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Synthesis, characterization and cytotoxic activity evaluation of ginsengdiol oxidation and nitrogen hybrid derivatives†

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Panaxadiol (PD), a diol-type ginseng saponin, with a dammarane skeleton plays a potential role in the apoptosis of tumor cells. In this study, 28 oxidation and nitrogen hybrid derivatives of PD were synthesized, of which 20 were novel compounds. All the obtained compounds were screened for their cytotoxic activity in six cell lines. As compared with the positive control, some compounds showed better anti-proliferative activities while having much weaker effect on the growth of normal cells. Among them, ring-A fused pyrazoline of PD (**1j**) displayed impressive cytotoxic activity with IC_{50} 9.62 ± 1.34, 11.65 ± 1.71, and 13.45 ± 1.60 μM against A549, HeLa and 8901 cell lines, respectively. Additionally, compound **2f** exhibited the most potent activity with an IC_{50} value of 8.93 ± 1.11 μM against cell line A549. Therefore, our results indicated that **1j** and **2f** can be promising lead candidates for further studies.

Introduction

Ginseng has been traditionally used as a medicine in Eastern Asia for thousands of years. Ginsenosides are the main bioactive components of *Panax ginseng*, which own a range of biological activities including anticancer,[1–3] antioxidative,[4] immunomodulatory,[5] analgesic and anti-inflammatory.[6,7] Especially regarding cancer chemoprevention, ginsenosides are considered to be the main bioactive constituents.[8] It is classified into three subclasses: panaxadiol, panaxatriol, and oleanic acid-type ginsenosides.[9] Moreover, recent studies have found that the protopanaxadiol saponin possessed excellent anti-tumor activity with low toxicity and side effects.[10] In our previous studies, we found that many panaxadiol derivatives possessed more potent anti-tumor activities, such as 25-methoxylprotopanaxadiol and 25-hydroxyprotopanaxadiol.[11,12]

Panaxadiol (PD), a diol-type ginseng saponin, with a dammarane skeleton plays a potential role in the apoptosis of tumor cells.[13,14] It has been reported that PD in combination with anti-cancer drugs such as cyclophosphamide[15] and 5-fluorouracil[16] could significantly increase its anti-tumor activity in human cancer cell lines. Moreover, previous studies have shown that PD derivatives (3β-acetoxy-PD, 3β-palmitic acid aceloxy-PD and 3β-octadecanoic-PD) possessed more potent anti-tumor activity than PD.[17] Additionally, synthetic panaxadiol monoside possesses higher physiological activity than the original panaxadiol.[18] Therefore, PD derivatives as potential effective anti-tumor agents need to be developed.

In our previous study, some modified ginsenosides with a series of small molecular compounds were obtained, and some of them displayed potent or similar cytotoxic activities. Based on the structure–activity relationship of these derivatives, we concluded that C-3 was an active site of dammarane-type sapogenins and the hydroxyl substitutions at C-3 were crucial for biological activity.[19,20] Among the natural products, nitrogen heterocyclic compounds mainly distributed in sea worms possess outstanding cytotoxic effects.[21] It was reported that pyrazine and benzopyrazine compounds have significant antitumor effects.[22] Interestingly, the azole compounds of betulinic acid were extremely sensitive to HCT116 cells and had a low effect on human normal cells.[23] Mallavadhani *et al.* found that pyrimidine compounds possessed general inhibitory activity in breast cancer cells during the structural modification of oleanolic acid, and its activity was superior to the positive drug etoposide.[24] Meanwhile, it was reported that the oxidation and A ring-opening products of betulinic acid exhibited good activity in inhibiting tumor proliferation, while the oxidation product of PD and the ring-opening product of A ring can inhibit HIV and HCV protease.[25,26] Therefore, we have carried out a series of beneficial synthetic

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reactions on the available PD in order to improve its anti-tumor activity.

Results and discussion

Screening for cytotoxicity of the tested compounds (Table 1) in U87 cell line revealed that compounds **1a**, **3**, and **3d** were more potent than the positive control. Compound **3** showed stronger anti-proliferative activity with IC_{50} = 19.51 ± 1.00 μM. In addition, it exhibited more potent anti-cancer activities than panaxadiol, similar to the results of previous studies.[12] The values obtained for other compounds showed weak activity.

Data on the MCF-7 cell line showed similar results. Compounds **1a**, **1j**, and **2** showed superior anti-tumor activity than 5-fluorouracil (29.4 ± 0.70 μM) and the parent compound PD (52.62 ± 2.39 μM) with IC_{50} values of 17.73–23.58 μM. IC_{50} values of other compounds were greater than 50 μM.

For 8901 and A549, compounds **1j**, **1k**, and **2f** displayed excellent anti-proliferative activity than 5-fluorouracil (23.5–33.27 μM), with IC_{50} values of 8.93–13.45 μM. In particular, compound **2f** (8.93 ± 1.11 μM) possessed excellent activity, which was 2.6-fold that of the positive control 5-fluorouracil.

For HeLa, compounds **1j**, **1k**, **2**, and **3b** exhibited better activity with IC_{50} values of 11.65–19.60 μM, wherein compound **1j** showed an anti-proliferative activity which is 2.5-fold that of 5-fluorouracil. In addition, most of the compounds showed low toxicity in IOSE144 cell line.

In this study, 28 oxidation and ring-A fused nitrogen heterocycle derivatives of PD were designed, synthesized, and evaluated for their anti-tumor activity. Some of these compounds displayed potent or similar cytotoxic activity *in vitro* compared with PD and 5-fluorouracil. Interestingly, among the synthesized compounds, the pyrazoline compound **1j** showed much higher activity than the other derivatives with IC_{50} values of 9.62 ± 1.34, 11.65 ± 1.71, and 13.45 ± 1.60 μM against A549, HeLa, and 8901 cell lines, respectively. We speculated that it was related with its special ring-A fused pyrazoline structure. Furthermore, according to the SAR analysis, the pyrazine compound (**2f**) with C (17)-fused chain fragment owned excellent antiproliferative activity with IC_{50} 8.93–17.73 μM than C (17)-fused cycle fragment (**1d**). Moreover, the effect of compound **2f** was greatly improved compared to **2g**, which may be due to its C-12 position being attached to the hydroxyl group instead of the carbonyl group. In addition, compared with the parent compound PD, the activity of **1k** underwent beneficial changes due to its C-2 position linked with the cyano group.

Table 1 Anti-proliferative activity (IC_{50} ± SD values, μM, 48 h) of compounds in different human tumor cell lines

		IC_{50} (μM)					
No.	Compounds	U87	MCF-7	8901	A549	HeLa	IOSE144
1	**1**	89.99 ± 4.01	78.06 ± 2.87	>100	>100	66.43 ± 3.56	>100
2	**1a**	20.17 ± 1.01	23.58 ± 2.34	50.11 ± 3.11	39.99 ± 2.88	25.03 ± 1.98	>100
3	**1b**	>100	>100	>100	>100	>100	>100
4	**1c**[b]	76.43 ± 3.23	91.90 ± 7.72	>100	86.36 ± 3.97	>100	>100
5	**1d**[b]	>100	>100	>100	>100	>100	>100
6	**1e**	>100	>100	>100	>100	>100	>100
7	**1f**[b]	>100	>100	>100	>100	>100	>100
8	**1g**[b]	>100	>100	>100	>100	>100	>100
9	**1h**[b]	30.55 ± 1.23	28.31 ± 2.03	30.12 ± 2.52	18.47 ± 2.67	31.92 ± 3.00	>100
10	**1i**[b]	>100	>100	>100	67.35 ± 4.55	>100	>100
11	**1j**[b]	26.23 ± 2.69	18.90 ± 1.42	13.45 ± 1.60	9.62 ± 1.34	11.65 ± 1.71	>100
12	**1k**[b]	41.60 ± 1.19	29.65 ± 3.34	25.68 ± 1.28	12.93 ± 1.67	12.69 ± 2.56	>100
13	**1m**[b]	82.64 ± 3.14	27.95 ± 1.60	45.69 ± 3.09	26.89 ± 2.99	69.39 ± 3.35	>100
14	**1n**[b]	86.34 ± 2.77	82.95 ± 4.74	>100	>100	>100	>100
15	**2**	25.06 ± 1.40	49.58 ± 5.42	46.47 ± 2.72	27.91 ± 2.14	19.60 ± 2.23	>100
16	**2a**	>100	65.10 ± 2.87	>100	89.98 ± 4.29	>100	>100
17	**2b**[b]	>100	>100	66.48 ± 3.57	50.57 ± 2.72	>100	>100
18	**2c**	>100	66.62 ± 3.64	>100	25.74 ± 3.54	>100	>100
19	**2d**[b]	45.39 ± 2.48	46.22 ± 4.05	87.83 ± 5.61	24.31 ± 3.88	50.92 ± 2.68	>100
20	**2e**[b]	>100	>100	>100	>100	>100	>100
21	**2f**[b]	38.09 ± 2.09	17.73 ± 1.82	13.15 ± 2.11	8.93 ± 1.11	>100	>100
22	**2g**[b]	>100	39.10 ± 2.11	>100	19.70 ± 2.51	>100	>100
23	**3**	19.51 ± 1.00	44.59 ± 2.14	38.53 ± 1.90	29.28 ± 2.28	29.36 ± 3.06	>100
24	**3a**[b]	>100	98.51 ± 6.63	>100	>100	>100	>100
25	**3b**[b]	32.74 ± 1.38	44.90 ± 2.89	81.32 ± 3.12	37.12 ± 6.59	18.03 ± 1.99	>100
26	**3c**[b]	>100	50.34 ± 2.94	78.60 ± 2.87	39.94 ± 1.11	>100	>100
27	**3d**[b]	20.71 ± 1.58	35.69 ± 1.25	25.91 ± 3.33	21.57 ± 1.20	39.00 ± 2.79	>100
28	**3e**[b]	>100	>100	>100	85.00 ± 2.83	>100	>100
29	**PD**	>100	52.62 ± 2.39	>100	44.57 ± 3.01	>100	>100
30	**5-Fu**[a]	22.00 ± 0.98	29.41 ± 0.70	33.27 ± 1.37	23.57 ± 1.80	28.22 ± 1.91	34.80 ± 2.23

[a] Positive control. [b] Novel compound.

