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Original article

# Discovery, synthesis, and structure–activity relationships of 20(*S*)-protopanaxadiol (PPD) derivatives as a novel class of AMPK$\alpha2\beta1\gamma1$ activators

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ABSTRACT

Adenosine 5′-monophosphate-activated protein kinase (AMPK) has been demonstrated as a promising drug target due to its regulatory function in glucose and lipid metabolism. 20(*S*)-protopanoxadiol (PPD) was firstly identified from high throughput screening as a small molecule activator of AMPK subtype $\alpha2\beta1\gamma1$. In order to enhance its potency on AMPK, a series of PPD derivatives were synthesized and evaluated. Structure–activity relationship study showed that the amine derivatives at the 24-position (groups I–VI) can improve the potency ($EC_{50}$: 0.7–2.3 μM) and efficacy (fold: 2.5–3.8). Among them, compounds **12** and **13** exhibited the best potency ($EC_{50}$: 1.2 and 0.7 μM) and efficacy (fold: 3.7 and 3.8). Further study suggested the mechanism of AMPK activation may functioned at the allosteric position, resulting the inhibition of the lipid synthesis in HepG2 cell model.



## 1. Introduction

Adenosine 5′-monophosphate-activated protein kinase (AMPK) is a highly-reserved serine/threonine protein kinase that is composed by a catalytic $\alpha$ subunit and two regulatory subunits, $\beta$ and $\gamma$. These subunits include multiple isoforms ($\alpha1$, $\alpha2$; $\beta1$, $\beta2$; $\gamma1$, $\gamma2$, $\gamma3$) and are expressed in various tissues and subcellular locations [1,2]. The major function of AMPK is the cellular energy regulation and maintains the energy balance in the whole body. Upon activation, AMPK stimulates ATP generation, glucose uptake and fatty acid oxidation, while it simultaneously down-regulates the ATP-consuming anabolic pathways and inhibits the syntheses of hepatic triacylglycerol, cholesterol, protein and glycogen [3–7]. Due to its central role in the regulation of body weight, systemic glucose homeostasis, lipid metabolism, and mitochondrial biogenesis [8,9], recent study postulated that whether the activation of AMPK would be a promising therapeutics against type 2 diabetes as well as other metabolic syndromes [10]. For example, two first-class antidiabetic drugs, biguanides and thiazolidinediones, were proved to be related to the AMPK activation [11,12].

Although the treatment with the root of *Panax ginseng* C.A. Meyer (Araliaceae) has been practiced as an omnipotent remedy in eastern Asia over 2000 years [13], it is still questionable that eating ginseng itself relates to cure any diseases from the viewpoint of modern medicine. However, ginsenosides, the major active constituents in ginseng, appear to be mostly responsible for the activities of ginseng, including antihyperlipidemic [14,15], antidiabetic effects [16], antioxidation [17], immunostimulation [18], antistress [19], anticancer [20], as well as the learning and memory ameliorating agents in the central nervous system [21]. Specific to the antihyperlipidemic and antidiabetic effects, ginsenosides Rb1, Re, Rg3, Rb2, Rh2, and compound K have displayed positive effects in animal models [14,22–24]; besides, some studies argued that these effects are ascribed to the AMPK activation [15,24–26]. Since 20(*S*)-protopanoxadiol (PPD, Fig. 1) is the main metabolite of protopanoxadiol type ginsenosides in rats [27], we suspected whether PPD could have positive effect on AMPK pathway. Our ensuing screen verified that PPD activated the AMPK heterotrimer $\alpha2\beta1\gamma1$ by 3.2 fold with an $EC_{50}$ of 2.5 μM. Based on this result and our previous finding in new AMPK activators from medicinal plants [28,29], we herein report a series of PPD derivatives and their biological evaluations on AMPK related pathway.

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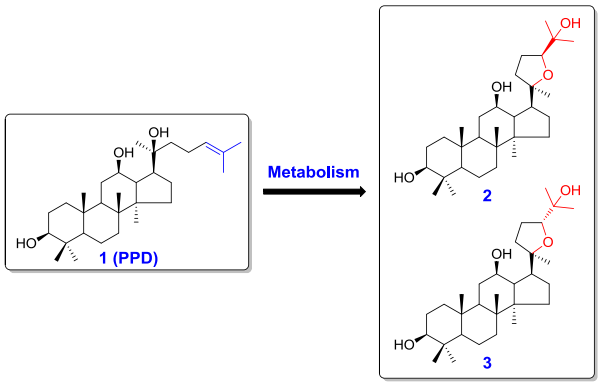



**Fig. 1.** The major metabolites of 20(*S*)-protopanaxadiol in humans.

## 2. Design and syntheses

On the basis of previous literature [30–32], the predominant metabolic pathway of PPD was the epoxidation and cyclization of the 24,25-double bond to **2** and **3** (Fig. 1). Therefore, the 24,25-double bond was primarily investigated in two methods. The major metabolites of 20(*S*)-protopanaxadiol (**2** and **3**) were firstly synthesized to test their effects on AMPK$\alpha2\beta1\gamma1$ activation (Scheme 1). Second, the double bond reduction product **4**, amide-substituted PPD derivatives (**6**–**8**), and amine-substituted PPD derivative (**9**) were synthesized to block the predominant metabolic pathway (Scheme 1). The preliminary results showed that amine-substituted PPD derivative **9** showed better potency ($EC_{50}$: 1.5 μM) and efficacy (fold: 3.3) on the activation of AMPK heterotrimer $\alpha2\beta1\gamma1$ than PPD ($EC_{50}$: 3.2 μM, fold: 2.5). We then designed a set of amine-substituted PPD derivatives **9**–**31** (Scheme 2) to further understand the structure–activity relationships. According to the chemical structure, the set is divided into six groups, including aliphatic acyclic amine (group I), aliphatic cyclic amine (group II), heterocycle-aliphatic amine (group III), polar aliphatic amine (group IV), aromatic amine (group V), and aromatic substituted aliphatic amine (group VI) groups.

## 3. Results and discussion

### 3.1. Chemistry

The synthetic route of PPD derivatives (**2**–**8**) has been shown in Scheme 1. Compounds **2** and **3** were obtained via the epoxidation with mCPBA. The relative configuration of **2** and **3** were elucidated by NMR spectroscopy with comparison of literature data [25]. Compound **4** was prepared by catalytic hydrogenation, while lactone **5** was achieved by oxidative cleavage and carboxylation with $KMnO_4$ and $NaIO_4$. Continuous aminolysis afforded the corresponding amide-substituted PPD derivatives **6**–**8**. Amine-substituted PPD derivatives **9**–**31** were conceived by ozonolysis and reducts amination with different amines.

### 3.2. Biochemistry

#### 3.2.1. Allosteric activation of AMPK$\alpha2\beta1\gamma1$ by PPD serial compounds

Two major AMPK assays were reported, homogeneous time resolved fluorescence (HTRF) and radiometric AMPK filter assay. As shown in the literature, HTRF assay is based on antigen–antibody interaction and its AMP titration curves were almost identical to the filter assays [33]. We also got similar $EC_{50}$ values of A769662 and AMP on AMPK activation using parallel HTRF and radioactive filter assays (See supporting information s1). However, HTRF assays showed higher sensitivity (For A769662 and AMP, AMPK activity increased 4.3-fold and 3.2-fold in HTRF assays, but 3.5-fold and 2-fold in Filter assays) and better adaptability in 384-wells plate high-throughput screening. Therefore, HTRF assay was employed in the evaluation of PPD derivatives (**1**–**31**). The $EC_{50}$ and efficacy values (fold) were shown in Table 1. PPD (**1**) activated the AMPK heterotrimer $\alpha2\beta1\gamma1$ by 3.2 fold with an $EC_{50}$ of 2.5 μM. PPD derivatives, including the reduced product **4**, lactone **5**, and amide-substituted PPD derivatives (**6**–**8**) had no activity. On contrary, the major metabolites **2**–**3** and amine-substituted PPD **9**-**31** showed activation of the AMPK heterotrimer *α2β1γ1*. The screening results suggested the following SAR: (a) the 24,25-double bond is significant for the activation of the AMPK heterotrimer *α2β1γ1*, since the saturated product **4** totally lost activity; (b) amide and lactone substitutions at the 23 position (**6**–**8**) also resulted in activity loss; (c) amine substitutions, including aliphatic acyclic amine (group I, **9**–**13**), aliphatic cyclic amine (group II, **14**), heterocycle-aliphatic amine (group III, **15**–**16**), polar aliphatic amine (group IV, **17**), and aromatic substituted aliphatic amine (group VI, **19**–**31**) improved the potency (0.7–2.3 vs 3.2 μM) and efficacy (fold: 2.5–3.8 vs 2.5); (d) aniline (group V) at the 24 position (**18**) slightly decreased the potency ($EC_{50}$: 5.3 vs 3.2 μM) but enhanced efficacy (fold: 3.1 vs 2.5); (e) the electrical properties of the aromatic ring (group VI, **19**–**31**) had no significant impact on activity.

Among them, the most potent compounds (**12** and **13**) in both potency ($EC_{50}$: 1.2 and 0.7 μM) and efficacy (fold: 3.7 and 3.8) were selected for further investigation.

#### 3.2.2. Activation of AMPK by *12* and *13* in HepG2 cells

After observing the allosteric activation of AMPK, we examined whether these compounds were able to activate AMPK in HepG2 cells and consistent with the profile shown on molecular level. The results exhibited the increased phosphorylation level of both AMPK and acetyl-CoA carboxylase (ACC) in a dose-dependent manner after the treatment of **12** and **13** for 1 h in HepG2 cells (Fig. 2A). Compounds the **12** and **13** presented a more potent activation of AMPK than PPD, which is consistent with their $EC_{50}$ values of AMPK activation. The cellular results also showed that the AMPK activation effects occurred at 20 μM, whereas, **13** had a higher level of AMPK phosphorylation than **12** and A769662 at 40 μM (Fig. 2B).

