7.92 (d, J = 8 Hz, 1H), 7.85 (d, J = 8 Hz, 1H), 7.54–7.61 (m, 2H), 7.45–7.52 (m, 2H), 6.51 (s, 1H), 5.07 (s, 1H), 4.98 (s, 1H), 4.34 (s, 1H), 3.80 (d, J = 4 Hz, 1H), 3.64 (d, J = 12 Hz, 1H), 3.59 (d, J = 12 Hz, 1H), 3.23 (dd, J = 8 Hz, 16 Hz, 1H), 2.73 (d, J = 12 Hz, 1H), 2.52 (s, 1H), 2.19 (s, 1H), 1.58–1.77 (m, 8H), 1.52 (d, J = 12 Hz, 1H), 1.49 (s, 3H), 1.39 (s, 2H), 1.36–1.38 (m, 2H), 1.27 (s, 6H), 1.16 (d, J = 8 Hz, 1H), 1.14 (s, 1H), 1.11 (d, J = 8 Hz, 1H), 1.04–1.09 (m, 2H), 0.85–0.89 (m, 4H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 158.0, 134.7, 133.0, 131.1, 130.6, 129.0, 128.5, 128.1, 127.4, 127.0, 125.8, 125.2, 122.7, 106.3, 84.4, 81.5, 76.4, 55.7, 55.6, 51.3, 44.9, 41.6, 40.3, 38.4, 37.9, 36.1, 35.8, 33.9, 27.7, 20.3, 20.3, 19.3, 18.2, 17.7, 17.3; HR-EI-MS (m/z): calcd. for $C_{30}H_{38}N_2O_3$ $[M]^+$, 474.2882, found 474.2888.

*6.2.15.9. N-(Methyl)-N-(11β,15β-dihydroxy-kaur-16-ene-4α-yl) urea (**19f**).* Compound **19f** (17 mg, 97%brsm) was prepared from compound **18** (17 mg, 0.05 mmol) as white solid according to the synthetic procedure described for **19h**. ^{1}H-NMR (CD_3OD, 500 MHz) δ 5.10 (s, 1H), 5.02 (s, 1H), 4.33 (d, J = 4 Hz, 1H), 4.12 (s, 1H), 4.01 (d, J = 4 Hz, 1H), 3.86 (d, J = 8 Hz, 1H), 3.76 (d, J = 8 Hz, 1H), 2.74 (d, J = 12 Hz, 1H), 2.70 (d, J = 4 Hz, 1H), 2.63 (s, 1H), 2.06 (s, 1H), 1.93–1.96 (m, 3H), 1.77–1.86 (m, 4H), 1.62 (d, J = 12 Hz, 1H), 1.58 (s, 1H), 1.43–1.48 (m, 1H), 1.38 (s, 3H), 1.32 (d, J = 8 Hz, 1H), 1.24 (s, 1H), 1.06–1.17 (m, 4H), 1.04 (s, 3H); ^{13}C-NMR (CD_3OD, 125 MHz) δ 157.9, 157.9, 106.2, 82.2, 66.1, 55.6, 55.1, 54.3, 44.4, 43.0, 39.6, 38.9, 38.4, 37.3, 36.6, 36.1, 27.8, 27.1, 19.1, 17.8, 17.6; HR-EI-MS (m/z): calcd. for $C_{21}H_{34}N_2O_3$ $[M]^+$, 362.2569, found 362.2575.

*6.2.15.10. N-(Tert-butyl)-N-(11β,15β-dihydroxy-kaur-16-ene-4α-yl) urea (**19g**).* Compound **19g** (19 mg, 99%) was prepared from compound **18** (17 mg, 0.05 mmol) as white solid according to the synthetic procedure described for **19h**. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 5.06 (s, 1H), 4.97 (s, 1H), 3.97 (d, J = 4 Hz, 1H), 3.71 (s, 1H), 2.58 (d, J = 4 Hz, 2H), 2.11 (s, 1H), 1.88–1.95 (m, 3H), 1.69–1.73 (m, 3H), 1.57 (d, J = 20 Hz, 1H), 1.52 (s, 1H), 1.40–1.45 (m, 3H), 1.33 (s, 3H), 1.32 (s, 9H), 1.21 (s, 1H), 1.19 (s, 2H), 1.09–1.13 (m, 2H), 1.04 (s, 3H), 1.01 (s, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 157.2, 157.0, 106.0, 82.5, 76.4, 56.0, 51.3, 45.1, 41.7, 40.5, 38.3, 38.1, 36.6, 36.3, 34.0, 29.2, 29.0, 27.7, 20.2, 19.2, 19.0, 17.7; HR-EI-MS (m/z): calcd. for $C_{24}H_{40}N_2O_3$ $[M]^+$, 404.3039, found 404.3039.