Since few studies on oxidation and nitrogen hybrid or SAR in tetracyclic triterpene dammarane and as PD in previous study showed very low cytotoxicity against human normal cells, this study may provide useful data for researching and developing the study on structure–activity relationship of anticancer agents.

Experimental section

General

20(*R*)-20,25-Epoxy-12β-hydroxydammaran-3-one (1). Yield: 85.4%, Mp: 228–230 °C, $[\alpha]_D^{20}$ + 22.5 (0.10, MeOH); ESI-MS: *m*/*z* 459.4 $[M + H]^+$; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.55 (1H, td, *J* = 10.3, 5.0 Hz, H-12), 1.27 (3H, s, CH_3), 1.22 (3H, s, CH_3), 1.19 (3H, s, CH_3), 1.08 (3H, s, CH_3), 1.04 (3H, s, CH_3), 1.02 (3H, s, CH_3), 0.98 (3H, s, CH_3), 0.89 (3H, s, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 39.80 (C-1), 34.28 (C-2), 218.24 (C-3), 47.51 (C-4), 55.44 (C-5), 19.53 (C-6), 34.32 (C-7), 39.83 (C-8), 49.36 (C-9), 36.93 (C-10), 31.02 (C-11), 69.97 (C-12), 49.36 (C-13), 51.34 (C-14), 31.26 (C-15), 25.26 (C-16), 54.78 (C-17), 15.47 (C-18), 16.07 (C-19), 77.16 (C-20), 19.80 (C-21), 35.84 (C-22), 16.38 (C-23), 36.55 (C-24), 73.33 (C-25), 33.14 (C-26), 27.24 (C-27), 26.80 (C-28), 21.15 (C-29), 17.09 (C-30).

20(*R*)-20,25-Epoxy-2,12β-dihydroxydammaran-1-en-3-one (1a). Yield: 93.6%, Mp: 195–198 °C, $[\alpha]_D^{20}$ + 23.0 (0.10, MeOH); ESI-MS: *m*/*z* 473.4 $[M + H]^+$; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.52 (1H, s, H-1), 3.56 (1H, td, *J* = 10.4, 5.0 Hz, H-12), 1.27 (3H, s, CH_3), 1.23 (3H, s, CH_3), 1.20 (3H, s, CH_3), 1.19 (3H, s, CH_3), 1.18 (3H, s, CH_3), 1.12 (3H, s, CH_3), 1.04 (3H, s, CH_3), 0.88 (3H, s, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 129.02 (C-1), 144.03 (C-2), 201.29 (C-3), 44.18 (C-4), 54.72 (C-5), 19.51 (C-6), 34.52 (C-7), 40.67 (C-8), 45.16 (C-9), 38.73 (C-10), 30.62 (C-11), 69.72 (C-12), 49.40 (C-13), 51.38 (C-14), 31.11 (C-15), 25.23 (C-16), 54.61 (C-17), 16.18 (C-18), 18.89 (C-19), 77.16 (C-20), 20.34 (C-21), 35.83 (C-22), 16.37 (C-23), 36.55 (C-24), 73.35 (C-25), 33.16 (C-26), 27.30 (C-27), 27.28 (C-28), 21.71 (C-29), 17.05 (C-30).

20(*R*)-20,25-Epoxy-2,3-seco-12β-hydroxydammaran-2,3-dioic acid (1b). Yield: 40.3%, Mp: 219–221 °C, $[\alpha]_D^{20}$ + 17.2 (0.10, MeOH); ESI-MS: *m*/*z* 505.2 $[M - H]^-$; ^{1}H-NMR (400 MHz, pyridine-d_5): δ 3.90 (1H, td, *J* = 10.1, 4.8 Hz, H-12), 1.61 (3H, s, CH_3), 1.55 (3H, s, CH_3), 1.25 (3H, s, CH_3), 1.23 (3H, s, CH_3), 1.21 (3H, s, CH_3), 1.17 (3H, s, CH_3), 1.10 (3H, s, CH_3), 1.04 (3H, s, CH_3); ^{13}C-NMR (100 MHz, pyridine-d_5): δ 43.44 (C-1), 174.83 (C-2), 182.72 (C-3), 47.47 (C-4), 49.17 (C-5), 20.05 (C-6), 34.94 (C-7), 40.31 (C-8), 41.99 (C-9), 42.55 (C-10), 31.10 (C-11), 70.60 (C-12), 50.46 (C-13), 52.23 (C-14), 32.60 (C-15), 25.80 (C-16), 55.15 (C-17), 16.07 (C-18), 22.17 (C-19), 77.22 (C-20), 20.83 (C-21), 36.10 (C-22), 16.83 (C-23), 36.86 (C-24), 73.32 (C-25), 33.51 (C-26), 27.74 (C-27), 28.05 (C-28), 25.32 (C-29), 17.78 (C-30).

20(*R*)-12,25-Epoxy-20-hydroxydammaran-3-one (1c). Yield: 10.2%, Mp: 196–199 °C, $[\alpha]_D^{20}$ + 34.0 (0.07, MeOH); HR-ESI-TOF-MS: *m*/*z* 481.3656 $[M + Na]^+$ (calcd for $C_{30}H_{50}NaO_3$, 481.3760); ^{1}H-NMR (600 MHz, $CDCl_3$): δ 3.65 (1H, td, *J* = 10.3, 5.0 Hz, H-12), 1.59 (6H, s, 2 × CH_3), 1.18 (3H, s, CH_3), 1.09 (3H, s, CH_3), 1.05 (3H, s, CH_3), 1.04 (3H, s, CH_3), 0.99 (3H, s, CH_3), 0.91 (3H, s, CH_3); ^{13}C-NMR (150 MHz, $CDCl_3$): δ 39.86 (C-1), 34.18 (C-2), 217.91 (C-3), 47.51 (C-4), 55.44 (C-5), 18.90 (C-6), 34.82 (C-7), 39.77 (C-8), 49.56 (C-9), 36.92 (C-10), 31.11 (C-11), 71.34 (C-12), 48.70 (C-13), 51.80 (C-14), 31.74 (C-15), 26.85 (C-16), 49.97 (C-17), 15.50 (C-18), 16.09 (C-19), 74.55 (C-20), 22.11 (C-21), 42.81 (C-22), 19.78 (C-23), 46.67 (C-24), 70.98 (C-25), 32.52 (C-26), 32.72 (C-27), 26.43 (C-28), 21.16 (C-29), 17.19 (C-30).

20(*R*)-20,25-Epoxy-[2,3-*b*]-pyrazine-damran-12-ol (1d). Yield: 68.0%, Mp: 257–259 °C, HR-ESI-TOF-MS: *m*/*z* 495.3948 $[M + H]^+$ (calcd for $C_{32}H_{51}N_2O_2$, 495.3945); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.43 (1H, s, N–CH═), 8.32 (1H, s, N–CH═), 3.60 (1H, td, *J* = 10.3, 5.0 Hz, H-12), 3.14 (1H, d, *J* = 16.7 Hz, H-1), 2.54 (1H, d, *J* = 16.8 Hz, H-1), 1.32 (6H, s, 2 × CH_3), 1.28 (3H, s, CH_3), 1.24 (3H, s, CH_3), 1.20 (3H, s, CH_3), 1.06 (3H, s, CH_3), 0.93 (3H, s, CH_3), 0.87 (3H, s, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 48.80 (C-1), 150.81 (C-2), 159.89 (C-3), 39.80 (C-4), 54.86 (C-5), 20.26 (C-6), 34.18 (C-7), 39.74 (C-8), 49.46 (C-9), 36.86 (C-10), 31.10 (C-11), 69.90 (C-12), 48.36 (C-13), 51.43 (C-14), 31.28 (C-15), 25.32 (C-16), 53.60 (C-17), 15.30 (C-18), 16.29 (C-19), 76.80 (C-20), 19.59 (C-21), 35.92 (C-22), 16.44 (C-23), 36.63 (C-24), 73.31 (C-25), 33.21 (C-26), 27.30 (C-27), 31.71 (C-28), 24.15 (C-29), 17.15 (C-30), 141.38 (N–CH═), 142.47 (N–CH═).