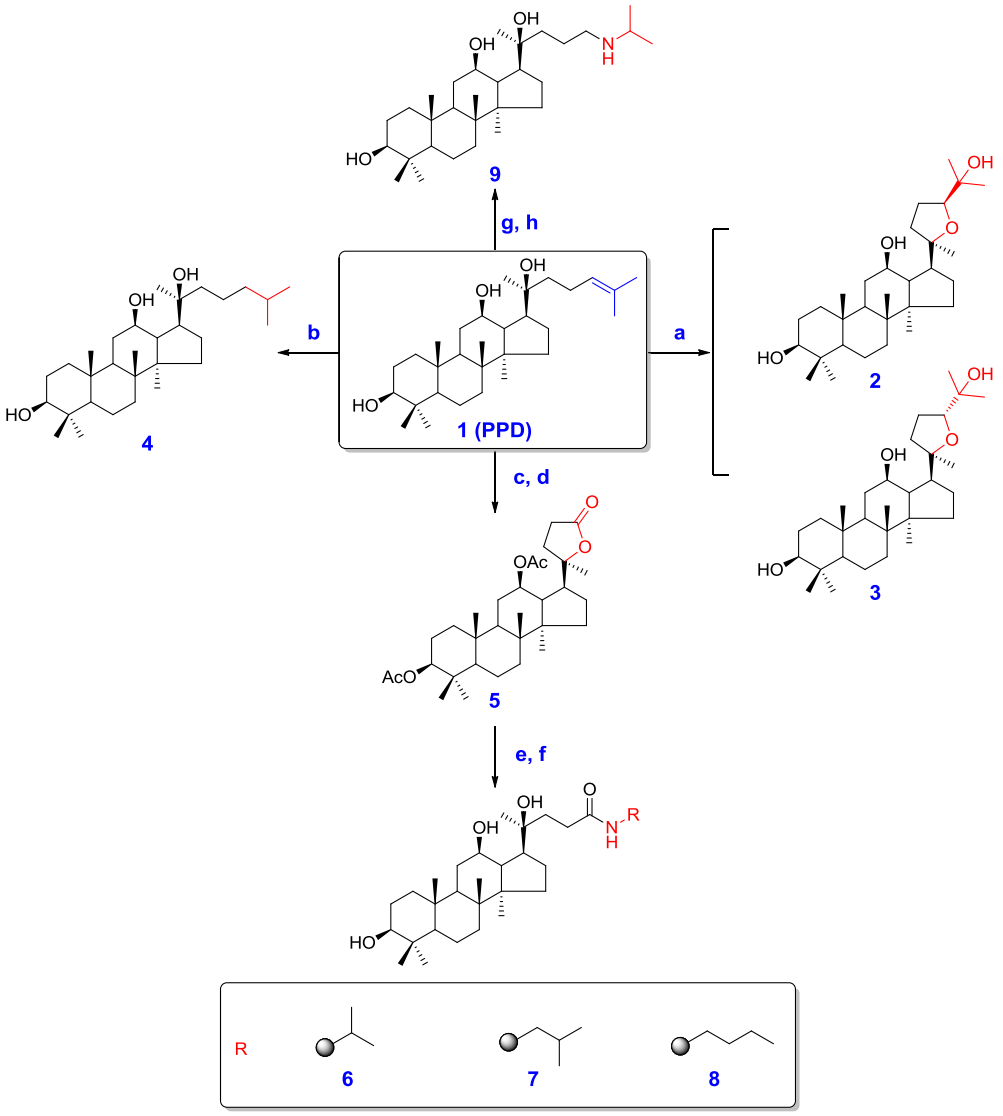
The equilibrium of AMP and ATP in cell is regulated by mitochondria and reflected by the mitochondrial membrane potential. Elevated AMP level leads to surplus AMP binding to the allosteric site of AMPK γ subunit, which is followed by AMPK activation. In our assay, PPD, **12** and **13** showed little influence on mitochondrial membrane potential (Fig. 2C), indicating that these compounds may directly binding to the AMPK allosteric position rather than affect mitochondrial function and the AMP/ATP ratio.

#### 3.2.3. *12* and *13* inhibit lipid synthesis in HepG2 cells

As ACC plays a key role in the regulation of fatty acid syntheses (FAS), activation of AMPK can down-regulated the level of malonyl-CoA by ACC phosphorylation and circuitously inhibited FAS. Alternatively speaking, the inhibition of lipid synthesis is correlated with the increased ACC phosphorylation. Since **12** and **13** were able to activate AMPK and promote ACC phosphorylation, we next confirmed they had better FAS inhibition than the parent compound **1** in HepG2 cells as well (Fig. 2D).

## 4. Conclusion

In summary, thirty PPD derivatives were synthesized and evaluated on the activation of AMPK$\alpha2\beta1\gamma1$. Improved AMPK activation




**Scheme 1.** Synthesis of compounds **2**–**9**. Reagents and conditions: (a) mCPBA, $CH_2Cl_2$, rt; (b) Pd/C, $H_2$, $CH_3OH$, rt; (c) $Ac_2O$, Py, rt; (d) $KMnO_4$, $NaIO_4$, acetone, rt; (e) $RNH_2$, THF, rt; (f) 2 N NaOH, THF; (g) $O_3$, $CH_2Cl_2$, -78 °C; (h) isopropylamine, $NaBH(OAc)_3$, 0 °C.

level was observed by introducing an amine group at 24 position. A detailed SAR studies suggested that aliphatic acyclic amine (group I, **9**–**13**), aliphatic cyclic amine (group II, **14**), heterocycle-aliphatic amine (group III, **15**, **16**), polar aliphatic amine (group IV, **17**), and aromatic substituted aliphatic amine (group VI, **19**–**31**) enhanced the activation of AMPK heterotrimer. Among all tested compounds, compounds **12** and **13** exhibited the best potency ($EC_{50}$: 1.2 and 0.7 μM) and efficacy (fold: 3.7 and 3.8). Further cellular experiments proved **12** and **13** obviously promoted AMPK activation and sequentially inhibited lipid synthesis in HepG2 cells. The unaffected mitochondrial potential suggested that their function may be possibly introduced to allosteric site of AMPK.

## 5. Experimental section

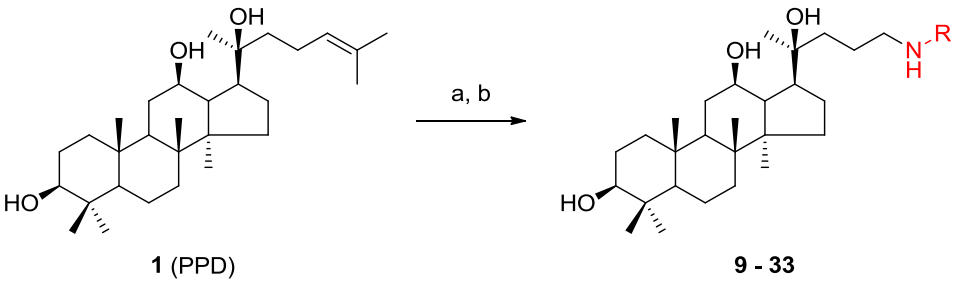
### 5.1. Chemistry

#### 5.1.1. General information

All final compounds are >95% pure based on HPLC. The reagents (chemicals) were purchased from Lancaster, Alfa Aesar, and Shanghai Chemical Reagent Co. and used without further purification. Nuclear magnetic resonance (NMR) spectroscopy was performed on Bruker AMX-300 or AMX-400 NMR (IS as TMS). Chemical shifts were reported in parts per million (ppm, $\delta$) downfield from tetramethylsilane. Proton coupling patterns were described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br). HRESIMS were determined on a Micromass Q-Tif Global mass spectrometer and ESIMS were run on a Bruker Esquire 3000 Plus Spectrometer. All reactions were monitored by thin-layer chromatography (TLC) on HSGF254 silica gel plates (150–200 μm thickness; Yantai Huiyou Co., China). Ozone was produced with a BGF-YQ ozone generator (2.0 L/min $O_2$, 100 V; Beijing ozone Co., China). Anhydrous solvents were purchased from commercial suppliers.

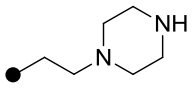
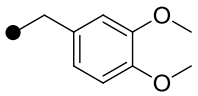
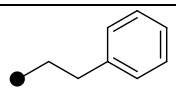
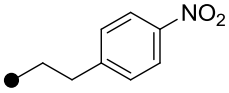
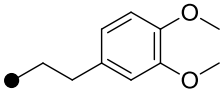
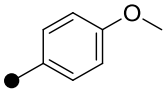
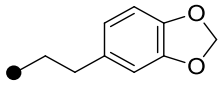
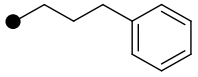
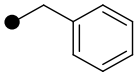
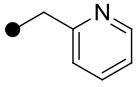
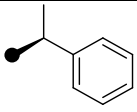
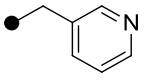
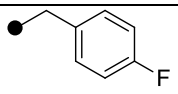
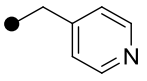
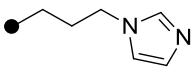
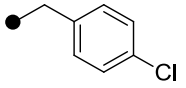
#### 5.1.2. General procedure for the synthesis of *2* and *3*

To a stirred solution of **1** (0.10 g, 0.22 mmol) in $CH_2Cl_2$ (5 mL), mCPBA (0.44 g, 0.26 mmol) was added. The resulting mixture was stirred at room temperature for 6 h. After completion of the reaction (TLC monitoring, PE/EtoAc, 1/2, V/V, on silica




**1** (PPD) **9 - 33**

Reagents and conditions: (a) $O_3$, $CH_2Cl_2$, -78 °C
(b) $RNH_2$, $NaBH(OAc)_3$, 0 °C

| Cmpd | R | | | | |
|---|---|---|---|---|---|
| | **Aliphatic Acyclic Group (I)** | **16** |  | **23** |  |
| **9** | *i*Pr | | **Polar Group (IV)** | **24** |  |
| **10** | Me | **17** | -$(CH_2)_3OH$ | **25** |  |
| **11** | Et | | **Aromatic Group (V)** | **26** |  |
| **12** | Pr | **18** |  | **27** |  |
| **13** | *t*Bu | | **Aromatic-aliphatic Group (VI)** | **28** |  |
| | **Aliphatic Cyclic Group (II)** | **19** |  | **29** |  |
| **14** |  | **20** |  | **30** |  |
| | **Heterocycle-aliphatic Group (III)** | **21** |  | **31** |  |
| **15** |  | **22** |  | | |

**Scheme 2.** Syntheses of amines derivatives **9**–**33**. Reagents and conditions: (a) $O_3$, $CH_2Cl_2$, − 78 °C, (b) $RNH_2$, $NaBH(OAc)_3$, 0 °C.

gel plate), the reaction mixture was diluted with $CH_2Cl_2$ (10 ml) and the mixture was washed by saturated $Na_2S_2O_3$ solution, saturated $Na_2CO_3$ solution, water, and brine. The organic layer was dried over anhydrous magnesium sulfate and the solvent removed under reduced pressure. The residue was purified by column chromatography (silica gel 60, 70–230 mesh ASTM, Merck) to afford **2** (41 mg, 40%) and **3** (46 mg, 45%).