*6.2.15.11. N-(Cyclohexyl)-N-(11β,15β-dihydroxy-kaur-16-ene-4α-yl) urea (**19h**).* Compound **19h** (41 mg, 95%) was prepared from compound **18** (33 mg, 0.1 mmol) as white solid according to the synthetic procedure described for **19h**. ^{1}H-NMR (MeOD, 500 MHz) δ 4.97 (s, 1H), 4.93 (s, 1H), 3.86 (d, J = 5 Hz, 1H), 3.72 (m, 6H), 3.25 (d, J = 15 Hz, 1H), 2.61 (d, J = 15 Hz, 1H), 2.51 (s, 1H), 1.90 (d, J = 10 Hz, 1H), 1.84 (d, J = 15 Hz, 1H), 1.79 (s, 2H), 1.75 (s, 2H), 1.67 (m, 4H), 1.51 (m, 1H), 1.46 (s, 1H), 1.35 (t, J = 15 Hz, 1H), 1.21 (d, J = 10 Hz, 2H), 1.01 (m, 4H), 0.97 (s, 3H), 0.92 (d, J = 15 Hz, 1H); ^{13}C-NMR (MeOD, 125 MHz) δ 157.4, 157.2, 106.0, 81.8, 65.3, 55.7, 54.7, 54.6, 44.4, 42.0, 39.4, 38.9, 38.4, 37.1, 36.8, 35.9, 33.6, 27.6, 25.3, 24.8, 18.9, 17.6, 17.1; HR-EI-MS (m/z): calcd. for $C_{26}H_{42}N_2O_3$ $[M]^+$, 430.3195, found 430.3185.

*6.2.15.12. N-(Tricyclo(3.3.1.1^{3,7})-dec-1-yl)-N-(11β,15β-dihydroxy-kaur-16-ene-4α-yl) urea (**19i**).* Compound **19i** (24 mg, 93%) was prepared from compound **18** (17 mg, 0.05 mmol) as white solid according to the synthetic procedure described for **19h**. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 5.10 (s, 1H), 5.01 (s, 1H), 4.01 (d, J = 4 Hz, 1H), 3.93 (s, 1H), 3.83 (d, J = 12 Hz, 2H), 3.75 (d, J = 12 Hz, 1H), 2.74 (d, J = 16 Hz, 1H), 2.62 (s, 1H), 2.03 (s, 3H), 1.95 (d, J = 12 Hz, 4H), 1.91 (s, 6H), 1.79 (m, 4H), 1.64 (s, 6H), 1.57 (s, 1H), 1.47 (m, 2H), 1.35 (s, 3H), 1.24 (s, 1H), 1.12 (m, 1H), 1.06 (s, 3H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 157.9, 156.9, 106.1, 82.2, 66.1, 55.7, 55.0, 54.2, 50.8, 44.4, 43.0, 42.5, 39.7, 38.8, 38.4, 37.3, 36.7, 36.3, 36.0, 29.5, 29.2, 27.9, 19.2, 17.8, 17.8; HR-EI-MS (m/z): calcd. for $C_{30}H_{46}N_2O_3$ $[M]^+$, 482.3508, found 482.3503.

*6.2.15.13. N-(Methyl, methoxy)-N-(11β,15β-dihydroxy-kaur-16-ene-4α-yl) urea (**19j**).* Compound **19j** (19 mg, 96%) was prepared from compound **18** (17 mg, 0.05 mmol) as white solid according to the synthetic procedure described for **19h**. ^{1}H-NMR (MeOD, 400 MHz) δ 5.16 (s, 1H), 5.15 (s, 1H), 4.05 (s, 1H), 3.87 (s, 1H), 3.79 (s, 3H), 3.44 (s, 1H), 3.12 (s, 3H), 2.74 (m, 2H), 2.14 (m, 2H), 2.00 (m, 3H), 1.88 (dd, J = 12 Hz, 4 Hz, 1H), 1.71 (s, 2H), 1.65 (d, J = 12 Hz, 4 Hz, 2H), 1.51 (s, 3H), 1.37 (m, 4H), 1.26 (dd, J = 12 Hz, 4 Hz, 2H), 1.23 (s, 3H), 0.98 (d, J = 8 Hz, 2H); ^{13}C-NMR (MeOD, 100 MHz) δ 161.2, 158.3, 107.5, 83.2, 66.4, 62. 5, 56.9, 56.4, 56.2, 43.0, 40.6, 40.6, 39.8, 38.5, 37.6, 37.2, 36.2, 29.9, 28.4, 20.3, 19.0, 18.5; HR-EI-MS (m/z): calcd. for $C_{22}H_{36}N_2O_4$ $[M]^+$, 392.2675, found 392.2675.