20(*R*)-20,25-Epoxy-[2,3-*b*]-indol-12β-dammaranol (1e). Yield: 39.3%, Mp: 206–208 °C. ESI-MS: *m*/*z* 530.1 $[M - H]^-$; ^{1}H-NMR (400 MHz, pyridine-d_5): δ 11.67 (1H, s, -NH), 7.75 (1H, m, Ar–H), 7.49 (1H, m, Ar–H), 7.30 (1H, m, Ar–H), 7.28 (1H, m, Ar–H), 3.89 (1H, td, *J* = 10.1, 5.0 Hz, H-12), 3.12 (1H, d, *J* = 14.9 Hz, H-1), 2.38 (1H, d, *J* = 14.8 Hz, H-1), 1.51 (3H, s, CH_3), 1.38 (3H, s, CH_3), 1.33 (3H, s, CH_3), 1.27 (3H, s, CH_3), 1.25 (3H, s, CH_3), 1.11 (3H, s, CH_3), 1.01 (3H, s, CH_3), 0.95 (3H, s, CH_3); ^{13}C-NMR (100 MHz, pyridine-d_5): δ 38.54 (C-1), 121.34 (C-2), 142.62 (C-3), 38.88 (C-4), 55.30 (C-5), 20.08 (C-6), 34.95 (C-7), 40.47 (C-8), 49.50 (C-9), 36.97 (C-10), 31.50 (C-11), 70.66 (C-12), 50.42 (C-13), 51.85 (C-14), 31.76 (C-15), 25.80 (C-16), 54.75 (C-17), 15.95 (C-18), 16.93 (C-19), 77.30 (C-20), 20.05 (C-21), 35.20 (C-22), 17.01 (C-23), 36.24 (C-24), 73.43 (C-25), 33.54 (C-26), 27.79 (C-27), 32.18 (C-28), 23.78 (C-29), 17.64 (C-30), 138.18 (Ar–C), 129.47 (Ar–C), 119.20 (Ar–C), 118.79 (Ar–C), 111.61 (Ar–C), 106.83 (Ar–C).

20(*R*)-20,25-Epoxy-[2,3-*b*]-quinoxaline-dammaran-12β-ol (1f). Yield: 15.0%, Mp: 228–230 °C, HR-ESI-TOF-MS: *m*/*z* 459.3829 $[M + H]^+$ (calcd for $C_{30}H_{51}O_3$, 459.3933); ^{1}H-NMR (600 MHz, $CDCl_3$): δ 3.54 (1H, td, *J* = 10.4, 5.1 Hz, H-12), 2.43 (1H, dd, *J* = 12.6, 1.9 Hz, H-1), 2.26 (1H, d, *J* = 13.3 Hz, H-3), 2.15 (1H, dd, *J* = 13.3, 1.9 Hz, H-3), 1.98 (1H, d, *J* = 12.8 Hz, H-1), 1.26 (3H, s, CH_3), 1.21 (3H, s, CH_3), 1.18 (3H, s, CH_3), 1.04 (3H, s, CH_3), 0.99 (3H, s, CH_3), 0.91 (3H, s, CH_3), 0.89 (3H, s, CH_3), 0.88 (3H, s, CH_3); ^{13}C-NMR (150 MHz, $CDCl_3$): δ 56.41 (C-1), 212.17 (C-2), 56.68 (C-3), 39.24 (C-4), 56.34 (C-5), 19.20 (C-6), 34.55 (C-7), 40.35 (C-8), 49.78 (C-9), 42.95 (C-10), 30.75 (C-11), 69.82 (C-12), 49.22 (C-13), 51.39 (C-14), 31.27 (C-15), 25.27 (C-16), 54.82 (C-17), 15.43 (C-18), 17.40 (C-19), 76.80 (C-20), 19.54 (C-21), 35.88 (C-22), 16.40 (C-23), 36.59 (C-24), 73.33 (C-

25), 33.51 (C-26), 27.25 (C-27), 33.17 (C-28), 23.18 (C-29), 17.18 (C-30).

20(*R*)-20,25-Epoxy-12β-hydroxy-dammar-3-one oxime (1g). Yield: 83.3%, Mp: 278–280 °C, HR-ESI-TOF-MS: *m*/*z* 474.3913 $[M + H]^+$ (calcd for $C_{30}H_{51}NO_3$, 474.3942); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.55 (1H, td, J = 10.5, 5.1 Hz, H-12), 3.00 (1H, m, H-2), 2.40 (1H, m, H-2), 1.26 (3H, s, CH_3), 1.21 (3H, s, CH_3), 1.20 (3H, s, CH_3), 1.18 (3H, s, CH_3), 1.11 (3H, s, CH_3), 1.00 (3H, s, CH_3), 0.97 (3H, s, CH_3), 0.86 (3H, s, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 38.90 (C-1), 27.53 (C-2), 169.38 (C-3), 39.95 (C-4), 56.06 (C-5), 18.02 (C-6), 34.55 (C-7), 40.65 (C-8), 49.59 (C-9), 37.31 (C-10), 30.95 (C-11), 69.96 (C-12), 49.38 (C-13), 51.35 (C-14), 31.25 (C-15), 25.29 (C-16), 54.83 (C-17), 15.65 (C-18), 15.95 (C-19), 77.16 (C-20), 19.56 (C-21), 35.89 (C-22), 16.42 (C-23), 36.60 (C-24), 73.30 (C-25), 33.17 (C-26), 27.29 (C-27), 22.95 (C-28), 19.25 (C-29), 17.11 (C-30).

20(*R*)-2-Formyl-20,25-epoxy-12β-hydroxy-dammar-3-one (1h). Yield: 67.9%, Mp: 237–240 °C, ESI-MS: *m*/*z* 485.1 $[M - H]^-$; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 14.87 (1H, d, J = 2.3 Hz, CHO), 8.59 (1H, d, J = 1.6 Hz, H-2), 3.57 (1H, td, J = 10.3, 5.0 Hz, H-12), 1.27 (3H, s, CH_3), 1.23 (3H, s, CH_3), 1.20 (3H, s, CH_3), 1.18 (3H, s, CH_3), 1.11 (3H, s, CH_3), 1.02 (3H, s, CH_3), 0.90 (3H, s, CH_3), 0.88 (3H, s, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 40.31 (C-1), 105.85 (C-2), 190.14 (C-3), 35.86 (C-4), 52.60 (C-5), 19.54 (C-6), 34.08 (C-7), 39.94 (C-8), 48.24 (C-9), 39.68 (C-10), 31.02 (C-11), 69.98 (C-12), 49.34 (C-13), 51.42 (C-14), 31.26 (C-15), 25.26 (C-16), 54.74 (C-17), 15.23 (C-18), 15.36 (C-19), 76.80 (C-20), 19.62 (C-21), 36.53 (C-22), 16.38 (C-23), 36.57 (C-24), 73.36 (C-25), 33.11 (C-26), 27.26 (C-27), 28.41 (C-28), 20.76 (C-29), 17.10 (C-30), 189.24 (CHO).

20(*R*)-20,25-Epoxy-[5,4-*b*]-isoxazole-12β-dammaranol (1i). Yield: 70.4%, Mp: 237–239 °C, HR-ESI-TOF-MS: *m*/*z* 484.3779 $[M + H]^+$ (calcd for $C_{31}H_{50}NO_3$, 484.3785); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.99 (1H, s, HC═N), 3.57 (1H, td, J = 10.3, 5.0 Hz, H-12), 2.52 (1H, d, J = 15.1 Hz, H-1), 2.01 (1H, d, J = 15.1 Hz, H-1), 1.30 (3H, s, CH_3), 1.27 (3H, s, CH_3), 1.23 (3H, s, CH_3), 1.21 (3H, s, CH_3), 1.20 (3H, s, CH_3), 1.03 (3H, s, CH_3), 0.90 (3H, s, CH_3), 0.86 (3H, s, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 36.23 (C-1), 109.23 (C-2), 173.04 (C-3), 35.00 (C-4), 54.15 (C-5), 18.96 (C-6), 34.19 (C-7), 39.98 (C-8), 48.73 (C-9), 39.00 (C-10), 31.11 (C-11), 70.00 (C-12), 49.33 (C-13), 51.48 (C-14), 31.47 (C-15), 25.28 (C-16), 54.88 (C-17), 15.36 (C-18), 16.20 (C-19), 76.80 (C-20), 19.58 (C-21), 35.88 (C-22), 16.40 (C-23), 36.58 (C-24), 73.33 (C-25), 33.16 (C-26), 27.28 (C-27), 28.87 (C-28), 21.75 (C-29), 17.12 (C-30), 150.54 (HC═N).

20(*R*)-20,25-Epoxy-[5,4-*b*]-pyrazole-12β-dammaranol (1j). Yield: 80.5%, Mp: 291–293 °C, HR-ESI-TOF-MS: *m*/*z* 505.3745 $[M + Na]^+$ (calcd for $C_{31}H_{50}N_2NaO_2$, 505.3764); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.57 (1H, td, J = 10.2, 4.9 Hz, H-12), 2.70 (1H, d, $J_{1,2}$ = 14.9 Hz, H-1), 2.05 (1H, d, J = 14.8 Hz, H-1), 1.30 (3H, s, CH_3), 1.27 (3H, s, CH_3), 1.23 (3H, s, CH_3), 1.21 (3H, s, CH_3), 1.20 (3H, s, CH_3), 1.04 (3H, s, CH_3), 0.91 (3H, s, CH_3), 0.85 (3H, s, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 33.73 (C-1), 112.96 (C-2), 150.09 (C-3), 35.91 (C-4), 54.00 (C-5), 18.96 (C-6), 34.28 (C-7), 39.91 (C-8), 48.73 (C-9), 38.62 (C-10), 31.13 (C-11), 70.03 (C-12), 49.43 (C-13), 51.45 (C-14), 31.38 (C-15), 25.32 (C-16), 54.81 (C-17), 15.36 (C-18), 16.03 (C-19), 76.84 (C-20), 19.58 (C-21), 36.62 (C-22), 16.43 (C-23), 36.94 (C-24), 73.28 (C-25), 33.18 (C-26), 27.28 (C-27), 31.13 (C-28), 24.07 (C-29), 17.16 (C-30), 132.74 (N═C).