*5.1.3. General procedure for the synthesis of **4***

Compound **1** (0.10 g, 0.22 mmol) was dissolved in methanol (10 mL) followed by the addition of 10% Pd–C (0.020 g) at ambient temperature. The mixture was stirred under hydrogen at 1 atm for 3 h and then filtered, concentrated under reduced pressure to give crude solid. The crude solid was purified by column chromatography (silica gel 60, 70–230 mesh ASTM, Merck) to afford 96 mg (95%) of **4** as a white powder.

**Table 1**
Allosteric activation of the AMPK heterotrimer $\alpha2\beta1\gamma1$ by PPD derivatives (**1**–**31**).[a]

| | Cmpd | $EC_{50}$ (μM) | Fold | | Cmpd | $EC_{50}$ (μM) | Fold[b] |
|---|---|---|---|---|---|---|---|
| | **1**(PPD) | 3.2 ± 0.2 | 2.5 | Polar Group (IV) | **17** | 2.0 ± 0.3 | 3.3 |
| Major Metabolites of PPD | **2** | 2.7 ± 0.5 | 3.2 | Aromatic Group (V) | **18** | 5.3 ± 0.4 | 3.1 |
| | **3** | 3.5 ± 0.1 | 3.0 | Aromatic-aliphatic Group (VI) | **19** | 1.2 ± 0.2 | 3.0 |
| Reduction Product | **4** | NA[c] | NA | | **20** | 1.9 ± 0.2 | 2.9 |
| | **5** | NA | NA | | **21** | 2.2 ± 0.5 | 3.3 |
| Amide-substituted | **6** | NA | NA | | **22** | 1.7 ± 0.3 | 3.5 |
| PPD Derivatives | **7** | NA | NA | | **23** | 1.9 ± 0.4 | 3.1 |
| | **8** | NA | NA | | **24** | 2.0 ± 0.2 | 3.5 |
| Aliphatic | **9** | 1.5 ± 0.2 | 3.3 | | **25** | 1.7 ± 0.2 | 2.5 |
| Acyclic Group (I) | **10** | 1.8 ± 0.2 | 3.2 | | **26** | 1.6 ± 0.2 | 3.1 |
| | **11** | 2.0 ± 0.4 | 3.2 | | **27** | 2.0 ± 0.2 | 2.7 |
| | **12** | 1.2 ± 0.2 | 3.7 | | **28** | 1.7 ± 0.2 | 3.3 |
| | **13** | 0.7 ± 0.2 | 3.8 | | **29** | 1.7 ± 0.2 | 2.5 |
| Aliphatic | **14** | 2.1 ± 0.2 | 3.3 | | **30** | 1.9 ± 0.3 | 3.0 |
| Cyclic Group (II) | | | | | | | |
| Heterocycle-aliphatic Group (III) | **15** | 2.1 ± 0.1 | 3.0 | | **31** | 2.3 ± 0.4 | 3.1 |
| | **16** | 1.5 ± 0.2 | 3.1 | Positive control | AMP | 1.4 ± 0.1 | 3.2 |

[a] See experimental section and supporting information S2.
[b] Fold, defined as activation fold relative to negative control.
[c] Not active, defined as Activation fold <1.5.

*5.1.4. General procedure for the synthesis of **5***

A solution of **1** (2.0 g, 4.35 mmol) in pyridine (15 mL) was mixed with $Ac_2O$ (4 mL), and the mixture was stirred at room temperature for 6 h. After evaporation of excess reagent, the residue was subjected to column chromatography on silica gel using PE/EtOAc (1/10, V/V) to yield white powder (2.25 g, 4.13 mmol, 95%). To a solution of the white powder (2.25 g, 4.13 mmol) in acetone (20 mL) was added dropwise the mixture of $KMnO_4$ (32.0 mg, 0.20 mmol), and $NaIO_4$ (2.21 g, 10.32 mmol) in acetone-$H_2O$ (7:3, 20 mL). After the reaction mixture was stirred at room temperature for 20 h, the solvent mixture was evaporated. The residue was purified by column chromatography on silica gel (PE/EtOAc, 6/1, V/V) to afford 1.27 g (60%) of **5** as a white solid.

*5.1.5. General procedure for the synthesis of **6**-**8***

A solution of **5** (0.10 g, 0.19 mmol) in THF (5 mL) was mixed with the appropriate amine ($RNH_2$, 0.21 mmol), and the mixture was stirred at room temperature for 6 h. After completion of the reaction (TLC monitoring, PE/EtoAc, 1/1, V/V, on silica gel plate), 2 N NaOH solution (5 mL) was added, and the reaction mixture was stirred at room temperature for 1 h. After evaporation of excess reagent, the residue was diluted with $CH_2Cl_2$ (10 ml) and the

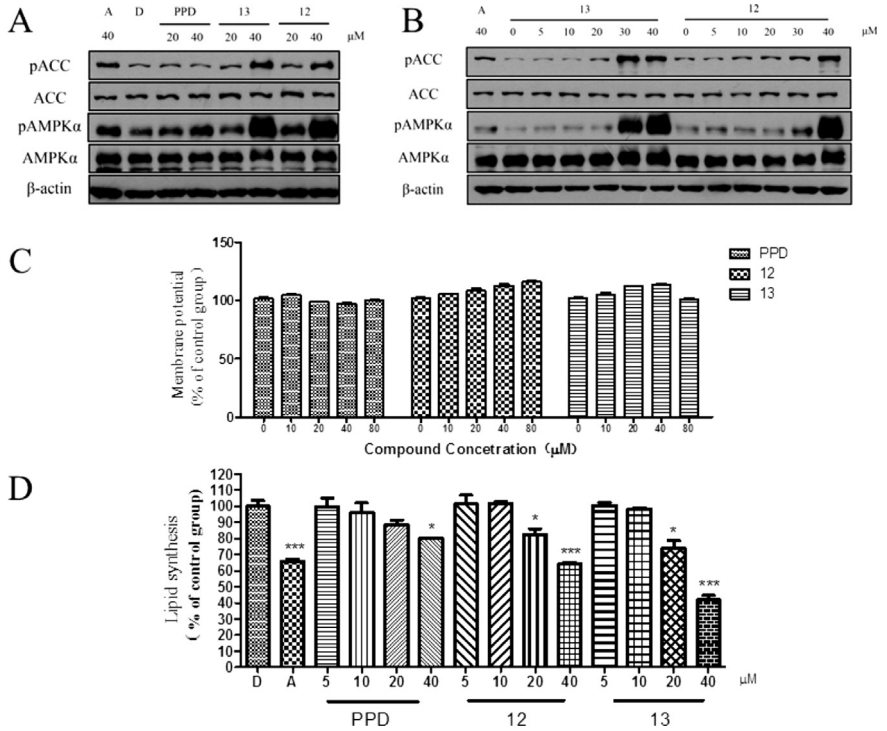



**Fig. 2.** The effects of PPD serial compounds in HepG2 cells. (A) Comparison of AMPK activation effect between PPD and its derivants in HepG2 cells after 1 h treatment. (B) Concentration response of the effects on AMPK and ACC phosphorylation due to **12** and **13** in HepG2 cells after 1 h treatment. (C) **12** and **13** did not reduce mitochondrial membrane potential in HepG2 cells after 1 h treatment ($n = 3$). (D) PPD and its derivants inhibit lipid synthesis in HepG2 cells after 6 h incubation in HepG2 cells. D is refer to DMSO as negative control and A is refer to A769662 (40 μM) as positive control ($n = 3$). *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$ compared with the negative control.

mixture was washed by water, and brine. The organic layer was dried over anhydrous magnesium sulfate and the solvent removed under reduced pressure. The residue was purified by column chromatography (silica gel 60, 70–230mesh ASTM, Merck) to afford the appropriate product (**6**–**8**).

*5.1.6. General procedure for the synthesis of **9**-**31***

Into a solution of PPD (**1**, 100 mg, 0.22 mmol) in $CH_2Cl_2$ (15 mL) and cooled to −78 °C, ozone was bubbled (at a flow rate of 2.0 L/min of oxygen containing 5% of ozone) with stirring. The mixture was maintained at −78 °C for 5 min. The reaction was monitored by thin-layer chromatography (TLC). The excess of ozone was eliminated by bubbling nitrogen into the solution. The appropriate amine ($RNH_2$) (0.1 mL), $NaBH(OAc)_3$ (368.8 mg, 1.7 mmol), and $CH_3OH$ (8 mL) were successively added, and the mixture was allowed to reach 0 °C. After completion of the reaction (TLC monitoring, $CH_2Cl_2/CH_3OH$, 30/1, V/V, on silica gel plate), water was added, and the product was isolated by extraction with dichloromethane. The organic phase was washed with water, brine, dried over anhydrous sodium sulfate, filtered, and concentrated under vacuum to obtain the desired crude products. The appropriate compounds (**9**–**31**) were obtained following purification by silica gel column chromatography.