*6.2.15.14. 4-Morpholine-carboxamide,N-(11β,15β-dihydroxy-kaur-16-ene-4α-yl) urea (**19k**).* Compound **19k** (19 mg, 99%) was prepared from compound **18** (17 mg, 0.05 mmol) as white solid according to the synthetic procedure described for **19h**. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 5.10 (s, 1H), 5.02 (s, 1H), 4.39 (s, 1H), 4.00 (d, J = 4 Hz, 1H), 3.77 (s, 1H), 3.68 (t, J = 4 Hz, 2H), 3.24 (t, J = 4 Hz, 2H), 2.77 (d, J = 16 Hz, 1H), 2.63 (d, J = 4 Hz, 1H), 1.94 (d, J = 12 Hz, 3H), 1.82 (m, 3H), 1.60 (s, 1H), 1.53 (m, 3H), 1.39 (s, 3H), 1.28 (dd, J = 8 Hz, 4 Hz, 1H), 1.24 (s, 1H), 1.17 (dd, J = 16 Hz, 4 Hz, 1H), 1.10 (s, 1H), 1.07 (s, 3H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 157.7, 156.6106.2, 82.1, 66.3, 65.9, 55.6, 54.3, 44.1, 43.0, 39.4, 38.8, 38.3, 37.2, 36.1, 35.9, 27.7, 27.7, 19.1, 18.0, 17.6; HR-EI-MS (m/z): calcd. for $C_{24}H_{38}N_2O_4$ $[M]^+$, 418.2832, found 418.2838.

*6.2.15.15. 1-Piperzaine-carboxamide,N-(11β,15β-dihydroxy-kaur-16-ene-4α-yl) urea (**19l**).* Compound **19l** (16 mg, 86%) was prepared from compound **18** (17 mg, 0.05 mmol) as white solid according to the synthetic procedure described for **19h**. ^{1}H-NMR (MeOD, 500 MHz) δ 5.02 (s, 1H), 5.01 (s, 1H), 3.92 (s, 1H), 3.74 (s, 1H), 3.34 (t, J = 5 Hz, 4H), 3.30–3.31 (m, 2H), 3.14 (dd, J = 10 Hz, 5 Hz, 4H), 2.91 (dd, J = 10 Hz, 5 Hz, 3H), 2.74 (d, J = 15 Hz, 1H), 2.59 (s, 1H), 2.03 (d, J = 10 Hz, 1H), 1.97 (d, J = 10 Hz, 1H), 1.86 (s, 2H), 1.83 (d, J = 10 Hz, 1H), 1.74 (t, J = 5 Hz, 1H), 1.57 (s, 2H), 1.48 (d, J = 10 Hz, 1H), 1.42 (d, J = 10 Hz, 1H), 1.37 (s, 3H), 1.15 (td, J = 10 Hz, 5 Hz, 2H), 1.10 (s, 3H), 1.08 (d, J = 15 Hz, 2H); ^{13}C-NMR (MeOD, 125 MHz) δ 157.7, 156.7, 106.8, 82.6, 65.9, 56.7, 55.8, 47.3, 45.4, 45.2, 44.0, 42.5, 40.2, 40.0, 39.3, 38.0, 37.0, 28.1, 19.7, 18.5, 18.4; HR-EI-MS (m/z): calcd. for $C_{24}H_{40}N_4O_3$ $[M]^+$, 432.3100, found 432.3123.