20(*R*)-20,25-Epoxy-2-cyano-2-ene-dammarane-3,12β-diol (1k). Yield: 85.0%, Mp: 236–238 °C, HR-ESI-TOF-MS: *m*/*z* 484.3824 $[M + H]^+$ (calcd for $C_{31}H_{50}NO_3$, 484.3785); ^{1}H-NMR (600 MHz, pyridine-d_5): δ 3.76 (1H, m, H-12), 2.32 (1H, d, J = 15.1 Hz, H-1), 1.93 (1H, d, J = 15.1 Hz, H-1), 1.34 (3H, s, CH_3), 1.29 (3H, s, CH_3), 1.24 (3H, s, CH_3), 1.23 (3H, s, CH_3), 1.21 (3H, s, CH_3), 0.99 (3H, s, CH_3), 0.90 (3H, s, CH_3), 0.89 (3H, s, CH_3); ^{13}C-NMR (150 MHz, pyridine-d_5): δ 42.84 (C-1), 80.12 (C-2), 172.90 (C-3), 39.51 (C-4), 53.35 (C-5), 20.01 (C-6), 34.57 (C-7), 40.12 (C-8), 48.80 (C-9), 36.55 (C-10), 31.93 (C-11), 70.38 (C-12), 50.06 (C-13), 51.40 (C-14), 31.65 (C-15), 25.72 (C-16), 55.25 (C-17), 15.74 (C-18), 16.12 (C-19), 77.25 (C-20), 20.18 (C-21), 36.21 (C-22), 16.90 (C-23), 36.94 (C-24), 73.43 (C-25), 33.51 (C-26), 27.73 (C-27), 28.41 (C-28), 20.40 (C-29), 17.51 (C-30), 121.57 (–CN).

20(*R*)-20,25-Epoxy-2′-amino-[6,5-*b*]-pyrimidine-12β-dammaranol (1m). Yield: 61.2%, Mp: 270–272 °C, HR-ESI-TOF-MS: *m*/*z* 510.4052 $[M + H]^+$ (calcd for $C_{32}H_{52}N_3O_2$, 510.4054); ^{1}H-NMR (400 MHz, pyridine-d_5): δ 8.16 (1H, s, –N═CH), 6.97 (2H, s, NH_2), 3.82 (1H, m, H-12), 2.63 (1H, d, J = 15.1 Hz, H-1), 2.07 (1H, d, J = 14.5 Hz, H-1), 1.42 (3H, s, CH_3), 1.33 (3H, s, CH_3), 1.31 (3H, s, CH_3), 1.26 (3H, s, CH_3), 1.25 (3H, s, CH_3), 1.05 (3H, s, CH_3), 0.92 (3H, s, CH_3), 0.80 (3H, s, CH_3); ^{13}C-NMR (100 MHz, pyridine-d_5): δ 42.43 (C-1), 116.77 (C-2), 173.17 (C-3), 39.96 (C-4), 54.12 (C-5), 20.82 (C-6), 34.77 (C-7), 40.21 (C-8), 48.84 (C-9), 36.95 (C-10), 31.48 (C-11), 70.53 (C-12), 50.36 (C-13), 51.79 (C-14), 31.68 (C-15), 25.76 (C-16), 55.28 (C-17), 15.78 (C-18), 15.97 (C-19), 77.28 (C-20), 20.04 (C-21), 36.22 (C-22), 16.91 (C-23), 36.80 (C-24), 73.43 (C-25), 33.56 (C-26), 27.74 (C-27), 32.05 (C-28), 24.06 (C-29), 17.57 (C-30), 164.42 (–C═N), 160.12 (–N═CH).

20(*R*)-20,25-Epoxy-2′-methyl-[6,5-*b*]-pyrimidine-12β-dammarol (1n). Yield: 18.0%, Mp: 291–293 °C, HR-ESI-TOF-MS: *m*/*z* 509.4107 $[M + H]^+$ (calcd for $C_{33}H_{53}N_2O_2$, 509.4102); ^{1}H-NMR (600 MHz, pyridine-d_5): δ 8.39 (1H, s, –N═CH), 3.84 (1H, td, J = 9.7, 5.0 Hz, H-12), 2.72 (1H, d, J = 15.7 Hz, H-1), 2.15 (1H, d, J = 15.4 Hz, H-1), 2.80 (3H, s, CH_3), 1.44 (3H, s, CH_3), 1.36 (3H, s, CH_3), 1.31 (3H, s, CH_3), 1.26 (3H, s, CH_3), 1.25 (3H, s, CH_3), 1.05 (3H, s, CH_3), 0.93 (3H, s, CH_3), 0.77 (3H, s, CH_3); ^{13}C-NMR (150 MHz, pyridine-d_5): δ 42.53 (C-1), 124.72 (C-2), 172.38 (C-3), 40.00 (C-4), 53.95 (C-5), 20.05 (C-6), 34.69 (C-7), 40.23 (C-8), 48.81 (C-9), 36.60 (C-10), 31.50 (C-11), 70.49 (C-12), 50.36 (C-13), 51.80 (C-14), 32.07 (C-15), 25.76 (C-16), 55.29 (C-17), 15.73 (C-18), 16.09 (C-19), 77.30 (C-20), 20.72 (C-21), 36.22 (C-22), 16.92 (C-23), 36.96 (C-24), 73.46 (C-25), 33.58 (C-26), 27.75 (C-27), 31.68 (C-28), 24.20 (C-29), 17.56 (C-30), 166.30 (–C═N), 158.89 (–N═CH), 26.47 (-CH_3).

20(*R*)-Dammarane-3β,12β,20,25-tetrol (25-OH-PPD) (2). Mp: 252–254 °C, HR-ESI-TOF: *m*/*z* 479.4022 $[M + H]^+$ (calcd for $C_{30}H_{55}O_4$, 479.4056); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 3.15 (1H, dd, H-3), 3.58 (1H, td, H-12), 1.24 (6H, s, 2 × CH_3), 1.15 (3H, s, CH_3), 1.00 (3H, s, CH_3), 0.98 (3H, s, CH_3), 0.90 (6H, s,

2 × CH_3), 0.78 (3H, s, CH_3); ^{13}C-NMR (75 MHz, pyridine-d_5): δ 40.3 (C-1), 28.0 (C-2), 79.5 (C-3), 40.0 (C-4), 57.3 (C-5), 18.9 (C-6), 35.9 (C-7), 40.9 (C-8), 50.9 (C-9), 38.2 (C-10), 32.0 (C-11), 71.9 (C-12), 49.5 (C-13), 52.6 (C-14), 32.0 (C-15), 27.1 (C-16), 51.3 (C-17), 16.3 (C-18), 16.8 (C-19), 74.7 (C-20), 22.4 (C-21), 44.0 (C-22), 19.4 (C-23), 45.4 (C-24), 71.5 (C-25), 29.4 (C-26), 29.1 (C-27), 28.6 (C-28), 16.2 (C-29), 17.4 (C-30).

20(*R*)-20,25-Dihydroxydammaran-3,12-dione (2a). Yield: 26.1%, Mp: 115–118 °C, $[\alpha]_D^{20}$ + 32.0 (0.09, MeOH); HR-ESI-TOF-MS: *m*/*z* 497.3602 $[M + Na]^+$ (calcd for $C_{30}H_{50}NaO_4$, 497.3607); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.22 (6H, s, 2 × CH_3), 1.21 (3H, s, CH_3), 1.09 (3H, s, CH_3), 1.06 (3H, s, CH_3), 1.03 (3H, s, CH_3), 1.00 (3H, s, CH_3), 0.81 (3H, s, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 39.10 (C-1), 33.28 (C-2), 217.01 (C-3), 47.52 (C-4), 55.24 (C-5), 18.07 (C-6), 33.90 (C-7), 40.15 (C-8), 52.65 (C-9), 37.20 (C-10), 39.17 (C-11), 214.47 (C-12), 56.71 (C-13), 54.61 (C-14), 30.89 (C-15), 25.19 (C-16), 43.14 (C-17), 15.61 (C-18), 15.61 (C-19), 73.70 (C-20), 21.92 (C-21), 44.11 (C-22), 19.76 (C-23), 44.49 (C-24), 71.25 (C-25), 29.48 (C-26), 29.36 (C-27), 26.64 (C-28), 21.19 (C-29), 17.50 (C-30).

20(*R*)-3β,20,25-Trihydroxydammaran-12-one (2b). Yield: 22.6%, Mp: 166–169 °C, $[\alpha]_D^{20}$ + 18.7 (0.07, MeOH); HR-ESI-TOF-MS: *m*/*z* 499.3776 $[M + Na]^+$ (calcd for $C_{30}H_{52}NaO_4$, 499.3763); ^{1}H-NMR (400 MHz, pyridine-d_5): δ 3.25 (1H, d, *J* = 9.9 Hz, H-3), 1.43 (6H, s, 2 × CH_3), 1.32 (3H, s, CH_3), 1.24 (3H, s, CH_3), 1.18 (3H, s, CH_3), 1.05 (3H, s, CH_3), 0.89 (3H, s, CH_3), 0.81 (3H, s, CH_3); ^{13}C-NMR (100 MHz, pyridine-d_5): δ 39.91 (C-1), 28.44 (C-2), 78.13 (C-3), 39.26 (C-4), 56.47 (C-5), 19.19 (C-6), 34.85 (C-7), 41.00 (C-8), 54.61 (C-9), 38.14 (C-10), 40.09 (C-11), 213.51 (C-12), 57.12 (C-13), 55.80 (C-14), 31.99 (C-15), 25.63 (C-16), 43.92 (C-17), 16.60 (C-18), 16.49 (C-19), 73.93 (C-20), 24.60 (C-21), 45.17 (C-22), 19.61 (C-23), 46.04 (C-24), 70.12 (C-25), 30.56 (C-26), 30.34 (C-27), 28.98 (C-28), 16.20 (C-29), 17.76 (C-30).