*5.1.7. (3S,8R,10R,12R,14R,17S)-17-((2R,5S)-5-(2-Hydroxypropan-2-yl)-2-methyltetrahydrofuran-2-yl)-4,4,8,10,14-pentamethylhexadecahydro-1H-cyclopenta[a]phenanthrene-3,12-diol (**2**)*

The product was purified by flash chromatography (PE/EtOAc, 1/1, V/V) to give a white powder (41 mg, 40%). ESI(+)-MS (*m/z*): 499.9 $[M+Na]^+$; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 5.74 (s, 1H), 3.87 (dd, $J$ = 10.8, 5.4 Hz, 1H), 3.52 (td, $J$ = 10.3, 4.7 Hz, 1H), 3.19 (dt, $J$ = 10.6, 5.1 Hz, 1H), 2.25 (td, $J$ = 10.4, 4.4 Hz, 1H), 1.27 (s, 3H), 1.23 (s, 3H), 1.10 (s, 3H), 1.00 (s, 3H), 0.97 (s, 3H), 0.91 (s, 3H), 0.88 (s, 3H), 0.77 (s, 3H), 0.73 (dd, $J$ = 10.9, 2.6 Hz, 1H); $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta$ 87.4, 87.1, 78.8, 70.5, 70.0, 60.4, 55.9, 52.2, 50.2, 48.9, 48.8, 39.7, 38.9, 37.2, 34.8, 32.2, 31.7, 31.6, 28.9, 28.6, 28.0, 27.5, 25.1, 24.2, 18.3, 17.8, 16.3, 15.5, 15.3, 14.2.

*5.1.8. (3S,8R,10R,12R,14R,17S)-17-((2R,5R)-5-(2-hydroxypropan-2-yl)-2-methyltetrahydrofuran-2-yl)-4,4,8,10,14-pentamethylhexadecahydro-1H-cyclopenta[a]phenanthrene-3,12-diol (**3**)*

The product was purified by flash chromatography (PE/EtOAc, 1/1, V/V) to give a white powder (46 mg, 45%). ESI(+)-MS (*m/z*): 477.9 $[M+H]^+$; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 3.84 (dd, $J$ = 8.9, 6.6 Hz, 1H), 3.50 (td, $J$ = 10.5, 4.5 Hz, 1H), 3.18 (dd, $J$ = 11.3, 4.9 Hz), 2.18 (td, $J$ = 10.3, 9.7, 3.6 Hz, 1H), 1.27 (s, 3H), 1.26 (s, 3H), 1.09 (s, 3H), 0.97 (s, 3H), 0.94 (s, 3H), 0.89 (s, 3H), 0.84 (s, 3H), 0.76 (s, 3H), 0.72 (dd, $J$ = 11.2, 2.7 Hz, 1H); $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta$ 86.5, 85.4, 78.9, 71.0, 70.1, 55.9, 52.0, 50.5, 49.4, 47.9, 39.7, 38.9, 38.9, 37.2, 34.8, 32.6, 31.3, 31.2, 28.6, 28.0, 27.9, 27.6, 27.5, 26.1, 25.0, 18.3, 18.2, 16.3, 15.4, 15.3.

*5.1.9. (3S,8R,10R,12R,14R,17S)-17-((S)-2-hydroxy-6-methylheptan-2-yl)-4,4,8,10,14-pentamethylhexadecahydro-1H-cyclopenta[a]phenanthrene-3,12-diol (**4**)*

The product was purified by flash chromatography (PE/EtOAc, 5/1, V/V) to give a white powder (96 mg, 95%). ESI(+)-MS (*m/z*): 463.4 $[M+H]^+$; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 3.61 (tt, $J$ = 10.2, 4.6 Hz, 1H), 3.26–3.17 (m, 1H), 2.05 (td, $J$ = 10.7, 7.1 Hz, 1H), 1.94–1.81 (m, 2H), 1.78–1.72 (m, 2H), 1.19 (s, 3H), 0.99 (d, $J$ = 4.0 Hz, 6H), 0.90 (s, 3H), 0.89 (s, 9H), 0.80 (s, 3H), 0.73 (d, $J$ = 8.7 Hz, 1H); $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta$ 78.9, 74.4, 70.9, 55.8, 53.5, 51.6, 50.1, 47.7, 39.8, 39.7, 38.9, 38.9, 37.1, 35.1, 34.7, 31.2, 30.9, 28.2, 28.0, 27.4, 27.1, 26.6, 22.7, 22.6, 21.3, 18.3, 16.9, 16.1, 15.6, 15.4.

*5.1.10. (3S,8R,10R,12R,14R,17S)-4,4,8,10,14-Pentamethyl-17-((R)-2-methyl-5-oxotetrahydrofuran-2-yl)hexadecahydro-1H-cyclopenta[a]phenanthrene-3,12-diyl diacetate (**5**)*

The product was purified by flash chromatography (PE/EtOAc, 6/1, V/V) to give a white powder (1.27 g, 60%). ESI(+)-MS (*m/z*): 517.3 $[M+H]^+$; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 4.88 (td, $J$ = 10.7, 5.2 Hz, 1H), 4.49 (dd, $J$ = 11.2, 4.8 Hz, 1H), 2.70–2.50 (m, 2H), 2.31 (td, $J$ = 10.3, 4.9 Hz, 1H), 2.08 (s, 3H), 2.05 (s, 3H), 1.39 (s, 3H), 1.27 (s, 3H), 1.01 (s, 3H), 0.98 (s, 3H), 0.89 (s, 3H), 0.86 (s, 3H); $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta$ 176.5, 170.9, 170.9, 89.5, 80.5, 74.5, 55.8, 52.5, 49.7, 49.1, 46.8, 39.6, 38.5, 37.9, 37.0, 34.4, 32.5, 31.3, 29.1, 28.5, 27.9, 26.4, 24.2, 23.5, 21.9, 21.3, 18.1, 17.6, 16.5, 16.1, 15.5.

*5.1.11. (4S)-4-((3S,8R,10R,12R,14R,17S)-3,12-Dihydroxy-4,4,8,10,14-pentamethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)-4-hydroxy-N-isopropylpentanamide (**6**)*

The product was purified by flash chromatography (PE/EtOAc, 1/2, V/V) to give a white powder (69 mg, 75%). ESI(+)-MS (*m/z*): 492.6 $[M+H]^+$; $^1H$ NMR (400 MHz, $CD_3OD$): $\delta$ 3.97 (p, $J$ = 6.6 Hz, 1H), 3.56 (td, $J$ = 10.4, 5.2 Hz, 1H), 3.17 (dd, $J$ = 11.1, 5.0 Hz, 1H), 2.35 (ddd, $J$ = 14.1, 10.4, 5.4 Hz, 1H), 2.24 (ddd, $J$ = 14.1, 10.4, 6.0 Hz, 1H), 2.08 (td, $J$ = 10.7, 7.1 Hz, 1H), 1.15 (d, $J$ = 1.2 Hz, 3H), 1.14 (d, $J$ = 1.2 Hz, 3H), 1.13 (s, 3H), 1.04 (s, 3H), 0.99 (s, 3H), 0.94 (s, 6H), 0.80 (s, 3H); $^{13}C$ NMR (100 MHz, $CD_3OD$): $\delta$ 174.5, 78.1, 72.5, 70.8, 55.9, 53.8, 51.2, 50.0, 47.5, 40.9, 39.6, 38.9, 38.6, 36.8, 34.5, 30.7, 30.6, 30.4, 30.3, 27.2, 26.6, 25.9, 24.9, 21.2, 18.0, 15.7, 15.4, 14.8, 14.7.

*5.1.12. (4S)-4-((3S,8R,10R,12R,14R,17S)-3,12-dihydroxy-4,4,8,10,14-pentamethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)-4-hydroxy-N-isobutylpentanamide (**7**)*

The product was purified by flash chromatography (PE/EtOAc, 1/2, V/V) to give a white powder (74 mg, 78%). ESI(+)-MS (*m/z*): 506.4 $[M+H]^+$; $^1H$ NMR (400 MHz, $CD_3OD$): $\delta$ 3.56 (td, $J$ = 10.4, 5.1 Hz, 1H), 3.17 (dd, $J$ = 11.1, 5.1 Hz, 1H), 3.01 (dd, $J$ = 6.7, 4.7 Hz, 2H), 2.40 (ddd, $J$ = 14.0, 10.6, 5.3 Hz, 1H), 2.29 (ddd, $J$ = 14.1, 10.5, 5.8 Hz, 1H), 2.09 (td, $J$ = 10.7, 6.9 Hz, 1H), 1.14 (s, 3H), 1.04 (s, 3H), 0.99 (s, 3H), 0.94 (d, $J$ = 2.4 Hz, 6H), 0.93 (s, 3H), 0.92 (s, 3H), 0.80 (s, 3H); $^{13}C$ NMR (100 MHz, $CD_3OD$): $\delta$ 175.5, 78.1, 72.5, 70.7, 55.9, 53.8, 51.2, 50.0, 47.5, 46.6, 39.6, 38.9, 38.6, 36.8, 34.6, 30.7, 30.6, 30.6, 30.3, 28.2, 27.3, 26.6, 25.9, 24.9, 19.2, 18.1, 15.8, 15.4, 14.8, 14.8.

*5.1.13. (4S)-N-butyl-4-((3S,8R,10R,12R,14R,17S)-3,12-dihydroxy-4,4,8,10,14-pentamethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)-4-hydroxypentanamide (**8**)*

The product was purified by flash chromatography (PE/EtOAc, 1/2, V/V) to give a white powder (73 mg, 79%). ESI(+)-MS (*m/z*): 505.3 $[M + H]^+$; $^1H$ NMR (400 MHz, $CD_3OD$): $\delta$ 3.55 (m, 1H), 3.17 (m, 3H), 2.38 (ddd, $J$ = 13.9, 10.6, 5.2 Hz, 1H), 2.26 (ddd, $J$ = 14.0, 10.5, 5.9 Hz, 1H), 2.08 (m, 1H), 1.14 (s, 3H), 1.04 (s, 3H), 0.99 (s, 3H), 0.97 (t, $J$ = 7.3 Hz, 3H), 0.94 (s, 6H), 0.80 (s, 3H); $^{13}C$ NMR (100 MHz, $CD_3OD$): $\delta$ 175.4, 78.1, 72.5, 70.7, 55.9, 53.8, 51.2, 50.0, 47.5, 39.6, 38.9, 38.8, 38.6, 36.8, 34.6, 31.1, 30.7, 30.6, 30.6, 30.2, 27.3, 26.6, 25.9, 24.9, 19.7, 18.0, 15.7, 15.4, 14.8, 14.7, 12.7.