*6.2.15.16. 1-Pyrrolidine-carboxamide,N-(11β,15β-dihydroxy-kaur-16-ene-4α-yl) urea (**19m**).* Compound **19m** (19 mg, 98%) was prepared from compound **18** (17 mg, 0.05 mmol) as white solid according to the synthetic procedure described for **19h**. ^{1}H-NMR (MeOD, 500 MHz) δ 4.56 (s, 1H), 4.55 (s, 1H), 3.46 (s, 1H), 3.27 (s, 1H), 2.88 (s,

1H), 2.81 (m, 5H), 2.29 (d, $J = 8$ Hz, 1H), 2.13 (d, $J = 4$ Hz, 1H), 1.58 (d, $J = 8$ Hz, 1H), 1.52 (d, $J = 8$ Hz, 1H), 1.46 (t, $J = 8$ Hz, 1H), 1.39 (m, 3H), 1.30 (dt, $J = 12$ Hz, 4 Hz, 1H), 1.18 (d, $J = 12$ Hz, 1H), 1.12 (s, 1H), 1.04 (d, $J = 12$ Hz, 1H), 0.99 (m, 1H), 0.93 (s, 3H), 0.69 (s, 3H), 0.63 (dd, $J = 8$ Hz, 4 Hz, 1H), 0.60 (d, $J = 8$ Hz, 1H); ^{13}C-NMR (MeOD, 125 MHz) δ 158.0, 157.6, 106.9, 82.8, 66.0, 56.9, 56.3, 56.1, 46.2, 45.5, 42.6, 40.3, 40.2, 39.5, 38.1, 37.1, 37.1, 28.4, 26.2, 19.8, 18.6, 18.4; HR-EI-MS (m/z): calcd. for $C_{24}H_{38}N_2O_3$ $[M]^+$, 425.2780, found 425.2782.

6.2.16. *11β,15β-dihydroxy-kaur-16-en-19-oic acid methyl ester (**17**)*

To a solution of compound **2** (17 mg, 0.05 mmol) in Et_2O (1.0 mL) at 0 °C under N_2 was added a freshly prepared solution of CH_2N_2 in Et_2O (1 N, 1.0 mL) dropwise. The mixture was stirred for 15 min until no starting material was detected by TLC judgment. Then HOAc (0.5 mL) was added to quench the reaction, and H_2O was added to dilute the mixture. The aqueous phase was extracted with EtOAc (10 mL × 3). The combined organic layers were washed with H_2O and brine successively, dried over anhydrous Na_2SO_4 and concentrated. The crude product was subjected to flash chromatography on silica gel (ethyl acetate/petrol ether = 1:4) to afford compound **17** as white foams (18 mg, 99%). The structure of compound 17 was characterized by comparing the spectra data with that reported in the literature [38].

6.3. Biological assay

6.3.1. *Scintillation proximity assay (SPA) for identification of 11β-HSD1 inhibitors [29]*

Inhibition of compounds on human 11β-HSD1 and mouse 11β-HSD1 enzymatic activities was determined by the scintillation proximity assay (SPA) using microsomes containing 11β-HSD1 according to previous studies [29]. Briefly, the full-length cDNAs of human 11β-HSD1 and mouse 11β-HSD1 were isolated from the cDNA libraries provided by NIH Mammalian Gene Collection and cloned into pcDNA3 expression vector. HEK-293 cells were transfected with the pcDNA3-derived expression plasmid and selected by cultivation in the presence of 700 μg/mL of G418. The microsomal fraction overexpressing 11β-HSD1 was prepared from the HEK-293 cells stable transfected with 11β-HSD1 and used as the enzyme source for SPA. Microsomes containing 11β-HSD1 was incubated with NADPH and [^{3}H] cortisone (Amersham), then the product, [^{3}H] cortisol was specifically captured by a monoclonal antibody coupled to protein A-coated SPA beads (GE). IC_{50} values were calculated by using Prism Version 4 (GRAPHPAD Software, San Diego, CA).

6.4. Molecular modeling of compound ***19a*** *with 11β-HSD1*

The compound **19a** was prepared (generating stereoisomers and valid single 3D conformers) by means of the Ligand Preparation module in Maestro. The crystal structures of mouse and human 11β-HSD1 were retrieved from the Protein Data Bank (PDB entry: 1Y5R and 2IRW). All crystallographic water molecules were removed from the coordinate set. Glide was used for docking. In the docking process, the standard docking score was used to rank the docking conformations. All the parameters were set as the default values. The grid-enclosing box was centered on the centroid of cocomplexed **19a** in 11β-HSD1 and defined so as to enclose residues located within 14.0 Å around the **19a** binding site, and default van der Waal scaling was used (1.0 for the receptor and 0.8 for the ligand). The RMSD between the top-scoring ligand orientation and the crystal ligand orientation was less than 2.0 A. The binding poses of compound **19a** were modeled by Glide (Schrödinger, Inc.) in SP mode with the same parameter settings [39,40].

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.05.010.

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