20(*R*)-12β,20,25-Trihydroxydammaran-3-one (2c). Yield: 28.6%, Mp: 215–218 °C, $[\alpha]_D^{20}$ + 19.6 (0.07, MeOH); HR-ESI-TOF-MS: *m*/*z* 499.3778 $[M + Na]^+$ (calcd for $C_{30}H_{52}NaO_4$, 499.3763); ^{1}H-NMR (400 MHz, pyridine-d_5): δ 3.93 (1H, td, *J* = 8.9, 4.9 Hz, H-12), 1.44 (9H, s, 3 × CH_3), 1.16 (3H, s, CH_3), 1.07 (3H, s, CH_3), 1.05 (3H, s, CH_3), 0.93 (3H, s, CH_3), 0.91 (3H, s, CH_3); ^{13}C-NMR (100 MHz, pyridine-d_5): δ 40.12 (C-1), 34.66 (C-2), 216.76 (C-3), 47.76 (C-4), 55.61 (C-5), 19.13 (C-6), 34.81 (C-7), 40.28 (C-8), 50.07 (C-9), 37.29 (C-10), 32.92 (C-11), 71.06 (C-12), 49.84 (C-13), 52.13 (C-14), 31.80 (C-15), 27.04 (C-16), 51.13 (C-17), 15.90 (C-18), 16.44 (C-19), 73.77 (C-20), 23.21 (C-21), 44.47 (C-22), 20.30 (C-23), 46.01 (C-24), 70.09 (C-25), 30.63 (C-26), 30.34 (C-27), 27.19 (C-28), 21.56 (C-29), 17.64 (C-30).

20(*R*)-2,12β,20,25-Tetrahydroxydammaran-1-en-3-one (2d). Yield: 85.7%, Mp: 222–224 °C, $[\alpha]_D^{20}$ + 24.4 (0.06, MeOH); HR-ESI-TOF-MS: *m*/*z* 513.3540 $[M + Na]^+$ (calcd for $C_{30}H_{50}NaO_5$, 513.3556); ^{1}H-NMR (400 MHz, pyridine-d_5): δ 6.77 (1H, s, H-1), 3.96 (1H, td, *J* = 10.0, 5.0 Hz, H-12), 1.45 (3H, s, CH_3), 1.44 (6H, s, 2 × CH_3), 1.27 (3H, s, CH_3), 1.18 (3H, s, CH_3), 1.13 (3H, s, CH_3), 1.07 (3H, s, CH_3), 0.90 (3H, s, CH_3); ^{13}C-NMR (100 MHz, pyridine-d_5): δ 130.16 (C-1), 146.15 (C-2), 201.51 (C-3), 46.15 (C-4), 54.98 (C-5), 19.11 (C-6), 35.01 (C-7), 41.06 (C-8), 45.26 (C-9), 39.08 (C-10), 32.56 (C-11), 70.93 (C-12), 49.88 (C-13), 52.16 (C-14), 31.65 (C-15), 26.99 (C-16), 51.22 (C-17), 16.54 (C-18), 23.08 (C-19), 73.79 (C-20), 22.29 (C-21), 44.42 (C-22), 19.62 (C-23), 46.00 (C-24), 70.08 (C-25), 30.32 (C-26), 30.63 (C-27), 28.27 (C-28), 21.14 (C-29), 17.57 (C-30).

20(*R*)-2,3-Seco-12β,20,25-trihydroxydammaran-2,3-dioc acid (2e). Yield: 32.1%, Mp: 154–157 °C, $[\alpha]_D^{20}$ + 18.0 (0.07, MeOH); HR-ESI-TOF-MS: *m*/*z* 523.3631 $[M-H]^-$ (calcd for $C_{30}H_{51}O_7$, 523.3635); ^{1}H-NMR (600 MHz, pyridine-d_5): δ 4.04 (1H, td, *J* = 8.6, 3.1 Hz, H-12), 1.62 (3H, s, CH_3), 1.56 (3H, s, CH_3), 1.43 (3H, s, CH_3), 1.42 (3H, s, CH_3), 1.16 (3H, s, CH_3), 1.14 (3H, s, CH_3), 1.05 (3H, s, CH_3); ^{13}C-NMR (150 MHz, pyridine-d_5): δ 43.55 (C-1), 174.76 (C-2), 182.78 (C-3), 47.53 (C-4), 49.19 (C-5), 19.13 (C-6), 34.95 (C-7), 40.31 (C-8), 42.62 (C-9), 42.44 (C-10), 30.89 (C-11), 71.24 (C-12), 49.76 (C-13), 52.78 (C-14), 31.87 (C-15), 27.15 (C-16), 51.07 (C-17), 16.08 (C-18), 20.93 (C-19), 73.53 (C-20), 23.31 (C-21), 44.34 (C-22), 19.62 (C-23), 46.01 (C-24), 70.10 (C-25), 30.57 (C-26), 30.33 (C-27), 28.05 (C-28), 25.37 (C-29), 17.90 (C-30).

20(*R*)-[2,3-*b*]-Pyrazine-dammarane-12β,20,25-triol (2f). Yield: 60.0%, Mp: 229–231 °C, HR-ESI-TOF-MS: *m*/*z* 513.4026 $[M + H]^+$ (calcd for $C_{32}H_{53}N_2O_3$, 513.4051); ^{1}H-NMR (600 MHz, $CDCl_3$): δ 8.42 (1H, d, *J* = 1.8 Hz, N–CH═), 8.26 (1H, d, *J* = 2.4 Hz, N–CH═), 3.67 (1H, td, *J* = 10.4, 5.0 Hz, H-12), 3.10 (1H, d, *J* = 16.5 Hz, H-1), 2.54 (1H, d, *J* = 15.1 Hz, H-1), 1.31 (3H, s, CH_3), 1.30 (3H, s, CH_3), 1.24 (3H, s, CH_3), 1.23 (3H, s, CH_3), 1.14 (3H, s, CH_3), 1.07 (3H, s, CH_3), 0.94 (3H, s, CH_3), 0.86 (3H, s, CH_3); ^{13}C-NMR (150 MHz, $CDCl_3$): δ 48.91 (C-1), 150.60 (C-2), 159.78 (C-3), 39.72 (C-4), 53.58 (C-5), 20.23 (C-6), 34.09 (C-7), 39.70 (C-8), 48.51 (C-9), 36.78 (C-10), 31.97 (C-11), 70.71 (C-12), 48.60 (C-13), 51.85 (C-14), 31.17 (C-15), 26.51 (C-16), 50.41 (C-17), 15.29 (C-18), 16.3 (C-19), 74.14 (C-20), 21.92 (C-21), 43.11 (C-22), 18.00 (C-23), 44.52 (C-24), 71.12 (C-25), 29.56 (C-26), 29.53 (C-27), 31.70 (C-28), 24.15 (C-29), 17.21 (C-30), 141.56 (N–CH═), 142.62 (N–CH═).

20(*R*)-20,25-Dihydroxy-[2,3-*b*]-pyrazine-dammar-12-one (2g). Yield: 69.4%, Mp: 229–231 °C, HR-ESI-TOF-MS: *m*/*z* 533.3701 $[M + Na]^+$ (calcd for $C_{32}H_{50}N_2NaO_3$, 533.3714); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.43 (1H, d, *J* = 1.8 Hz, N–CH═), 8.29 (1H, d, *J* = 2.3 Hz, N–CH═), 1.32 (6H, s, 2 × CH_3), 1.25 (3H, s, CH_3), 1.22 (6H, s, 2 × CH_3), 1.01 (3H, s, CH_3), 0.90 (3H, s, CH_3), 0.85 (3H, s, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 48.21 (C-1), 149.83 (C-2), 159.31 (C-3), 39.70 (C-4), 54.73 (C-5), 20.24 (C-6), 33.10 (C-7), 40.10 (C-8), 51.72 (C-9), 37.09 (C-10), 39.14 (C-11), 214.10 (C-12), 56.66 (C-13), 53.34 (C-14), 31.00 (C-15), 25.22 (C-16), 43.18 (C-17), 15.32 (C-18), 15.93 (C-19), 73.62 (C-20), 22.03 (C-21), 44.10 (C-22), 18.13 (C-23), 44.57 (C-24), 71.20 (C-25), 29.54 (C-26), 29.36 (C-27), 31.65 (C-28), 24.15 (C-29), 17.49 (C-30), 141.73 (N–CH═), 142.72 (N–CH═).