*5.1.14. (3S,8R,10R,12R,14R,17S)-17-((S)-2-hydroxy-5-(isopropylamino)pentan-2-yl)-4,4,8,10,14-pentamethylhexadecahydro-1H-cyclopenta[a]phenanthrene-3,12-diol (**9**)*

The product was purified by flash chromatography ($CH_2Cl_2/CH_3OH$/TEA, 60/1/0.5, V/V/V) to give a white powder (92 mg, 88%). ESI(+)-MS (*m/z*): 478.6 $[M+H]^+$; $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 3.57 (td, $J$ = 12.5, 6.0 Hz, 1H), 3.19 (dd, $J$ = 10.8, 5.2 Hz, 1H), 2.95 (d, $J$ = 12.6 Hz, 1H), 2.82–2.87 (m, 1H), 2.48–2.52 (m, 1H), 2.08–2.10 (m, 1H), 1.14 (s, 3H), 1.13 (d, $J$ = 7.2 Hz, 6H), 0.97 (s, 3H), 0.95 (s, 3H), 0.87 (s, 3H), 0.86 (s, 3H), 0.76 (s, 3H), 0.73 (d, $J$ = 10.9 Hz, 1H); $^{13}C$

NMR (100 MHz, $CDCl_3$): δ 79.1, 72.3, 70.5, 56.0, 53.3, 51.5, 50.2, 49.3, 48.3, 47.3, 39.9, 39.1, 37.3, 34.9, 34.3, 31.1, 30.8, 28.2, 27.6, 26.5, 24.0, 22.4, 21.4, 18.5, 16.9, 16.3, 15.9, 15.6.

#### 5.1.15. (3S,8R,10R,12R,14R,17S)-17-((S)-2-Hydroxy-5-(methylamino)pentan-2-yl)-4,4,8,10,14-pentamethylhexadecahydro-1H-cyclopenta[a]phenanthrene-3,12-diol (**10**)

The product was purified by flash chromatography ($CH_2Cl_2$/$CH_3OH$/TEA, 60/1/0.5, V/V/V) to give a white powder (83 mg, 85%). ESI(+)-MS (*m/z*): 450.7 $[M + H]^+$; $^1H$ NMR (300 MHz, $CDCl_3$): δ 4.09–4.16 (m, 1H), 3.52 (td, $J$ = 13.0, 6.2 Hz, 1H), 3.21 (dd, $J$ = 10.9, 5.0 Hz, 1H), 2.92–2.97 (m, 1H), 2.60–2.64 (m, 1H), 2.47 (s, 3H), 1.13 (s, 3H), 0.99 (s, 3H), 0.98 (s, 3H), 0.88 (s, 6H), 0.78 (s, 3H), 0.73 (d, $J$ = 11.0 Hz, 1H); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 78.8, 72.3, 70.7, 55.8, 53.3, 51.5, 50.9, 50.2, 48.1, 39.8, 38.9, 37.1, 34.8, 34.2, 32.8, 30.9, 30.8, 28.0, 27.4, 26.8, 26.4, 22.2, 18.3, 16.7, 16.2, 15.7, 15.4.

#### 5.1.16. (3S,8R,10R,12R,14R,17S)-17-((S)-5-(ethylamino)-2-hydroxypentan-2-yl)-4,4,8,10,14-pentamethylhexadecahydro-1H-cyclopenta[a]phenanthrene-3,12-diol (**11**)

The product was purified by flash chromatography ($CH_2Cl_2$/$CH_3OH$/TEA, 60/1/0.5, V/V/V) to give a white powder (87 mg, 86%). ESI(+)-MS (*m/z*): 464.7 $[M+H]^+$; $^1H$ NMR (300 MHz, $CDCl_3$): δ 3.55 (td, $J$ = 12.9, 6.3 Hz, 1H), 3.19 (dd, $J$ = 10.9, 4.9 Hz, 1H), 2.91–2.95 (m, 1H), 2.68–2.76 (m, 1H), 2.52–2.62 (m, 1H), 2.44–2.50 (m, 1H), 2.07–2.11 (m, 1H), 1.15 (s, 3H), 1.11 (t, $J$ = 7.2 Hz, 3H), 0.99 (s, 3H), 0.98 (s, 3H), 0.89 (s, 3H), 0.88 (s, 3H), 0.78 (s, 3H), 0.73 (d, $J$ = 10.7 Hz, 1H); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 78.9, 71.9, 70.2, 55.8, 52.9, 51.4, 50.0, 49.7, 48.1, 43.4, 39.8, 38.9, 37.1, 34.8, 34.3, 31.0, 30.6, 28.0, 27.5, 27.4, 26.4, 23.7, 18.3, 16.8, 16.2, 15.7, 15.4, 14.2.

#### 5.1.17. (3S,8R,10R,12R,14R,17S)-17-((S)-2-Hydroxy-5-(propylamino)pentan-2-yl)-4,4,8,10,14-pentamethylhexadecahydro-1H-cyclopenta[a]phenanthrene-3,12-diol (**12**)

The product was purified by flash chromatography ($CH_2Cl_2$/$CH_3OH$/TEA, 60/1/0.5, V/V/V) to give a white powder (91 mg, 87%). ESI(+)-MS (*m/z*): 478.7 $[M + H]^+$; $^1H$ NMR (300 MHz, $CDCl_3$): δ 3.55 (td, $J$ = 12.7, 6.2 Hz, 1H), 3.20 (dd, $J$ = 10.8, 5.2 Hz, 1H), 2.91–2.96 (m, 1H), 2.62–2.71 (m, 1H), 2.48–2.55 (m, 2H), 2.106–2.17 (m, 1H), 1.14 (s, 3H), 0.99 (s, 3H), 0.97 (s, 3H), 0.93 (t, $J$ = 7.3 Hz, 3H), 0.89 (s, 3H), 0.88 (s, 3H), 0.78 (s, 3H), 0.73 (d, $J$ = 10.9 Hz, 1H); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 78.9, 72.0, 70.4, 55.8, 53.0, 51.4, 50.8, 50.1, 49.8, 48.1, 39.8, 38.9, 37.1, 34.8, 34.1, 30.9, 30.9, 30.6, 28.0, 27.4, 27.4, 26.4, 23.4, 21.9, 18.3, 16.7, 16.2, 15.7, 15.4, 11.6.

#### 5.1.18. (3S,8R,10R,12R,14R,17S)-17-((S)-5-(Tert-butylamino)-2-hydroxypentan-2-yl)-4,4,8,10,14-pentamethylhexadecahydro-1H-cyclopenta[a]phenanthrene-3,12-diol (**13**)

The product was purified by flash chromatography ($CH_2Cl_2$/$CH_3OH$/TEA, 60/1/0.5, V/V/V) to give a white powder (95 mg, 88%). ESI(+)-MS (*m/z*): 492.7 $[M + H]^+$; $^1H$ NMR (300 MHz, $CDCl_3$): δ 5.30 (s, 1H), 3.57 (td, $J$ = 12.3, 6.1 Hz, 1H), 3.19 (dd, $J$ = 10.7, 5.3 Hz, 1H), 2.93 (d, $J$ = 10.8 Hz, 1H), 2.44 (t, $J$ = 11.5 Hz, 1H), 2.06–2.12 (m, 1H), 1.13 (s, 12H), 0.97 (s, 6H), 0.88 (s, 6H), 0.77 (s, 3H), 0.72 (d, $J$ = 10.8 Hz, 1H); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 78.9, 71.9, 70.2, 55.8, 53.1, 51.3, 50.0, 48.1, 42.5, 39.8, 38.9, 38.9, 37.2, 34.8, 34.2, 30.9, 30.6, 28.1, 28.0, 27.6, 27.5, 26.3, 24.3, 18.3, 16.7, 16.1, 15.7, 15.4.

#### 5.1.19. (3S,8R,10R,12R,14R,17S)-17-((S)-5-(Cyclopropylamino)-2-hydroxypentan-2-yl)-4,4,8,10,14-pentamethylhexadecahydro-1H-cyclopenta[a]phenanthrene-3,12-diol (**14**)

The product was purified by flash chromatography ($CH_2Cl_2$/$CH_3OH$/TEA, 60/1/0.5, V/V/V) to give a white powder (83 mg, 80%). ESI(+)-MS (*m/z*): 476.6 $[M + H]^+$; $^1H$ NMR (300 MHz, $CDCl_3$): δ 3.54 (td, $J$ = 12.6, 6.2 Hz, 1H), 3.20 (dd, $J$ = 10.7, 5.3 Hz, 1H), 3.05 (m, 1H), 2.95 (dd, $J$ = 11.0, 7.2 Hz, 1H), 2.56 (t, $J$ = 11.0 Hz, 1H), 2.09–2.13 (m, 1H), 2.05–2.09 (m, 1H), 1.11 (s, 3H), 0.97 (s, 3H), 0.96 (s, 3H), 0.87 (s, 6H), 0.76 (s, 3H), 0.72 (d, $J$ = 10.9 Hz, 1H); $^{13}C$ NMR (125 MHz, $CDCl_3$): δ 78.9, 72.4, 70.4, 55.8, 53.0, 51.4, 50.1, 50.1, 48.1, 39.8, 38.9, 37.1, 34.8, 34.1, 30.9, 30.6, 30.6, 28.0, 27.4, 27.4, 26.3, 23.7, 18.3, 16.7, 16.2, 15.7, 15.4, 5.6, 5.5.

#### 5.1.20. (3S,8R,10R,12R,14R,17S)-17-((S)-5-((3-(1H-imidazol-1-yl)propyl)amino)-2-hydroxypentan-2-yl)-4,4,8,10,14-pentamethylhexadecahydro-1H-cyclopenta[a]phenanthrene-3,12-diol (**15**)

The product was purified by flash chromatography ($CH_2Cl_2$/$CH_3OH$/TEA, 50/1/0.5, V/V/V) to give a white powder (83 mg, 70%). ESI(+)-MS (*m/z*): 544.7 $[M + H]^+$; $^1H$ NMR (300 MHz, $CDCl_3$): δ 7.49 (s, 1H), 7.05 (s, 1H), 6.92 (s, 1H), 4.00–4.04 (m, 2H), 3.55 (td, $J$ = 12.7, 6.0 Hz, 1H), 3.19 (dd, $J$ = 11.0, 5.2 Hz, 1H), 2.86–2.90 (m, 1H), 2.66–2.70 (m, 1H), 2.50–2.54 (m, 2H), 1.13 (s, 3H), 0.98 (s, 3H), 0.97 (s, 3H), 0.89 (s, 6H), 0.78 (s, 3H), 0.72 (d, $J$ = 10.9 Hz, 1H); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 137.2, 129.5, 118.8, 78.8, 72.4, 70.5, 55.8, 53.0, 51.4, 50.1, 48.1, 45.9, 44.7, 39.8, 38.9, 37.1, 34.8, 33.8, 31.0, 30.7, 30.1, 28.0, 27.4, 27.3, 26.5, 23.3, 18.3, 16.8, 16.2, 15.7, 15.4.

#### 5.1.21. (3S,8R,10R,12R,14R,17S)-17-((S)-2-Hydroxy-5-((2-(piperazin-1-yl)ethyl)amino)pentan-2-yl)-4,4,8,10,14-pentamethylhexadecahydro-1H-cyclopenta[a]phenanthrene-3,12-diol (**16**)

The product was purified by flash chromatography ($CH_2Cl_2$/$CH_3OH$/TEA, 45/1/0.5, V/V/V) to give a white powder (85 mg, 71%). ESI(+)-MS (*m/z*): 548.6 $[M+H]^+$; $^1H$ NMR (300 MHz, $CDCl_3$): δ 5.31 (d, $J$ = 1.4 Hz, 1H), 3.57 (td, $J$ = 13.0, 5.6 Hz, 1H), 3.20 (dd, $J$ = 10.9, 5.3 Hz, 1H), 2.80 (t, $J$ = 6.0 Hz, 2H), 2.46–2.48 (m, 12H), 1.14 (s, 3H), 0.98 (s, 6H), 0.89 (s, 6H), 0.78 (s, 3H), 0.73 (d, $J$ = 10.9 Hz, 1H); $^{13}C$ NMR (125 MHz, $CDCl_3$): δ 78.9, 72.1, 70.1, 59.6, 59.3, 55.8, 52.8, 52.3, 51.3, 49.9, 48.2, 39.8, 38.9, 38.9, 38.2, 37.1, 34.8, 30.9, 30.5, 28.0, 27.9, 27.4, 26.4, 20.8, 18.3, 16.7, 16.1, 15.7, 15.4.

#### 5.1.22. (3S,8R,10R,12R,14R,17S)-17-((S)-2-Hydroxy-5-((3-hydroxypropyl)amino)pentan-2-yl)-4,4,8,10,14-pentamethylhexadecahydro-1H-cyclopenta[a]phenanthrene-3,12-diol (**17**)

The product was purified by flash chromatography ($CH_2Cl_2$/$CH_3OH$/TEA, 40/1/0.5, V/V/V) to give a white powder (75 mg, 70%). ESI(+)-MS (*m/z*): 494.6 $[M+H]^+$; $^1H$ NMR (300 MHz, $CDCl_3$): δ 3.74 (t, $J$ = 5.7 Hz, 2H), 3.54 (td, $J$ = 12.6, 5.8 Hz, 1H), 3.20 (dd, $J$ = 10.8, 5.4 Hz, 1H), 2.83–2.94 (m, 2H), 2.74–2.80 (m, 1H), 2.56–2.63 (m, 1H), 1.15 (s, 3H), 0.98 (s, 3H), 0.97 (s, 3H), 0.88 (s, 3H), 0.86 (s, 3H), 0.78 (s, 3H), 0.72 (d, $J$ = 10.7 Hz, 1H); $^{13}C$ NMR (125 MHz, $CDCl_3$): δ 78.9, 72.3, 70.6, 61.7, 55.8, 53.2, 51.5, 50.2, 49.7, 48.1, 47.3, 39.6, 38.9, 37.1, 34.8, 33.2, 31.1, 30.8, 28.0, 27.4, 27.1, 26.5, 23.1, 18.3, 16.8, 16.2, 15.7, 15.4.

#### 5.1.23. (3S,8R,10R,12R,14R,17S)-17-((S)-2-Hydroxy-5-((4-methoxyphenyl)amino)pentan-2-yl)-4,4,8,10,14-pentamethylhexadecahydro-1H-cyclopenta[a]phenanthrene-3,12-diol (**18**)

The product was purified by flash chromatography ($CH_2Cl_2$/$CH_3OH$/TEA, 50/1/0.5, V/V/V) to give a white powder (107 mg, 90%). ESI(+)-MS (*m/z*): 542.6 $[M+H]^+$; $^1H$ NMR (300 MHz, $CDCl_3$): δ 6.76 (d, $J$ = 9.0 Hz, 2H), 6.68 (d, $J$ = 9.0 Hz, 2H), 3.71 (s, 3H), 3.54 (td, $J$ = 12.8, 6.3 Hz, 1H), 3.14 (dd, $J$ = 11.0, 5.2 Hz, 1H), 3.05 (t, $J$ = 6.4 Hz, 2H), 2.03–2.05 (m, 1H), 1.14 (s, 3H), 0.99 (s, 3H), 0.96 (s, 3H), 0.91 (s, 6H), 0.78 (s, 3H), 0.75 (d, $J$ = 10.6 Hz, 1H); $^{13}C$ NMR (125 MHz, $CDCl_3$): δ 152.8, 141.9, 115.4, 114.9, 78.8, 73.5, 70.9, 55.8, 55.8, 53.5, 51.6, 50.1, 47.8, 46.6, 39.7, 38.9, 38.9, 37.1, 34.8, 32.8, 31.2, 30.9, 28.0, 27.4, 27.0, 26.5, 23.9, 18.3, 16.8, 16.1, 15.7, 15.4.

*5.1.24. (3S,8R,10R,12R,14R,17S)-17-((S)-5-(Benzylamino)-2-hydroxypentan-2-yl)-4,4,8,10,14-pentamethylhexadecahydro-1H-cyclopenta[a]phenanthrene-3,12-diol (**19**)*

The product was purified by flash chromatography ($CH_2Cl_2$/$CH_3OH$/TEA, 50/1/0.5, V/V/V) to give a white powder (103 mg, 90%). ESI(+)-MS (*m/z*): 526.5 $[M+H]^+$; $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 7.30–7.32 (m, 5H), 3.74 (s, 2H), 3.57 (td, $J$ = 12.9, 6.0 Hz, 1H), 3.20 (dd, $J$ = 10.8, 5.2 Hz, 1H), 2.97 (d, $J$ = 12.3 Hz, 1H), 2.78 (dd, $J$ = 12.3, 7.2 Hz, 1H), 2.52 (t, $J$ = 11.0 Hz, 1H), 2.10–2.14 (m, 1H), 1.15 (s, 3H), 0.99 (s, 3H), 0.97 (s, 3H), 0.90 (s, 3H), 0.89 (s, 3H), 0.78 (s, 3H), 0.73 (d, $J$ = 10.7 Hz, 1H); $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta$ 138.2, 128.7, 128.4, 127.5, 78.9, 72.2, 70.2, 55.9, 53.7, 52.8, 51.4, 50.1, 48.2, 46.2, 39.8, 38.9, 37.2, 34.8, 34.5, 31.1, 30.7, 28.1, 27.7, 27.5, 26.4, 23.8, 18.3, 16.8, 16.2, 15.7, 15.4.

*5.1.25. (3S,8R,10R,12R,14R,17S)-17-((S)-2-Hydroxy-5-(((S)-1-phenylethyl)amino)pentan-2-yl)-4,4,8,10,14-pentamethylhexadecahydro-1H-cyclopenta[a]phenanthrene-3,12-diol (**20**)*

The product was purified by flash chromatography ($CH_2Cl_2$/$CH_3OH$/TEA, 50/1/0.5, V/V/V) to give a white powder (106 mg, 90%). ESI(+)-MS (*m/z*): 540.9 $[M+H]^+$; $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 7.23–7.37 (m, 5H), 3.82 (q, $J$ = 6.7 Hz, 1H), 3.56 (td, $J$ = 12.8, 5.9 Hz, 1H), 3.19 (dd, $J$ = 10.8, 5.3 Hz, 1H), 2.66–2.71 (m, 1H), 2.36–2.44 (m, 1H), 2.06–2.16 (m, 1H), 1.40 (d, $J$ = 6.7 Hz, 3H), 1.18 (s, 3H), 0.97 (s, 3H), 0.96 (s, 3H), 0.89 (s, 3H), 0.87 (s, 3H), 0.77 (s, 3H), 0.77 (d, $J$ = 10.8 Hz, 1H); $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta$ 142.6, 128.8, 127.6, 126.7, 78.9, 72.4, 70.4, 57.5, 55.9, 52.9, 51.4, 50.1, 48.1, 47.1, 39.8, 38.9, 38.9, 37.1, 34.8, 33.9, 31.0, 30.8, 28.0, 27.6, 27.4, 26.5, 23.2, 22.7, 18.3, 16.8, 16.2, 15.7, 15.4.

*5.1.26. (3S,8R,10R,12R,14R,17S)-17-((S)-5-((4-Fluorobenzyl)amino)-2-hydroxypentan-2-yl)-4,4,8,10,14-pentamethylhexadecahydro-1H-cyclopenta[a]phenanthrene-3,12-diol (**21**)*

The product was purified by flash chromatography ($CH_2Cl_2$/$CH_3OH$/TEA, 50/1/0.5, V/V/V) to give a white powder (105 mg, 88%). ESI(+)-MS (*m/z*): 544.6 $[M+H]^+$; $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 7.32 (dd, $J$ = 8.6, 5.4 Hz, 2H), 7.03 (dd, $J$ = 8.6, 5.4 Hz, 2H), 5.30 (s, 1H), 3.84 (s, 2H), 3.53 (td, $J$ = 12.9, 6.0 Hz, 1H), 3.20 (dd, $J$ = 11.0, 5.2 Hz, 1H), 2.96–3.00 (m, 1H), 2.62–2.67 (m, 1H), 2.06–2.11 (m, 1H), 1.12 (s, 3H), 0.98 (s, 6H), 0.88 (s, 6H), 0.78 (s, 3H), 0.73 (d, $J$ = 10.8 Hz, 1H); $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta$ 163.6 and 161.6 ($^1J_{CF}$ = 245 Hz), 147.1, 130.9 and 130.8 ($^3J_{CF}$ = 8.7 Hz), 115.9 and 115.7 ($^2J_{CF}$ = 21.3 Hz), 78.9, 72.6, 70.8, 55.8, 53.2, 51.7, 51.5, 50.2, 48.5, 48.1, 39.8, 38.9, 37.1, 34.8, 33.0, 31.0, 30.8, 28.0, 27.4, 26.9, 26.5, 23.1, 22.4, 18.3, 16.7, 16.2, 15.7, 15.4.