20(*R*)-25-Methoxy-dammarane-3β,12β,20-triol (3). Mp: 232–234 °C, $[\alpha]_D^{20}$ + 21.0 (0.5, MeOH); HR-ESI-TOF: *m*/*z* 493.4168 $[M + H]^+$ (calcd for $C_{31}H_{56}O_4$, 499.3763); ^{1}H-NMR (300 Hz, $CDCl_3$): δ 3.20 (1H, dd, H-3), 3.58 (1H, td, H-12), 3.12 (3H, s, –OCH_3), 1.42 (3H, s, CH_3), 1.24 (3H, s, CH_3), 1.15 (3H, s, CH_3), 0.91 (3H, s, CH_3), 1.10 (3H, s, CH_3), 1.04 (3H, s, CH_3),

0.95 (3H, s, CH_3), 0.91 (3H, s, CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 38.9 (C-1), 27.4 (C-2), 78.9 (C-3), 39.7 (C-4), 55.9 (C-5), 18.3 (C-6), 34.8 (C-7), 38.9 (C-8), 49.9 (C-9), 37.1 (C-10), 31.2 (C-11), 70.8 (C-12), 49.1 (C-13), 51.6 (C-14), 31.0 (C-15), 26.3 (C-16), 50.1 (C-17), 15.6 (C-18), 16.1 (C-19), 74.4 (C-20), 21.8 (C-21), 43.1 (C-22), 17.3 (C-23), 40.5 (C-24), 74.8 (C-25), 24.9 (C-26), 24.9 (C-27), 28.0 (C-28), 15.3 (C-29), 17.1 (C-30), 48.5 (–OCH_3).

20(*R*)-25-Methoxy-20-hydroxydammaran-3,12-dione (3a). Yield: 20.4%, Mp: 128–131 °C, $[\alpha]_D^{20}$ + 45.6 (0.10, MeOH); HR-ESI-TOF-MS: *m*/*z* 489.3928 $[M + H]^+$ (calcd for $C_{31}H_{53}O_4$, 489.3944); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.18 (3H, s, –OCH_3), 1.22 (3H, s, CH_3), 1.15 (6H, s, 2 × CH_3), 1.10 (3H, s, CH_3), 1.06 (3H, s, CH_3), 1.03 (3H, s, CH_3), 1.00 (3H, s, CH_3), 0.81 (3H, s, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 39.21 (C-1), 33.34 (C-2), 216.90 (C-3), 47.53 (C-4), 55.30 (C-5), 19.80 (C-6), 33.92 (C-7), 39.14 (C-8), 52.70 (C-9), 37.24 (C-10), 40.19 (C-11), 214.21 (C-12), 56.72 (C-13), 54.03 (C-14), 30.97 (C-15), 25.23 (C-16), 44.06 (C-17), 15.63 (C-18), 15.63 (C-19), 73.65 (C-20), 21.98 (C-21), 43.22 (C-22), 17.70 (C-23), 40.56 (C-24), 74.88 (C-25), 25.13 (C-26), 25.17 (C-27), 26.68 (C-28), 21.21 (C-29), 17.48 (C-30), 49.25 (–OCH_3).

20(*R*)-25-Methoxy-3β,20-dihydroxydammaran-12-one (3b). Yield: 13.9%, Mp: 141–143 °C, $[\alpha]_D^{20}$ + 29.3 (0.10, MeOH); HR-ESI-TOF-MS: *m*/*z* 491.4119 $[M + H]^+$ (calcd for $C_{31}H_{55}O_4$, 491.4100); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.20 (1H, dd, *J* = 11.5, 4.8 Hz, H-3), 3.18 (3H, s, –OCH_3), 1.17 (3H, s, CH_3), 1.15 (6H, s, 2 × CH_3), 0.99 (6H, s, 2 × CH_3), 0.92 (3H, s, CH_3), 0.80 (6H, s, 2 × CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 39.05 (C-1), 27.24 (C-2), 78.68 (C-3), 39.10 (C-4), 55.80 (C-5), 18.46 (C-6), 34.01 (C-7), 38.54 (C-8), 53.31 (C-9), 37.56 (C-10), 40.25 (C-11), 215.04 (C-12), 56.60 (C-13), 54.58 (C-14), 30.88 (C-15), 25.25 (C-16), 44.06 (C-17), 15.94 (C-18), 15.97 (C-19), 73.60 (C-20), 21.98 (C-21), 43.32 (C-22), 17.70 (C-23), 40.54 (C-24), 74.90 (C-25), 15.17 (C-26), 25.13 (C-27), 28.13 (C-28), 15.45 (C-29), 17.61 (C-30), 49.24 (–OCH_3).

20(*R*)-25-Methoxy-12β,20-dihydroxydammaran-3-one (3c). Yield: 34.2%, Mp: 176–179 °C, $[\alpha]_D^{20}$ + 39.9 (0.07, MeOH); HR-ESI-TOF-MS: *m*/*z* 491.4114 $[M + H]^+$ (calcd for $C_{31}H_{55}O_4$, 491.4100); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.51 (1H, td, *J* = 10.3, 5.1 Hz, H-12), 3.07 (3H, s, –OCH_3), 1.05 (9H, s, 3 × CH_3), 0.98 (3H, s, CH_3), 0.94 (3H, s, CH_3), 0.93 (3H, s, CH_3), 0.88 (3H, s, CH_3), 0.80 (3H, s, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 39.86 (C-1), 34.21 (C-2), 217.90 (C-3), 47.51 (C-4), 55.45 (C-5), 19.80 (C-6), 34.21 (C-7), 39.79 (C-8), 49.48 (C-9), 36.94 (C-10), 31.58 (C-11), 70.81 (C-12), 49.26 (C-13), 51.75 (C-14), 31.13 (C-15), 26.44 (C-16), 49.89 (C-17), 16.07 (C-18), 16.07 (C-19), 74.92 (C-20), 21.98 (C-21), 43.16 (C-22), 17.42 (C-23), 40.61 (C-24), 74.92 (C-25), 25.10 (C-26), 25.10 (C-27), 26.85 (C-28), 21.17 (C-29), 17.19 (C-30), 48.80 (–OCH_3).

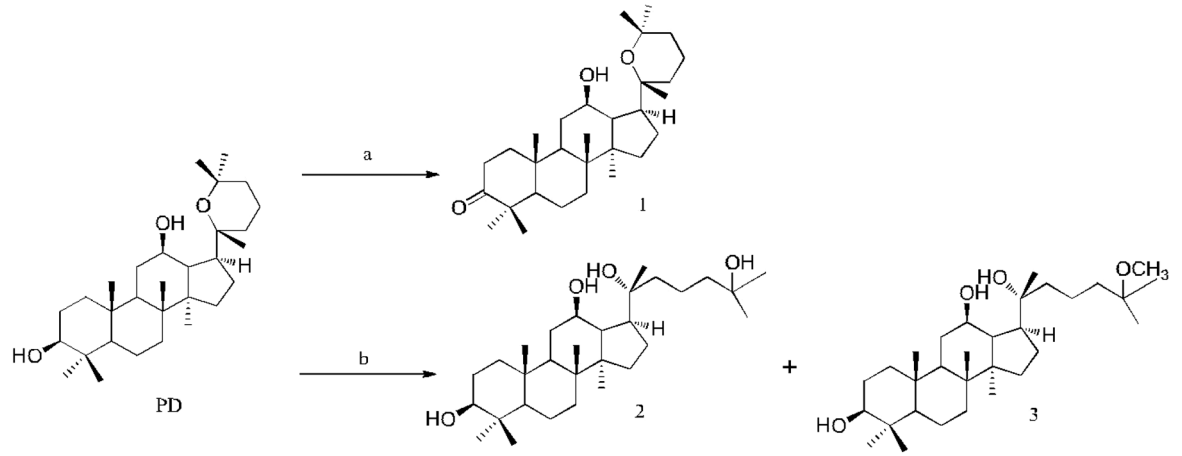
20(*R*)-25-Methoxy-2,12β,20-trihydroxydammaran-1-en-3-one (3d). Yield: 62.3%, Mp: 178–181 °C, $[\alpha]_D^{20}$ + 38.1 (0.07, MeOH); HR-ESI-TOF-MS: *m*/*z* 527.3733 $[M + Na]^+$ (calcd for $C_{31}H_{52}NaO_5$, 527.3712); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.50 (1H, s, H-2), 3.71 (1H, td, *J* = 9.8, 4.4 Hz, H-12), 3.19 (3H, s, –OCH_3), 1.22 (3H, s, CH_3), 1.20 (6H, s, 2 × CH_3), 1.16 (6H, s, 2 × CH_3), 1.13 (3H, s, CH_3), 1.07 (3H, s, CH_3), 0.90 (3H, s, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 128.68 (C-1), 144.08 (C-2), 201.19 (C-3), 45.30 (C-4), 54.63 (C-5), 18.87 (C-6), 34.47 (C-7), 40.65 (C-8), 44.20 (C-9), 38.71 (C-10), 31.22 (C-11), 70.43 (C-12), 49.26 (C-13), 51.77 (C-14), 30.94 (C-15), 26.40 (C-16), 50.01 (C-17), 16.24 (C-18), 20.32 (C-19), 74.77 (C-20), 21.94 (C-21), 43.20 (C-22), 17.40 (C-23), 40.61 (C-24), 74.95 (C-25), 25.07 (C-26), 25.07 (C-27), 27.27 (C-28), 21.73 (C-29), 17.19 (C-30), 48.91 (–OCH_3).

20(*R*)-25-Methoxy-2,3-seco-12β,20-dihydroxydammaran-2,3-dioc acid (3e). Yield: 42.3%, Mp: 161–163 °C, $[\alpha]_D^{20}$ + 17.6 (0.10, MeOH); HR-ESI-TOF-MS: *m*/*z* 537.3771 $[M - H]^-$ (calcd for $C_{31}H_{53}O_7$, 537.3791); ^{1}H-NMR (400 MHz, pyridine-d_5): δ 4.05 (1H, td, *J* = 9.5, 4.4 Hz, H-12), 3.15 (3H, s, –OCH_3), 1.62 (3H, s, CH_3), 1.56 (3H, s, CH_3), 1.42 (3H, s, CH_3), 1.16 (3H, s, CH_3), 1.15 (9H, s, 3 × CH_3), 1.07 (3H, s, CH_3); ^{13}C-NMR (100 MHz, pyridine-d_5): δ 43.57 (C-1), 174.81 (C-2), 182.80 (C-3), 47.50 (C-4), 49.19 (C-5), 18.45 (C-6), 34.93 (C-7), 40.31 (C-8), 42.60 (C-9), 42.43 (C-10), 31.86 (C-11), 71.23 (C-12), 49.72 (C-13), 52.70 (C-14), 31.80 (C-15), 27.15 (C-16), 51.11 (C-17), 16.08 (C-18), 20.91 (C-19), 73.40 (C-20), 23.65 (C-21), 44.12 (C-22), 19.60 (C-23), 41.51 (C-24), 74.91 (C-25), 25.52 (C-26), 25.52 (C-27), 28.14 (C-28), 25.34 (C-29), 17.87 (C-30), 49.30 (–OCH_3).

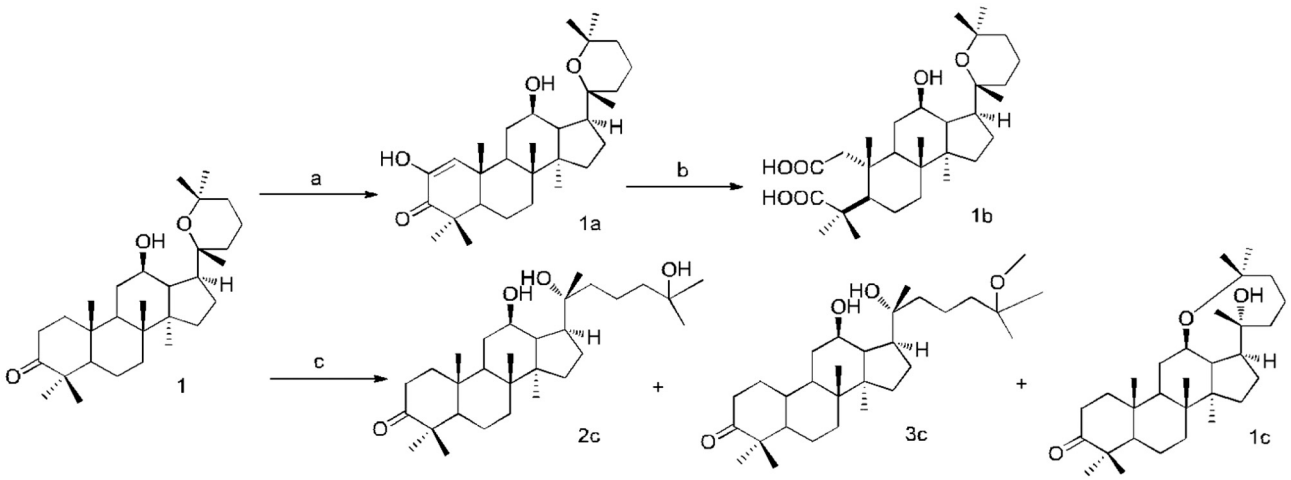
Materials and methods

The schemes and methods of compounds

PD was derived from laboratory preparation with a purity >97%. In this study, a series of ketone, enol, pyrazine, pyrimidine and azole derivatives were synthesized and the synthetic routes of their preparation are illustrated in Schemes 1–5. The structural characterizations of these compounds were conducted using 1D-, 2D-NMR and MS (ESI†). All compounds were successfully obtained in the end by a series of reactions. It is worth noting that **20** compounds such as **1c**, **1d**, **1f–1n**, **2b**, **2d–2g**, and **3a–3e** were novel compounds. According to the reported methods,[25,26] PD was added to a solution of hydrochloric acid in dry methanol. The reactants were kept at 40 °C under ultrasonic reaction conditions for 30 min to afford **2** and **3**.[27,28] PD, compounds **2** and **3** were oxidized with pyridine chlorochromate (PCC) in CH_2Cl_2 to obtain compound **1**, **2a**, **2b**, **2c**, **3a**, **3b**, and **3c**. In addition, compound **1c** was obtained in the same way as shown in Scheme 1. Further, derivatives **1a**, **2d**, and **3d** were obtained by introducing air into *tert*-butyl alcohol solutions of the 3-oxo derivatives, in the presence of potassium *tert*-butoxide. Compounds **1a**, **2d**, and **3d** were then oxidized by the combined action of hydrogen peroxide and KOH in refluxing methanol to obtain diacid derivatives **1b**, **2e**, and **3e**. Compound **1** was added to morpholine, stirred and dissolved; then, sublimed sulfur, ethylenediamine and *o*-phenylenediamine were added to obtain compounds **1d** and **1f**. Compound **1** was added to a glacial acetic acid solution of phenylhydrazine, and the reactants were heated at 120 °C under reflux conditions for 6 h to yield **1e**. In the presence of methanol, triethylamine and hydroxylamine hydrochloride



Scheme 1 The synthesis of intermediates. Reagents and conditions: (a) PCC, CH_2Cl_2, rt, 3 h; (b) CH_3OH, HCl, ultrasonic, 1.5 h.

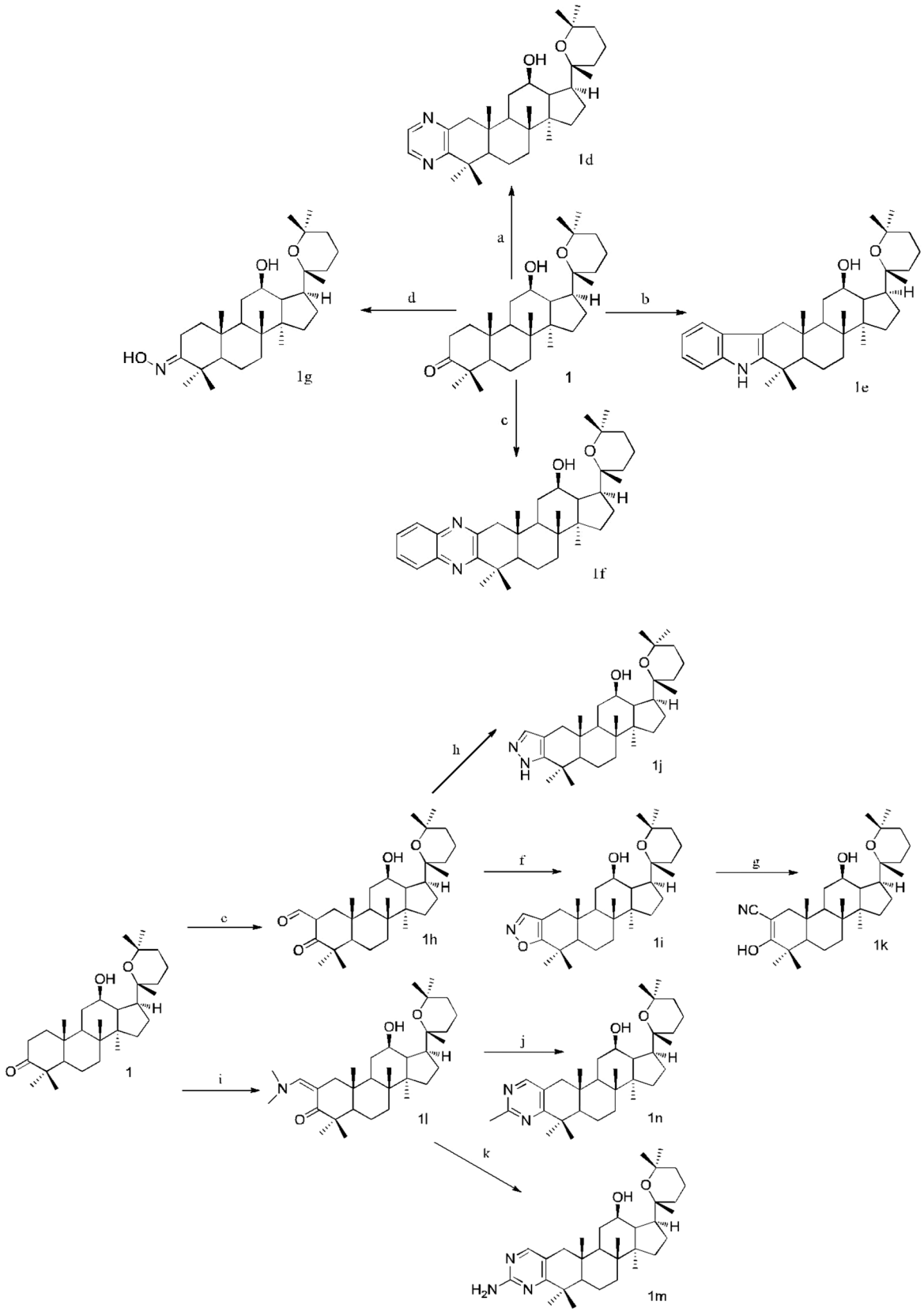


Scheme 2 The synthesis of oxidation products of intermediate 1. Reagents and conditions: (a) O2, *t*-BuOK, *t*-BuOH, 40 °C, 2 h; (b) H_2O_2, KOH, CH_3OH, reflux, 100 min; (c) CH_3OH, HCl, ultrasonic, 1.5 h.