*5.1.27. (3S,8R,10R,12R,14R,17S)-17-((S)-5-((4-chlorobenzyl)amino)-2-hydroxypentan-2-yl)-4,4,8,10,14-pentamethylhexadecahydro-1H-cyclopenta[a]phenanthrene-3,12-diol (**22**)*

The product was purified by flash chromatography ($CH_2Cl_2$/$CH_3OH$/TEA, 50/1/0.5, V/V/V) to give a white powder (106 mg, 87%). ESI(+)-MS (*m/z*): 560.7 $[M+H]^+$; $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 7.29 (d, $J$ = 6.1 Hz, 2H), 7.20 (d, $J$ = 6.1 Hz, 2H), 3.84 (s, 1H), 3.71 (s, 2H), 3.56 (td, $J$ = 12.9, 5.9 Hz, 1H), 3.19 (dd, $J$ = 10.9, 5.3 Hz, 1H), 2.94 (m, 1H), 2.52 (t, $J$ = 10.1 Hz, 1H), 2.06–2.14 (m, 1H), 1.13 (s, 3H), 0.98 (s, 3H), 0.97 (s, 3H), 0.89 (s, 3H), 0.87 (s, 3H), 0.77 (s, 3H), 0.74 (d, $J$ = 10.7 Hz, 1H); $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta$ 136.4, 133.5, 129.8, 128.9, 78.9, 72.3, 70.3, 55.9, 52.9, 52.8, 51.4, 50.1, 50.0, 48.2, 39.8, 38.9, 37.1, 34.8, 34.3, 31.1, 30.7, 28.0, 27.6, 27.4, 26.5, 23.7, 18.3, 16.8, 16.2, 15.7, 15.4.

*5.1.28. (3S,8R,10R,12R,14R,17S)-17-((S)-5-((3,4-dimethoxybenzyl)amino)-2-hydroxypentan-2-yl)-4,4,8,10,14-pentamethylhexadecahydro-1H-cyclopenta[a]phenanthrene-3,12-diol (**23**)*

The product was purified by flash chromatography ($CH_2Cl_2$/$CH_3OH$/TEA, 50/1/0.5, V/V/V) to give a white powder (118 mg, 92%). ESI(+)-MS (*m/z*): 586.6 $[M+H]^+$; $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 6.93 (s, 1H), 6.86 (d, $J$ = 8.4 Hz, 1H), 6.84 (d, $J$ = 8.4 Hz, 1H), 5.30 (s, 1H), 3.89 (s, 3H), 3.87 (s, 3H), 3.82 (s, 2H), 3.53 (td, $J$ = 12.8, 6.0 Hz, 1H), 3.20 (dd, $J$ = 10.8, 5.3 Hz, 1H), 2.94–2.98 (m, 1H), 2.67–2.71 (m, 1H), 2.06–2.10 (m, 1H), 1.12 (s, 3H), 0.98 (s, 6H), 0.88 (s, 6H), 0.78 (s, 3H), 0.71 (d, $J$ = 10.8 Hz, 1H); $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta$ 149.2, 148.9, 127.2, 121.5, 112.2, 111.1, 78.9, 72.6, 70.8, 55.9, 55.9, 55.8, 53.2, 51.9, 51.5, 50.2, 48.2, 48.0, 39.7, 38.9, 37.1, 34.8, 32.7, 31.0, 30.9, 28.0, 27.4, 26.8, 26.5, 23.5, 22.2, 18.3, 16.7, 16.2, 15.7, 15.4.

*5.1.29. (3S,8R,10R,12R,14R,17S)-17-((S)-2-Hydroxy-5-(phenethylamino)pentan-2-yl)-4,4,8,10,14-pentamethylhexadecahydro-1H-cyclopenta[a]phenanthrene-3,12-diol (**24**)*

The product was purified by flash chromatography ($CH_2Cl_2$/$CH_3OH$/TEA, 50/1/0.5, V/V/V) to give a white powder (106 mg, 90%). ESI(+)-MS (*m/z*): 540.8 $[M+H]^+$; $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 7.17–7.29 (m, 5H), 3.56 (td, $J$ = 13.1, 6.1 Hz, 1H), 3.20 (dd, $J$ = 10.9, 4.9 Hz, 1H), 2.76–2.87 (m, 5H), 2.50–2.57 (m, 1H), 2.00–2.12 (m, 1H), 1.13 (s, 3H), 0.98 (s, 3H), 0.97 (s, 3H), 0.89 (s, 3H), 0.88 (s, 3H), 0.77 (s, 3H), 0.71 (d, $J$ = 10.9 Hz, 1H); $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta$ 138.7, 128.8, 128.7, 126.5, 78.9, 72.2, 70.4, 55.9, 53.0, 51.4, 50.1, 50.0, 49.7, 48.1, 39.8, 38.9, 37.1, 34.9, 34.8, 34.0, 31.0, 30.7, 28.0, 27.4, 27.3, 26.4, 23.3, 18.3, 16.8, 16.2, 15.7, 15.4.

*5.1.30. (3S,8R,10R,12R,14R,17S)-17-((S)-2-Hydroxy-5-((4-nitrophenethyl)amino)pentan-2-yl)-4,4,8,10,14-pentamethylhexadecahydro-1H-cyclopenta[a]phenanthrene-3,12-diol (**25**)*

The product was purified by flash chromatography ($CH_2Cl_2$/$CH_3OH$/TEA, 50/1/0.5, V/V/V) to give a white powder (106 mg, 83%). ESI(+)-MS (*m/z*): 585.9 $[M+H]^+$; $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 8.16 (d, $J$ = 8.8 Hz, 2H), 7.40 (d, $J$ = 8.8 Hz, 2H), 3.44–3.51 (m, 2H), 3.16–2.22 (m, 2H), 3.05–3.11 (m, 4H), 2.78–2.82 (m, 1H), 1.10 (s, 3H), 1.00 (s, 3H), 0.97 (s, 3H), 0.88 (s, 6H), 0.77 (s, 3H), 0.72 (d, $J$ = 11.0 Hz, 1H); $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta$ 147.0, 145.3, 129.7, 123.9, 78.8, 72.8, 71.3, 55.8, 53.3, 51.6, 50.3, 49.4, 49.1, 48.0, 39.8, 38.9, 38.9, 37.1, 34.7, 33.5, 32.9, 30.9, 30.8, 28.0, 27.4, 26.7, 26.5, 22.1, 18.3, 16.6, 16.2, 15.7, 15.4.

*5.1.31. (3S,8R,10R,12R,14R,17S)-17-((S)-5-((3,4-Dimethoxyphenethyl)amino)-2-hydroxypentan-2-yl)-4,4,8,10,14-pentamethylhexadecahydro-1H-cyclopenta[a]phenanthrene-3,12-diol (**26**)*

The product was purified by flash chromatography ($CH_2Cl_2$/$CH_3OH$/TEA, 50/1/0.5, V/V/V) to give a white powder (122 mg, 93%). ESI(+)-MS (*m/z*): 600.7 $[M+H]^+$; $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 6.80 (d, $J$ = 8.2 Hz, 1H), 6.73 (d, $J$ = 8.2 Hz, 1H), 6.71 (s, 1H), 5.30 (s, 1H), 3.86 (s, 3H), 3.85 (s, 3H), 3.56 (td, $J$ = 12.8, 6.0 Hz, 1H), 3.20 (dd, $J$ = 10.9, 5.3 Hz, 1H), 2.72–2.87 (m, 5H), 2.52 (t, $J$ = 9 Hz, 1H), 1.13 (s, 3H), 0.98 (s, 3H), 0.97 (s, 3H), 0.90 (s, 3H), 0.89 (s, 3H), 0.77 (s, 3H), 0.73 (d, $J$ = 11.0 Hz, 1H); $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta$ 148.9, 147.6, 131.3, 120.6, 111.9, 111.4, 78.9, 72.1, 70.3, 55.9, 55.9, 53.0, 51.4, 50.3, 50.1, 49.9, 48.1, 39.8, 38.9, 37.1, 34.8, 34.7, 34.2, 31.0, 30.7, 28.0, 27.5, 27.4, 26.4, 23.6, 18.3, 16.8, 16.2, 15.7, 15.4.

*5.1.32. (3S,8R,10R,12R,14R,17S)-17-((S)-5-((2-(Benzo[d][1,3]dioxol-5-yl)ethyl)amino)-2-hydroxypentan-2-yl)-4,4,8,10,14-pentamethylhexadecahydro-1H-cyclopenta[a]phenanthrene-3,12-diol (**27**)*

The product was purified by flash chromatography ($CH_2Cl_2$/$CH_3OH$/TEA, 50/1/0.5, V/V/V) to give a white powder (117 mg, 92%). ESI(+)-MS (*m/z*): 584.9 $[M+H]^+$; $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 6.72 (d, $J$ = 7.8 Hz, 1H), 6.65 (d, $J$ = 1.6 Hz, 1H), 6.61 (dd, $J$ = 7.8, 1.6 Hz, 1H), 5.91 (s, 2H), 3.56 (td, $J$ = 12.8, 5.8 Hz, 1H), 3.19 (dd, $J$ = 10.9, 5.3 Hz, 1H), 2.66–2.91 (m, 6H), 2.44–2.51 (m, 1H), 2.02–2.15 (m,

1H), 1.13 (s, 3H), 0.98 (s, 3H), 0.97 (s, 3H), 0.89 (s, 3H), 0.87 (s, 3H), 0.77 (s, 3H), 0.73 (d, $J$ = 10.7 Hz, 1H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 147.8, 146.2, 132.6, 121.7, 109.0, 108.4, 100.9, 78.9, 72.1, 70.3, 55.9, 52.9, 51.4, 50.2, 50.1, 49.9, 48.1, 46.0, 39.8, 38.9, 37.1, 34.9, 34.8, 34.3, 31.0, 30.7, 28.0, 27.5, 27.4, 26.4, 23.6, 18.3, 16.8, 16.2, 15.7, 15.4.