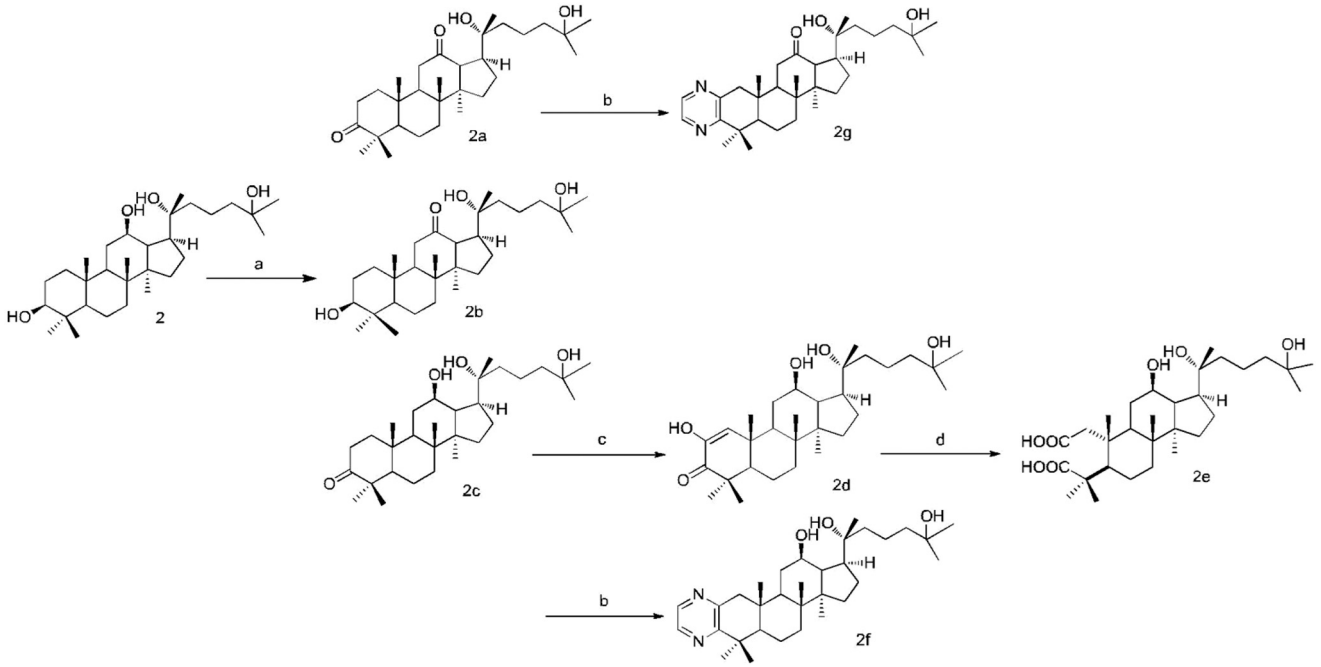
were added to PD-2, and after 24 h, **1g** was obtained. Compound **1** was dissolved in dimethylformamide, followed by the addition of ethyl formate and sodium hydride; after 3 h of reaction, compound **1h** was obtained. Hydroxylamine hydrochloride or hydrazine dihydrochloride was added to the ethanol solution of **1h**, and refluxed at 85 °C for 2 h to obtain compound **1i** and **1j**, respectively. Compound **1i** was dissolved in methanol at 0 °C, and then diethyl ether and sodium methoxide were added and the temperature was gradually raised to room temperature, and reaction was carried out for 2 h to obtain compound **1k**. Dimethylformamide acetal, sodium methoxide and triethylamine were added to the methanol solution of compound **1**, and refluxed for 24 h to obtain compound **1l**. Interestingly, when guanidine hydrochloride or acetyl hydrochloride was added to **1l**, **1m** and **1n** were obtained, respectively. Moreover, the synthesis method of the compounds **2g** and **2f** are similar to that of compound **1d**. All products were purified from the reaction mixture by silica gel column chromatography.

Cytotoxicity assay for human cancer cell and normal cell

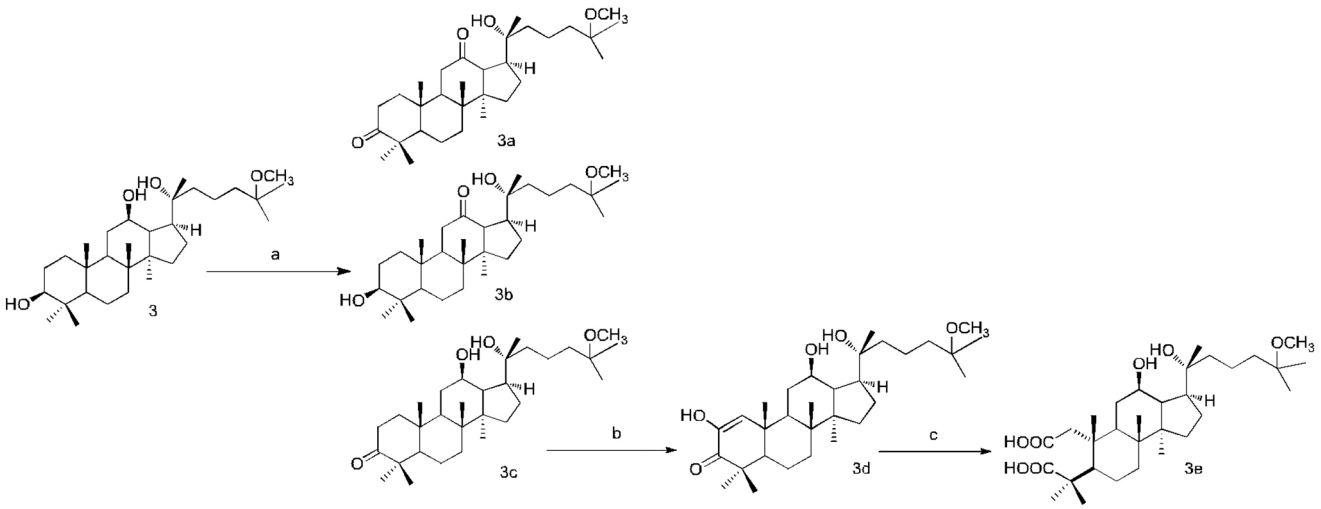
The MTT assay was used to test the ability of each compound to inhibit the proliferation of the human lung cancer A549 cells, human breast cancer MCF-7 cells, human glioma cancer U87 cells, human cervical cancer HeLa cells, human ovarian cancer 8901 cells and one normal cell line IOSE144. In addition, all of the cell lines were obtained from the Cell Bank of Chinese Academy of Sciences (Shanghai, China). In short, 5×10^3 cells per well were seeded into 96-well plates; 24 h later, the cells were treated with serial dilutions of the compounds (0–100 μM) for another 48 h. Then 10 μL of the MTT solution (5 mg mL^{-1}) was added to each well, and the tumor cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2 for 4 h. At the end of the incubation period, the growth medium was removed and replaced with 100 μL DMSO (Sigma Chemical Co., Ltd, USA) at room temperature. After agitating for 10 min using a vortex mixer, and the absorbance was determined at 492 nm using a Bio-Rad



Scheme 3 The synthesis of pyrazine, pyrimidine and azole compounds of intermediate 1. Reagents and conditions: (a) ethylenediamine, morpholine, sulfur, reflux, 5 h; (b) phenylhydrazine, AcOH, reflux, 6 h; (c) *o*-phenylenediamine, morpholine, sulfur, reflux, 5 h; (d) MeOH, $NH_2OH{\cdot}HCl$, Et_3N, rt, 24 h; (e) HCOOEt, NaH, DMF, rt, 4 h; (f) EtOH, $NH_2OH{\cdot}HCl$, reflux, 2 h; (g) CH_3OH, CH_3ONa, EtOH, 0 °C-rt, 2 h; (h) EtOH, $H_2NNH_2{\cdot}2HCl$, reflux, 2 h; (i) *N*,*N*-dimethylformamide dimethyl acetal, TEA, toluene, reflux, 24 h; (j) guanidine hydrochloride, NaOMe, EtOH, reflux, 12 h; (k) ethanimidamide hydrochloride, NaOMe, EtOH, reflux, 12 h.



Scheme 4 The synthesis of oxidation products and pyrazine compounds of intermediate 2. Reagents and conditions: (a) PCC, CH_2Cl_2, rt, 3 h; (b) Ethylenediamine, morpholine, sulfur, reflux, 8 h; (c) O_2, *t*-BuOK, *t*-BuOH, 40 °C, 2 h; (d) H_2O_2, KOH, MeOH, reflux, 100 min.



Scheme 5 The synthesis of oxidation products of intermediate 3. Reagents and conditions: (a) PCC, CH_2Cl_2, rt, 3 h; (b) O_2, *t*-BuOK, *t*-BuOH, 40 °C, 2 h; (c) H_2O_2, KOH, MeOH, reflux, 100 min.

(model 550) microplate reader to calculate the 50% inhibition concentration (IC_{50}). Results of cytotoxicity studies were expressed by the IC_{50} values (Table 1). PD and 5-Fluorouracil were used as the positive control. It was clear that all of the obtained compounds showed different cytotoxic activities and lower toxicity for IOSE144 cells (normal cell).

Conflicts of interest

All authors have no conflict of interest to declare.

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