#### 5.1.33. (3S,8R,10R,12R,14R,17S)-17-((S)-2-Hydroxy-5-((3-phenylpropyl)amino)pentan-2-yl)-4,4,8,10,14-pentamethylhexadecahydro-1H-cyclopenta[a]phenanthrene-3,12-diol (**28**)

The product was purified by flash chromatography ($CH_2Cl_2$/$CH_3OH$/TEA, 50/1/0.5, V/V/V) to give a white powder (109 mg, 90%). ESI(+)-MS ($m/z$): 554.9 $[M+H]^+$; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 7.14–7.29 (m, 5H), 3.53 (td, $J$ = 13.1, 6.5 Hz, 1H), 3.19 (dd, $J$ = 10.8, 5.3 Hz, 1H), 2.88–2.93 (m, 1H), 2.49–2.80 (m, 6H), 2.04–2.09 (m, 1H), 1.10 (s, 3H), 0.98 (s, 3H), 0.97 (s, 3H), 0.88 (s, 3H), 0.87 (s, 3H), 0.72 (s, 3H), 0.72 (d, $J$ = 10.7 Hz, 1H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 141.2, 128.4, 128.3, 126.0, 78.9, 72.2, 70.4, 55.8, 53.0, 51.4, 50.1, 49.8, 48.5, 48.1, 46.1, 39.8, 38.9, 37.1, 34.8, 33.9, 33.4, 31.0, 30.7, 30.1, 28.0, 27.4, 27.3, 26.4, 23.2, 18.3, 16.7, 16.2, 15.7, 15.4.

#### 5.1.34. (3S,8R,10R,12R,14R,17S)-17-((S)-2-Hydroxy-5-((pyridin-2-ylmethyl)amino)pentan-2-yl)-4,4,8,10,14-pentamethylhexadecahydro-1H-cyclopenta[a]phenanthrene-3,12-diol (**29**)

The product was purified by flash chromatography ($CH_2Cl_2$/$CH_3OH$/TEA, 30/1/0.5, V/V/V) to give a white powder (94 mg, 82%). ESI(+)-MS ($m/z$): 527.8 $[M + H]^+$; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 8.53 (d, $J$ = 4.9 Hz, 1H), 7.64 (t, $J$ = 4.9 Hz, 1H), 7.20 (d, $J$ = 4.9 Hz, 1H), 7.16 (t, $J$ = 4.9 Hz, 1H), 3.85 (s, 1H), 3.83 (s, 1H), 3.55 (d, $J$ = 12.8, 5.9 Hz, 1H), 3.19 (dd, $J$ = 11.0, 5.1 Hz, 1H), 2.87–2.99 (m, 1H), 2.45–2.49 (m, 1H), 2.02–2.10 (m, 1H), 1.15 (s, 3H), 0.98 (s, 3H), 0.97 (s, 3H), 0.88 (s, 6H), 0.77 (s, 3H), 0.73 (d, $J$ = 10.9 Hz, 1H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 156.3, 149.4, 136.9, 122.9, 122.7, 78.9, 72.3, 70.5, 55.8, 53.6, 53.1, 51.4, 50.1, 49.7, 48.1, 39.8, 38.9, 37.1, 34.8, 33.9, 31.0, 30.7, 28.0, 27.4, 27.3, 26.4, 23.3, 18.3, 16.7, 16.2, 15.7, 15.4.

#### 5.1.35. (3S,8R,10R,12R,14R,17S)-17-((S)-2-Hydroxy-5-((pyridin-3-ylmethyl)amino)pentan-2-yl)-4,4,8,10,14-pentamethylhexadecahydro-1H-cyclopenta[a]phenanthrene-3,12-diol (**30**)

The product was purified by flash chromatography ($CH_2Cl_2$/$CH_3OH$/TEA, 30/1/0.5, V/V/V) to give a white powder (93 mg, 81%). ESI(+)-MS ($m/z$): 527.8 $[M + H]^+$; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 8.52 (s, 1H), 8.43 (d, $J$ = 4.7 Hz, 1H), 7.85 (d, $J$ = 7.4 Hz, 1H), 7.41 (dd, $J$ = 7.4, 4.7 Hz, 1H), 3.79 (s, 2H), 3.53 (d, $J$ = 13.1, 5.7 Hz, 1H), 3.14 (dd, $J$ = 10.8, 5.2 Hz, 1H), 2.60–2.65 (m, 2H), 1.99–2.05 (m, 1H), 1.14 (s, 3H), 1.00 (s, 3H), 0.96 (s, 3H), 0.91 (s, 6H), 0.77 (s, 3H), 0.74 (d, $J$ = 10.7 Hz, 1H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 149.6, 149.1, 136.1, 133.8, 123.8, 78.9, 72.4, 70.2, 55.9, 52.8, 51.4, 51.1, 50.3, 50.0, 48.2, 39.8, 38.9, 37.1, 34.8, 34.4, 31.1, 30.7, 28.0, 27.7, 27.4, 26.5, 23.9, 18.3, 16.8, 16.2, 15.7, 15.4.

#### 5.1.36. (3S,8R,10R,12R,14R,17S)-17-((S)-2-Hydroxy-5-((pyridin-4-ylmethyl)amino)pentan-2-yl)-4,4,8,10,14-pentamethylhexadecahydro-1H-cyclopenta[a]phenanthrene-3,12-diol (**31**)

The product was purified by flash chromatography ($CH_2Cl_2$/$CH_3OH$/TEA, 30/1/0.5, V/V/V) to give a white powder (93 mg, 81%). ESI(+)-MS ($m/z$): 527.7 $[M + H]^+$; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 8.56 (dd, $J$ = 4.4, 1.6 Hz, 2H), 7.23 (dd, $J$ = 4.4, 1.6 Hz, 2H), 3.77 (s, 2H), 3.58 (td, $J$ = 12.9, 5.8 Hz, 1H), 3.21 (dd, $J$ = 11.0, 5.3 Hz, 1H), 2.94–2.98 (m, 1H), 2.52–2.60 (m, 1H), 2.08–2.18 (m, 1H), 1.17 (s, 3H), 0.99 (s, 3H), 0.98 (s, 3H), 0.89 (s, 3H), 0.87 (s, 3H), 0.78 (s, 3H), 0.78 (d, $J$ = 10.8 Hz, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 150.1, 146.9, 123.1, 78.9, 72.5, 70.3, 55.8, 52.8, 52.4, 51.4, 50.2, 50.0, 48.1, 39.8, 38.9, 37.1, 34.8, 34.1, 31.0, 30.7, 28.0, 27.5, 27.4, 26.5, 23.7, 18.3, 16.8, 16.2, 15.7, 15.4.

### 5.2. Biology

#### 5.2.1. Recombinant AMPK heterotrimers construction, expression and purification

The coding sequences of different $\alpha/\beta/\gamma$ subunit that form AMPK$\alpha2\beta1\gamma1$ heterotrimers were amplified from cDNA of each human AMPK subunit and constructed into pET28b vector, and then expression and purified as previously described [34].

#### 5.2.2. Phosphorylation and activation of AMPK

AMPK protein was completely phosphorylated by incubation with CaMKK$\beta$ at 30 °C for 4 h as previously described [35]. The AMPK activity and the activation of compounds on AMPK were test with the STK substrate 1-biotin, XL-665 and STK-Antibody of HTRF® KinEASE™-STK1 Kit. The reaction was carried out in 384-well plates in a reaction volume of 10 μL containing 32 mmol/l Tris–HCl, pH 7.5, 4 mmol/l $MgCl_2$, 0.8 mmol/l DTT, 160 μmol/l substrate-1 peptide and 4 μmol/l ATP. The reaction was initiated by addition of 1.6 nmol/l recombinant AMPK into the well, followed by incubation for 45 min at 30 °C. The reaction were terminated by addition of detection reagent contains 57.5 nmol/l XL-665 and STK-Antibody labeled with $Eu^{3+}$-Cryptate. The fluorescence is measured at 615 nm (Cryptate) and 665 nm (XL665). A ratio is calculated (665/620) for each well and represents the AMPK activity.

#### 5.2.3. Cell culture and compound treatment

Cell culture was performed as previously described [36]. Before compound incubation, the cells were deprived of serum for 2 h, then incubated with compounds of indicated concentration for indicated time. Before Western blotting analysis, the cells were washed with phosphate-buffered saline and lysed using SDS-PAGE loading buffer.

#### 5.2.4. Western blot

Samples were subjected to 8% SDS-PAGE, and proteins were transferred onto polyvinylliidene difluoride membranes. The amount of protein and the level of phosphorylation level were detected by the respective antibodies.

#### 5.2.5. Lipid synthesis

The experimental procedure is adaptation of the method described previously [37]. HepG2 cells were plated at $4 \times 10^4$ cells per well on white-walled 96-well plates in HG-DMEM supplemented with 10% FBS. Cells were deprived of serum for 2 h followed by a 6hr compound incubation with serum-free medium containing $^{14}$C acetic sodium (0.1 $\mu$Ci/ml, Perkin Elmer). The plates were rinsed with cold PBS, The final wash was replaced with 0.25 M NaOH, after protein concentration measurement, the Microscint20 was added into wells and radioactivity incorporated into lipid was monitored on a Wallac Microbeta plate reader and amended with protein concentration.

#### 5.2.6. Mitochondria membrane potential assay

HepG2 cells were plated at $4 \times 10^4$ cells per well on black-walled 96-well plates in HG-DMEM supplemented with 10% FBS, Cells were deprived of serum for 2 h followed by a 1hr compound incubation with serum-free medium, after incubation, 100 μL fresh medium containing 0.2 μg JC-1 was added to each well and incubated for another 30 min, the plates were rinsed three times with 100 μL Krebs–Ringer phosphate HEPES buffer. Fluorescence was measured as described previously.

*5.2.7. Statistical analysis*

The results are presented as the mean ± SEM. The differences between the two groups were analyzed using paired student's *t*-test. $P < 0.05$ was regarded as statistically significant.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.ejmech.2014.04.010. These data include MOL files and InChiKeys of the most important compounds described in this article.

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